STRUCTURAL AND BIOSYNTHETIC STUDIES OF THE
ANTIBIOTICS TETRACENOMYCIN X AND STREPTONIGRIN.

A THESIS PRESENTED FOR
THE DEGREE OF
DOCTOR OF PHILOSOPHY
in the
AUSTRALIAN NATIONAL UNIVERSITY

by
CORINNE KHOO LAY YING
DECLARATION

This thesis contains no material previously submitted for a degree in any other University, and to the best of my knowledge and belief, contains no material published or written by another person, except where due reference is made in the text.

Corinne Khoo Lay Ying
ACKNOWLEDGEMENTS

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Finally, my sincere thanks to my family for their unfailing support and to my colleagues for their friendship.
We are continually faced by great opportunities brilliantly disguised as insoluble problems.
SUMMARY

The present work has examined particular areas of natural product chemistry. The structural and biosynthetic aspects of two antibiotics, tetracenomycin X (36) and streptonigrin (80) have been investigated using a combination of spectroscopic, chemical and biosynthetic techniques, and are described in two parts.

The first part begins with a survey of the literature pertaining to the isolation, structure elucidation, possible biosynthesis and biological activity of the tetracenomycin and elloramycin antibiotics (Chapter 1). In the second chapter, our work on the structure determination of tetracenomycin X (36), a new antibiotic isolated from Nocardia mediterranea (strain NT 19), is described. This compound was established to be the 12a-O-methyl derivative of tetracenomycin C (1). An incorporation experiment with [2-13C]acetate established the biogenetic origin of tetracenomycin X (36), and by analogy of the tetracenomycin and elloramycin antibiotics, to be from acetate units via the polyketide pathway. The same experiment also determined the D-ring orientation of the antibiotic (36). The position of the O-methyl angular group, and the relative and absolute stereochemistry of the three chiral centres at C-4, C-4a and C-12a were subsequently determined using chemical and spectroscopic techniques. The biological activity of tetracenomycin X (36) against a range of microorganisms was found to be similar to that of tetracenomycin C. In Chapter 3, we have determined, using [18O2]oxygen, that a total of three oxygens in tetracenomycin X were derived by metabolic oxidation. The 18O2 labelling regiochemistry was established, using a combination of mass spectroscopy and a chemical transformation, to be at C-4, C-5 and C-12a. The C-5 and C-12a oxygen atoms were also established to have been derived from different molecules of oxygen by mass spectral analysis of the labelled compound produced by feeding a known [16O2]- and [18O2]oxygen mixture to the Nocardia strain.

In the second part, a brief survey of the streptonigrin group of antibiotics, (80)-(83), concerning their structure determination, syntheses, biosyntheses and biological activities is described (Chapter 5). In the subsequent chapter (Chapter 6), we have examined
various aspects, not yet understood, of the biosynthesis of streptonigrin (80). The 5-amino-4-phenylpyridine moiety of streptonigrin (80) was determined to have been derived by cleavage of a β-carboline intermediate (133) derived from tryptophan (130). Two possible mechanisms, both involving a hydrolytic process, were studied using a model system, β-carboline-3-carboxylic acid (159). The biosynthetic conversion of streptonigrin (80) or a β-carboline precursor (133) to the 2-pyridone analogue, streptonigrone (82), has also been investigated, using several approaches, as a biomimetic model and as a means of correlating the chirality of the two compounds, (80) and (82). These investigations were studied with the model systems, 2-pyridine carboxylic acid (190), methyl 4-phenyl-2-pyridine carboxylate (227) and β-carboline-3-carboxylic acid methyl ester (159).

In Chapter 7, an investigation of the enantiomeric purity of streptonigrin (80) and streptonigrone (82) is discussed. Two approaches were examined; one involved the use of a chiral lanthanide shift reagent, while the other involved the use of esters of O-methylmandelic acid. An approach to determine the absolute configuration of streptonigrin (80) and streptonigrone (82), involving the use of O-methylmandelate derivatives in the presence of lanthanide shift reagents, is also described.
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<td>4-dimethylaminopyridine</td>
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<td>THF</td>
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<td>tlc</td>
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<td>UV</td>
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CHAPTER 1

INTRODUCTION TO THE TETRACROMYCN AND ELLORAMYCN ANTIBIOTICS

1.1 GENERAL

A new group of antibiotics, the tetracromycins (1) and (2-6), was isolated from the microorganism Streptomyces griseus by Zameck and coworkers\(^\text{1,2}\) as a result of screening tests. The main component of this mixture (listed in Table 1), tetracromycin C (1), is a narrow spectrum antibiotic like the previous. The structures of some of the minor components (3-6) have been determined, while others (7-9) have been suggested to be tetracromycin antibiotics by virtue of their close occurrence with tetracromycin C (1). Tetracromycin C (1) has also been independently isolated by Tanaka from a fungal species (H-581), an antibiotic being named "antibiotic H-581".

Recently, the tetraamycin group of antibiotics was joined by a related group, the ellomycins (10-20), see Table 2 in section 1.3, which were isolated from Streptomyces albidos by Zameck et al.\(^\text{3,4}\) These compounds differ from the tetracromycins only in the substituents. In particular, the ellomycins have a C-8 perimethylated L-phenol in a phenolic acetal linkage.

Hence, to date, a total of fourteen antibiotics belonging to these two groups of antibiotics have been isolated (Tables 1 and 2) from several microorganisms. Most of the chemistry of these compounds has been carried out in connection with structure determination and with structure-activity studies. Defined structures established using a combination of degradation, chemical and spectroscopic techniques, have been assigned to ten of the antibiotics mentioned. Two of these structures, (1) and (29) have been verified by X-ray analysis.\(^\text{9,10}\) The isolation, structure determination, possible biosynthesis and biological activity of these compounds will be discussed in this chapter. For convenience, these antibiotics and their derivatives will be referred to by their trivial names, although the systematic names have been used in the literature.
CHAPTER 1: INTRODUCTION TO THE TETRACENOMYCIN AND ELLORAMYCIN ANTIBIOTICS

1.1 GENERAL

A new group of antibiotics, the tetracenomycins, (1) and (3-8), was isolated from the microorganism *Streptomyces glaucescens* by Zeeck and coworkers\(^1-4\) as a result of screening tests. The main component of this mixture (listed in Table 1), tetracenomycin C (1), is a narrow spectrum anthracycline-like antibiotic. The structures of some of the minor components (3-6) have been determined, whilst others (7-8) have been suggested to be tetracenomycin antibiotics by virtue of their co-occurrence with tetracenomycin C (1). Tetracenomycin C (1) has also been independently isolated by Ye *et al.*\(^5\) from a *Streptomyces* species (H-881), the antibiotic being named "antibiotic H-881".

Recently, the tetracenomycin group of antibiotics was joined by a related group, the elloramycins [(24-29), see Table 2 in section 1.3] which were isolated from *Streptomyces olivaceus* by Zeeck *et al.*\(^6-9\) These compounds differ from the tetracenomycins only in the substituents; in particular, the elloramycins have a C-8 permethylated *L*-rhamnose in a phenolic \(\alpha\)-glycoside linkage.

Hence, to date, a total of fourteen substances belonging to these two groups of antibiotics have been isolated (Tables 1 and 2) from various microorganisms. Most of the chemistry of these compounds has been carried out in connection with structure determination and with structure-activity studies. Defined structures, established using a combination of degradative, chemical and spectroscopic techniques, have been assigned to ten of the antibiotics mentioned. Two of these structures, (1) and (24), have been verified by X-ray analysis.\(^8,9\) The isolation, structure determination, possible biosynthesis and biological activity of these compounds will be discussed in this chapter. For convenience, these antibiotics and their derivatives will be referred to by their trivial names, although the systematic names have been used in the literature.
Table 1: The Tetracenomycin Antibiotics

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracenomycin C</td>
<td>(1) ( R=\text{Me} )</td>
<td><em>Streptomyces glaucescens,</em> <em>Streptomyces species H-881,</em> ethionine-resistant <em>S. glaucescens</em></td>
</tr>
<tr>
<td>3-Demethoxy-3-ethoxy-tetracenomycin C</td>
<td>(2) ( R=\text{Et} )</td>
<td></td>
</tr>
<tr>
<td>Tetracenomycin A_2</td>
<td>(3) ( R^1=\text{CO}_2\text{Me}, \ R^2=R^3=\text{Me} )</td>
<td><em>S. glaucescens</em></td>
</tr>
<tr>
<td>Tetracenomycin D</td>
<td>(4) ( R^1=R^2=R^3=\text{H} )</td>
<td><em>S. glaucescens</em></td>
</tr>
<tr>
<td>Tetracenomycin B_1</td>
<td>(5) ( R^1=R^3=\text{H}, \ R^2=\text{Me} ) or ( R^1=R^2=\text{H}, \ R^3=\text{Me} )</td>
<td><em>S. glaucescens</em></td>
</tr>
<tr>
<td>Tetracenomycin B_2</td>
<td>(6) ( R^1=\text{CO}_2\text{Me}, \ R^2=\text{Me}, \ R^3=\text{H} ) or ( R^1=\text{CO}_2\text{Me}, \ R^2=\text{H}, \ R^3=\text{Me} )</td>
<td><em>S. glaucescens</em></td>
</tr>
<tr>
<td>Tetracenomycin A_1</td>
<td>(7) Not determined</td>
<td><em>S. glaucescens</em></td>
</tr>
<tr>
<td>Tetracenomycin E</td>
<td>(8) Not determined</td>
<td><em>S. glaucescens</em></td>
</tr>
</tbody>
</table>
1.2 THE TETRACENOMYCIN ANTIBIOTICS

Fermentation of *Streptomyces glaucescens* and extraction of the mycelium and culture filtrate produced a mixture of several lipophilic antibiotics.\(^1\)-\(^4\) The structure of the main and most biologically active component, tetracenomycin C,\(^1\)-\(^5\) was largely determined using a combination of degradative and spectral methods, as well as by the synthesis of derivatives and analysis of their spectral data. A single crystal X-ray analysis subsequently proved the structure (1) for tetracenomycin C.\(^8\) The structures of the other antibiotics were determined by comparison of their physical data with those of tetracenomycin C (1).\(^1\)-\(^4\)

1.2.1 The Structure of Tetracenomycin C

Elemental analysis of this compound established a molecular formula of \(\text{C}_{23}\text{H}_{20}\text{O}_{11}\). A partial structure, including the various functional groups as shown in Fig. 1, was proposed on the basis of the spectroscopic data and some chemical transformations of tetracenomycin C. In the IR spectrum, absorptions were observed at 3521 (OH) and between 1600 and 1750 (C=O) cm\(^{-1}\). In addition, the UV spectrum in methanolic hydrochloric acid and methanolic sodium hydroxide was pH dependent, indicating the presence of an ionizable group.

**Figure 1**

- \(\text{Me}\)
- \(\text{C}=\text{O}\)
- \(\text{CO}_2\text{Me}\)
- 2 x \(\text{C}=\text{O}\)
- 2 x \(-\text{OMe}\)
- 2 x \(\text{Ar}-\text{H}\)
- \(\text{C}=\text{CH}\)
- \(\text{CH}-\text{OH}\)

\[\text{HO} \quad \text{C} \quad \text{H}\]
\[\text{HO} \quad \text{C} \quad \text{H}\]
The UV spectrum in chloroform (absorptions at 410 and 391 nm) indicated a close similarity between the chromophore of tetracenomycin C and that of 2,3-dihydro-5-hydroxy-anthracene-1,4-dione (9) \(^3\) (absorptions at 390 and 370 nm).

![Chemical Structure](image)

The presence of one olefinic \((C=CH)\) and two aromatic \((Ar-H)\) protons and the identity of the various functional groups, \textit{i.e.} three carbonyls \((C=O)\), one methoxycarbonyl \((-CO_2Me)\), two tertiary hydroxyls \((C-OH)\), one secondary hydroxyl group \((CH-OH)\), one phenolic hydroxyl \((Ar-OH)\), two methoxyls \((-OMe)\) and one methyl group \((-Me)\) were further deduced by analysis of the \(^1\)H and \(^{13}\)C NMR data. The \(^1\)H NMR data also demonstrated that the olefinic proton experienced a long range coupling \((J = 2 \text{ Hz})\) with the proton of the secondary alcohol \((CH-OH)\), and hence established the structural element as shown in Fig. 1.

Of the fourteen sites of unsaturation (as determined from the molecular formula), it was established from the above observations that four of these were due to carbonyl functions, another four to the tetracyclic ring system and six to aromatic and olefinic double bonds.

Stepwise acetylation of tetracenomycin C with acetic anhydride and pyridine gave a diacetate, a triacetate, and finally a tetraacetate, thus confirming the presence of four hydroxyl groups. [The diacetate, triacetate and tetraacetate were subsequently assigned the structures (10), (11) and (12) respectively.] The \(^1\)H NMR spectrum of the diacetate showed that the phenolic and secondary hydroxyl groups had been acetylated. In the mass spectrum of this diacetate \(^i\) (C\(_{27}\)H\(_{24}\)O\(_{13}\), m/z 556), loss of a methoxyl (mass 31) and characteristic losses of ketene (mass 42) and acetic acid (mass 60) were observed in the higher mass region.

\(^i\) The mass spectrum of this diacetate (10) is discussed in greater detail in Chapter 2 (section 2.3.2).
More importantly, a major fragmentation [subsequently determined to be a retro-diene fragmentation characteristic of the tetracenomycin1-4 and elloramycin5-9 antibiotics] giving the ions at m/z 401 (C_{20}H_{17}O_9) and at m/z 156 (C_7H_8O_4), both of which on loss of ketene gave the ions at m/z 359 (C_{18}H_{15}O_8, 100%) and at m/z 114 (C_5H_6O_3) respectively, was observed (refer Fig.2).

Figure 2
Treatment of tetracenomycin C with 2,2-dimethoxypropane in the presence of p-toluenesulphonic acid, afforded an acetonide in which the secondary alcohol and one of the tertiary hydroxyl functions had reacted. This result indicated that the secondary hydroxyl was in close proximity to one of the tertiary hydroxyl groups. [The structure of the acetonide [either (13) or (14)] has not been defined.]

Therefore, on the basis of all the above observations and chemical transformations, the partial structure consisting of four linearly-annulated, six-membered rings, and the D-ring substitution pattern as shown in Fig.3, was established.

**Figure 3**

The chemical information and spectroscopic data for the derivatives and parent compound, tetracenomycin C, were, however, not sufficient to determine the substitution pattern of the A-ring. Hence, Siebers$^3$ further investigated the structure of this compound by using degradative techniques. Chromic acid oxidation, followed by treatment of the resulting acid with diazomethane gave a pentasubstituted benzene with the various substituents indicated in Fig.4.
Analysis of the off-resonance $^{13}$C NMR data and $^1$H NMR increment calculations for defining the substitution patterns of benzenoid systems narrowed the choice of ten possible isomers to three, i.e. (15), (16) and (17). The identity of the degradation product was then established as (15) by the synthesis of trimethyl 5-methoxy-3-methyl-1,2,4-benzenetricarboxylate and comparison of spectroscopic data (IR, UV, $^1$H NMR and mass spectra) and a mixed melting point.

On the basis of the above information, two partial structures [either (18) or (18a)] were possible for the ABC-rings of tetracenomycin C. Analysis of long range coupling in the $^{13}$C NMR spectrum established that the direction of annulation of the A- and B-rings, as indicated by structure (18) in Fig. 5, was correct. Long range (4-bond) coupling was observed between both aromatic protons [Ar-H] and that of the carbon bearing the hydroxyl group [Ar(C)-OH] as expected for structure (18). Instead of the 3- and 4-bond couplings between the aromatic protons [Ar-H] and the carbon [Ar(C)-OH] as expected for structure (18a).
However, the direction of the annulation of the D-ring could not be determined by analysis of $^{13}$C NMR data (long range coupling) and $^{13}$C increment calculations. Nevertheless, of the two possible orientations of the D-ring, as shown by (1) and (1a), the former structure was favoured on biogenetic grounds (refer Scheme 2 and section 2.4.1 in Chapter 2).

An X-ray structure analysis subsequently established the D-ring orientation as shown in structure (1) to be correct for tetracenomycin C. Originally, no inference
regarding the stereochemistry of the secondary and tertiary hydroxyl groups was drawn from the synthesis of the acetonide [either (13) or (14)]. Therefore, the X-ray analysis also established the relative cis stereochemistry of the three hydroxyl functions.

The absolute configuration of the secondary (C-4) alcohol was determined using the method of Helmchen \(^\text{10}\) (vide infra Chapter 2, section 2.4). The synthesis of the 4-o-2'-phenylbutyryl esters, (52) and (53) of tetracenomycin C with (R)- and (R)(S)-2-phenylbutyric acid, and subsequent analysis of their \(^1\)H NMR data established the absolute configuration at C-4 as (R). Accordingly, the absolute stereochemistry at C-4a and C-12a was determined to be also (R). Hence, the complete structure, as indicated by (1), was established for tetracenomycin C.

1.2.2 Other Tetracenomycin Antibiotics

The minor components of the tetracenomycin mixture, isolated by Zeeck and coworkers \(^{1-4}\) from \textit{Streptomyces glaucescens}, were found to be very lipophilic and to have very low solubilities in water and organic solvents. The structures of tetracenomycins \(A_2\) (3) and \(D\) (4) have been established by analysis of their spectral data and comparison with those of tetracenomycin C (1). The main difference between these two antibiotics and tetracenomycin C (1) is the absence of the angular hydroxyl groups.

Two structures each were proposed as possibilities for the compounds, tetracenomycins \(B_1\) (5) and \(B_2\) (6). In contrast, tetracenomycins \(A_1\) (7) and \(E\) (8), were thought to be tetracenomycin antibiotics by virtue of their co-occurrence with
tetracenomycin C (1) and their intense red-orange colour on thin layer chromatographic plates.

Another analogue, 3-demethoxy-3-ethoxy-tetracenomyin C (2), was isolated from a mutant strain, an ethionine-resistant *S. glaucescens* cultivated in the presence of DL-ethionine.4 This compound, not produced by the wild type *S. glaucescens*, can also be obtained by chemical modification of tetracenomycin C (1).

1.2.3 Biological Activity

The tetracenomycin group of antibiotics displayed a relatively narrow spectrum of biological activity. In disc diffusion assays against a range of microorganisms, tetracenomycin C (1) was found to have little or no activity against Gram-negative bacteria and yeasts.1-4 This antibiotic was, however, highly active against some strains of Gram-positive bacteria such as *Streptomyces*. Tetracenomycin C (1) also showed strong cytotoxity towards fibroblasts of chicken embryos and mice, and towards P-388 leukemia cells.2 The mode of action of tetracenomycin C (1) appeared to be via interaction with DNA, presumably by intercalation. The interaction of tetracenomycin C (1) with DNA and preferential inhibition of RNA polymerase is similar to the mode of action of the antibiotics aclacinomycin A (19)11a,b and neothramycin (20),11c and may be the reason for its cytotoxicity and its antitumour activity.
The biological activity of various derivatives of tetracenomycin C (1) was also investigated. The acetate derivatives, (10) and (12), and 2-chloro compound (21), had little or no antimicrobial activity. The 6-bromo and 6-chloro analogues, (22) and (23), on the other hand, exhibited a ten-fold increase in activity, whilst the antimicrobial activity of 3-demethoxy-3-ethoxy-tetracenomycin C (2) was similar to that of the parent compound (1). The other naturally-occurring tetracenomycins, (3)-(8), showed only very poor antimicrobial activity against some *Arthrobacter* strains.

![Chemical Structure](image)

(21) $R^1=\text{Cl}, R^2=\text{H}$
(22) $R^1=\text{H}, R^2=\text{Br}$
(23) $R^1=\text{H}, R^2=\text{Cl}$

### 1.3 THE ELLORAMYCIN ANTIBIOTICS

A new group of antibiotics, the elloramycins (24)-(29), was detected during screening tests by Zeeck and coworkers as metabolic products of the microorganism *Streptomyces olivaceus*. The main component of this mixture of compounds, elloramycin (24), is an anthracycline-like antibiotic. Its structure was established by comparison of its spectroscopic data with those of the known tetracenomycin C (1), by analysis of its hydrolytic products and its derivatives, and by an X-ray structure analysis. The structures of the other antibiotics in the elloramycin group were determined by comparison of their spectroscopic data with those of elloramycin (24).

#### 1.3.1 The Structure of Elloramycin

Elemental analysis and high resolution mass measurements established a molecular formula of $C_{32}H_{36}O_{15}$ for elloramycin (24).
Table 2: The Elloramycin Antibiotics

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elloramycin</td>
<td><img src="24" alt="Elloramycin Structure" /></td>
<td>S. olivaceus</td>
</tr>
<tr>
<td>Elloramycin B</td>
<td>(25) $R^4 = R^2 = R^3 = H$, $R^4 = Me$</td>
<td>S. olivaceus</td>
</tr>
<tr>
<td>Elloramycin C</td>
<td>(26) $R^4 = H$, $R^2 = R^3 = Me$</td>
<td>S. olivaceus</td>
</tr>
<tr>
<td>Elloramycin D</td>
<td>(27) $R^4 = H$, $R^2 = R^4 = Me$</td>
<td>S. olivaceus</td>
</tr>
<tr>
<td>Elloramycin F</td>
<td>(29) $R^1 = OH$, $R^2 = R^3 = R^4 = Me$</td>
<td>S. olivaceus</td>
</tr>
<tr>
<td>Elloramycin E</td>
<td><img src="28" alt="Elloramycin E Structure" /></td>
<td>S. olivaceus</td>
</tr>
</tbody>
</table>
The UV absorption maxima (at different pH values) and the IR spectrum (in the region of 1800-1200 cm\(^{-1}\)) of elloramycin demonstrated similarity of the chromophore of the new compound to that of the known antibiotic, tetracenomycin C (1). The \(^1\)H and \(^{13}\)C NMR and mass spectra of elloramycin (24), in comparison with the data of tetracenomycin C (1), also showed an extensive similarity of the tetracyclic carbon skeleton and its substituents.

In the \(^1\)H NMR spectrum of elloramycin, only the 7-H signal exhibited a significant shift relative to the proton resonances observed for tetracenomycin C (1). An aliphatic methoxyl resonance was also observed at \(\delta 3.65\) ppm. On comparison with published \(^1\)H NMR data for methyl glycosides, additional signals in the \(^1\)H NMR spectrum of elloramycin were assigned to a methylated 6-deoxyhexapyranose. These observations were confirmed by analysis of the \(^{13}\)C NMR data of elloramycin, and by the loss, in the mass spectrum, of a \(C_9H_{16}O_4\) unit (m/z 188) from the molecular ion \((C_{32}H_{36}O_{15}, m/z 660)\), giving a peak at m/z 472 corresponding to the molecular weight of tetracenomycin C (1).

Hydrolysis of elloramycin (24) in aqueous trifluoroacetic acid gave the aglycone, elloramycinone (30) and a sugar, subsequently identified as the permethylated (L)-rhamnose (31) (Scheme 1). From the spectroscopic data, it was clear that elloramycinone (30) differed from tetracenomycin C (1) only in the methylation pattern. Indeed, on treatment with methyl iodide and silver oxide, both compounds, (30) and (1), yielded permethyl ethers (32) which were identical in all respects, including their CD spectra (Scheme 1), and thus established that elloramycinone (30) and tetracenomycin C (1) had the same carbon framework, similar substituents and a corresponding stereochemistry.

Analysis of the UV, \(^1\)H NMR and mass spectra of elloramycinone (30) and elloramycin (24) established the position of the glycosidic linkage to the sugar as C-8. The authors attempted to solve the remaining ambiguities, namely the positions of the angular methoxyl and hydroxyl groups at C-4a and C-12a by oxidative degradation of elloramycinone, but were unsuccessful. On the other hand, acceptable yields were obtained for the synthesis of an acetonide derivative (35) of elloramycin.
Molecular model studies suggested that the energetically favoured 5-membered 4,4a-acetonide (35) was the most probable derivative, and that the angular methoxyl and hydroxyl groups were attached at C-12a and C-4a, respectively. Therefore, from these observations and from the known stereochemistry of tetracenomycin C (1), the structure (30) was established for elloramycinone. An X-ray structure analysis of elloramycin (24) subsequently supplied the proof for the above conclusion.
On comparison of $^1$H and $^{13}$C NMR data of elloramycin (sugar portion) with those of methyl glycosides given in literature, a permethylated rhamnose was presupposed for the sugar moiety of elloramycin (24). The identity and absolute configuration of the sugar moiety was established as (31) when the methyl glycoside [synthesised by treatment of compound (31) with acidic methanol] was found to be identical with a sample of methyl 2,3,4-tri-$O$-methyl-$\alpha$-L-rhamnopyranoside (34).

The final proof of the structure (24) for elloramycin was obtained by an X-ray structure analysis of the compound.\(^9\)

### 1.3.2 Other Elloramycin Antibiotics

The other five antibiotics, elloramycins B-F, (25)-(29) (refer Table 2), were isolated from the same organism, although in smaller amounts.\(^6\)-\(^8\) The structures (25)-(29) were established for elloramycins B, C, D, E and F, respectively, by comparison of their spectroscopic data with those of elloramycin (24).

The similarity of the UV and CD spectra indicated that the new antibiotics possessed the same chromophore and the same stereochemistry as elloramycin (24). In the EI mass spectra, key fragments at m/z 472 (elloramycins C and D), 470 (elloramycin E) and 458 (elloramycin B) were assigned to the aglycones of the various elloramycins. The sugar moieties were observed at m/z 189 (elloramycins E and F) and at m/z 175 (elloramycins B-D). These fragments in the mass spectra indicated the extent of methylation of the aglycones or sugar moieties with respect to elloramycin (24).
The change of a methoxy group to a hydroxyl group resulted in significant downfield shifts of the adjacent proton, i.e. 2'-H for elloramycins B (25) and D (27), and 3'-H for elloramycin C (26) in the $^1$H NMR spectra. The absence of a 12a-methoxy signal in elloramycin B (25) and the absence of a 6-H signal in combination with an additional hydroxyl (hydrogen-bonded, aromatic) signal for elloramycin F (29) proved the given structures.

1.3.3 Biological Activity

The sensitivities of various bacteria and fungi (for example, *Escherichia coli*, *Bacillus subtilis*, *Micrococcus roseus*, *S. glaucescens*, *S. lavendulae*, *S. prasinus*, *Borrelia cinerea* and *Mucor miehei*), in disc diffusion assays, towards elloramycin (24) were compared to those of tetracenomycin C (1).\(^6\)\(^-\)\(^8\) The results showed that the antimicrobial activity of elloramycin (24) against Gram-positive bacteria such as *Sireptomyces*, was similar to that of tetracenomycin C (1). A similar mode of action by intercalation of the molecule into the DNA was assumed.

Elloramycin (24), however, exhibited weaker *in vitro* antitumour activity in comparison to tetracenomycin C (1), and no *in vivo* antitumour activity was detected. The high level of methylation of this antibiotic (24) may be one reason for its lower cytotoxic activity.

The biological activities of various derivatives of elloramycin were also tested. In the disc diffusion assay, the activities of elloramycinone (30), elloramycinone-8-methyl ether (36) \(^ii\) and isoelloramycin (37), against various bacteria and fungi, were comparable to that of elloramycin (24).

\(^{ii}\) This compound, also named tetracenomycin X, has been isolated from *Nocardia mediterranea* and its structure determination is discussed in Chapter 2.
In the \textit{in vitro} test against L-1210 leukemia cells, elloramycinone-8-methyl ether (36) also showed activity comparable to that of elloramycin (24), whilst elloramycinone (30) and isoelloramycin (37) showed very little activity.

Structure-activity considerations suggested that the less methylated (\textit{i.e.} less lipophilic) elloramycin antibiotics would have better solubility necessary for uptake by living cells, and that additional polar groups would possibly enhance antitumour activity by increasing interaction with DNA. Hence, the other elloramycin antibiotics, (25)-(29), were also tested against a variety of microorganisms and were found to be strongly active against \textit{Streptomyces} species, including the producer strain, \textit{S. olivaceus}. In particular, the less methylated elloramycin B (25) was found to have higher activity against Gram-positive bacteria than elloramycin (24).

1.4 BIOSYNTHESIS OF THE TETRACENOMYCIN AND ELLORAMYCIN ANTIBIOTICS

Prior to our present studies (refer Chapters 2 and 3), no work on the biosynthesis of the tetracenomycin and elloramycin antibiotics had been reported. A possible scheme for the biogenesis of the tetracenomycins involving the formation of polyketide intermediates was proposed by Siebers\textsuperscript{3} in an unpublished dissertation (Scheme 2). A similar biosynthetic scheme was also proposed independently, together with an alternative, in the present work before Siebers’ unpublished dissertation became available (see Chapters 2 and 3).

The proposed polyketide pathway could be considered in terms of a primary or assembly phase, followed by a secondary or modification phase. In essence, ten acetate units, possibly activated as their coenzyme A thioesters, could be condensed in a head-to-tail fashion to form enzyme-bound polyketide intermediates, for example, decaketide (38). The polyketide intermediate (38) could then undergo stabilizing reactions, typically intramolecular cyclization and aromatization to the tetracyclic intermediate (40).
Scheme 2

10 x MeCO₂H → Condensation → Enz-S-O

Cyclization →

Oxidation / Hydroxylation →

Methylation → other tetracenomycins

Glycosidation and Methylation →

and other elloramycins
Further functionalisation of the primary products would then give the various tetracenomycin antibiotics. Hence, oxidation and hydroxylation of the tetracyclic intermediate (40), followed by methylation of the acid (42) would give tetracenomycin C (1). On the other hand, oxidation of the intermediate (40) followed by methylation of the acid (41) would lead to the various tetracenomycin antibiotics (Scheme 2).

The biosynthesis of the related group of antibiotics, the elloramycins, is assumed to be similar to that of the tetracenomycins (refer Scheme 2). Head-to-tail condensation of acetate units, followed by cyclisation, aromatisation and oxidation would give a precursor (41) common to both the elloramycins and the tetracenomycins. At this point, divergence of the biosynthetic pathway by different modes of functionalization would then give the respective antibiotics. For example, glycosidation and methylation of compound (41) would give elloramycin (24), whereas tetracenomycin C (1) would be derived from methylation of the common precursor (41).

1.5 THE PRESENT WORK

The isolation, structure determination and biogenesis of tetracenomycin X (36), a new member of the tetracenomycin group of antibiotics isolated from *Nocardiopsis mediterranea* strain NT 19, has been investigated in the present work.

At the time the present work commenced, Zeeck and coworkers\(^1\)\(^-\)\(^4\) had established the partial structures, as shown in Fig.5, for tetracenomycin C. However, they had been unable to determine the D-ring orientation of the tetracyclic system and thus to distinguish between the two possible structures, (1) and (1a). Of the two possible structures, however, structure (1) was favoured on biogenetic grounds.

An attempt by Siebers\(^3\) to establish the orientation of the D-ring by X-ray structure analysis had been unsuccessful due to difficulties in resolving the X-ray crystal data. Other attempts by the same author\(^3\) to determine the direction of annulation of the D-ring using long range coupling in the \(^{13}\)C NMR spectrum and \(^{13}\)C increment calculations had also been unsuccessful. In addition, no details were given
concerning the stereochemistry (relative and absolute) of the secondary and tertiary hydroxyl functions.

We initially isolated, from *Nocardia mediterranea* strain NT 19, a compound responsible for the strong antibiotic activity as shown by cultures of this organism, and established that it was an α-methyl derivative of tetracenomycin C (1). Accordingly, we named this compound tetracenomycin X. The additional methyl group was shown to be located on one of the angular hydroxyl groups at C-4a or C-12a. We then faced the problem already confronting Zeeck and coworkers, viz that of the direction of annulation of the D-ring of the tetracenomycins. Our solution was to study the pattern of labelling in tetracenomycin X resulting from biosynthetic incorporation of [1-13C]acetate. This experiment (discussed in Chapter 2) verified the expected biogenetic origin of the tetracenomycin (and elloramycin) antibiotics. More significantly, however, it provided conclusive evidence for the D-ring orientation of tetracenomycin X, and of the other tetracenomycins isolated by Zeeck and his collaborators, thus establishing (1) as the correct structure for tetracenomycin C. The angular α-methyl group of tetracenomycin X was subsequently located at C-12a using spectroscopic techniques, and is described in Chapter 2.

The relative and absolute stereochemistry of the C-4 and C-4a hydroxyls were determined using a combination of chemical and spectroscopic methods, and are discussed in Chapter 2. All the above work was presented at the 5th National Royal Australian Chemical Institute Conference in May, 1986. Attempts to determine the relative (and absolute) stereochemistry of the C-12a α-methyl group were, however, unsuccessful.

In 1984, Rohr reported, in an unpublished dissertation, the X-ray structure analysis for tetracenomycin C (1), and established the absolute configuration of the C-4 secondary alcohol (and accordingly, of the C-4a and C-12a hydroxyls). The same author and his collaborators subsequently reported the isolation of elloramycin and its congeners, a new group of antibiotics produced by *S. olivaceus*. The structure (24) for elloramycin was established by comparison of its spectroscopic data with those of tetracenomycin C (1), by analysis of its hydrolytic products and its derivatives, and subsequently confirmed by an X-ray structure analysis. In particular, the cis
stereochemistry at C-4, C-4a and C-12a of the aglycone, elloramycinone (30), was
determined by synthesising the tetramethyl derivative, which on comparing spectral
data, proved to be identical to the tetramethyl derivative (32) of the tetracenomycin C
(vide supra section 1.3.1). Hence, the relative (and absolute) stereochemistry of the C-
12a o-methyl group of tetracenomycin X was subsequently established in our
laboratories by synthesising the trimethyl derivative, and comparing its spectroscopic
data, viz the CD spectrum, with those of tetramethyl tetracenomycin C (or tetramethyl
ellaromycinone) (32). This aspect of our work is also described in Chapter 2.

Finally, we have examined the biosynthetic origin of the oxygen atoms of
tetracenomycin X (36), in particular of the vicinal diol system at C-4a and C-12a, and
of the C-4 hydroxyl and the C-5 carbonyl groups by fermentation of Nocardia
mediterranea strain NT19 under an atmosphere enriched with $^{18}O_2$ oxygen. This study
is described in Chapter 3.
CHAPTER 2 - ISOLATION AND STRUCTURE DETERMINATION OF TETRACYCLIN X

2.1 INTRODUCTION

This chapter deals with the isolation and structure determination of tetracyclin X (43) from Micrococcus radiodurans strain ST 19, from the collection of the Commonwealth Serum Laboratories, Melbourne. Originally, Holder and coworkers\textsuperscript{11} reported the isolation of the antibiotic tetracyclin TV (40)\textsuperscript{12} from this microorganism. Their identification of the isolated compound as tetracyclin TV \(\text{(40)}\) was based on spectral data and observations. Firstly, the mobility of the isolated compound in several solvents was identical to that of a tetracyclin TV standard. Each sample examined spectrophotometrically also showed an absorption maximum at 445 nm corresponding to one of those of tetracyclin TV (40) (446, 376, and 273 nm). Secondly, the isolated compound, when assayed by the cell culture plate method using a penicillin-resistant strain of \(B. subtilis\) (strain T2, a Gram-positive bacterium), had an activity of a range equivalent to that of tetracyclin TV at 10 \(\mu\)g/ml. Thirdly, crude concentrated material was active in vivo against \(B. subtilis\) sub-dermal.

In subsequent work by Richards and coworkers\textsuperscript{12} however, the \(B. subtilis\) strain, grown under identical conditions to those reported by Ormerod \textit{et al.}\textsuperscript{12} failed to produce tetracyclin TV (43). Instead, a yellow compound which lost an antimicrobial activity
CHAPTER 2: ISOLATION AND STRUCTURE DETERMINATION OF TETRACENOMYCIN X

2.1 INTRODUCTION

This chapter deals with the isolation and structure determination of tetracenomycin X (36) from Nocardia mediterranea, strain NT 19 from the collection of the Commonwealth Serum Laboratories, Melbourne. Originally, Birner and coworkers\textsuperscript{13} reported the isolation of the antibiotic rifamycin SV (43)\textsuperscript{14a-c} from this Nocardia strain. Their identification of the isolated compound as rifamycin SV (43) was based on several tests and observations. Firstly, the mobility of the isolated compound, in several solvent systems, corresponded to that of a rifamycin SV standard. Eluted samples examined spectrometrically also showed an absorption maximum at 445 nm corresponding to one of those of rifamycin SV (43) (445, 314 and 223 nm). Secondly, the isolated compound, when assayed by the cylinder plate method using a penicillin-resistant strain of Staphylococcus aureus (a Gram-positive bacterium), had an activity in a range equivalent to that of rifamycin SV at 15-35 \( \mu \)g/mL. Thirdly, crude concentrated material was active \textit{in vitro} against Mycobacterium tuberculosis.

In subsequent work by Rickards and coworkers,\textsuperscript{15} however, the Nocardia strain, grown under identical conditions to those reported by Birner \textit{et al.},\textsuperscript{13} failed to produce rifamycin SV (43). Instead, a yellow compound which had no antimicrobial activity
in disc assays on seeded agar plates against a Gram-negative bacterium (*Bacillus subtilis*) and a yeast (*Saccharomyces cerevisiae*), but was highly active against some strains of *Streptomyces* (Gram-positive bacteria) such as *S. aureofaciens* and *S. fragilis* (refer Table 8), was isolated. We were interested in this yellow compound because of its narrow spectrum of activity.

In the present work, it is shown that this compound, named tetracenomycin X, is a new antibiotic, the 12α-O-methyl derivative of the antibiotic tetracenomycin C (1).\(^1\)\(^-\)\(^5\)\(^,\)\(^8\) It is possible that the microorganism *Nocardia mediterranea* had, by this stage, undergone mutation, and thereby no longer produced rifamycin SV (43) as reported by Birner and his collaborators.\(^13\) On the other hand, however, although several tests were conducted, the identification of the isolated compound as rifamycin SV (43) was not based on detailed comparison with published spectral data.\(^14\)\(^a\)\(^-\)\(^c\) In addition, none of the various test results reported by Birner and coworkers\(^13\) were in disagreement with those observed for tetracenomycin X. For example, tetracenomycin X exhibited an absorption maximum at 440 nm in methanolic sodium hydroxide [compared to the absorption maximum at 445 nm observed for the eluted samples (*vide supra*), and was also active against Gram-positive bacteria. It is therefore possible that the yellow compound originally produced by this *Nocardia* strain\(^13\) was wrongly identified as rifamycin SV (43).\(^14\)\(^a\)\(^-\)\(^c\)

### 2.2 ISOLATION

The organism was originally grown on a complex liquid medium in still culture following the description provided by Birner *et al.*\(^13\) However, the yield of the antibiotic was very low (2 mg/L). In later fermentations, improved production of the antibiotic (12 mg/L) was obtained by growth of the *Nocardia* strain in baffled conical flasks in a modified production medium containing glucose (20 g/L) and Bovril (20 g/L) in distilled water.\(^3\) The flasks were incubated at 28°C on a rotary shaker. After fermentation for 70 hours, the aqueous culture was acidified with dilute hydrochloric acid, centrifuged and filtered. The filtrate was extracted with ethyl acetate and the
Scheme 3: Isolation of Tetracenomycin X (36)

*Nocardia mediterranea* (strain NT 19)
broth (3.5 L, pH 7.2)

- acidified to pH 3
- centrifuged
- filtered
-(cell mass)

- extracted with acetone
  - acetone extracts
  - extracted with ethyl acetate
  - ethyl acetate extracts

- combined and concentrated
- redissolved in CHCl₃
- 2% sodium bicarbonate

- (sodium bicarbonate soluble fraction)

- Sephadex LH20 chromatography
- HPLC

tetracenomycin X (36)
(42 mg)
mycelium was dried and extracted repeatedly with acetone. The ethyl acetate and mycelium acetone extracts were combined, dried and evaporated. The residue was partitioned between chloroform and 2% aqueous sodium bicarbonate. The chloroform solution containing the bicarbonate insoluble fraction was evaporated to give a brown tar which was purified by chromatography on three successive Sephadex LH20 columns. Final purification was achieved by reverse-phase HPLC using 70% methanol in water as the solvent system (refer Scheme 3).

2.3 GENERAL PROPERTIES AND COMPARISON WITH TETRACENOMYCIN C

2.3.1 General Properties

The new antibiotic, which we have named tetracenomycin X, was isolated as a yellow amorphous solid which was stable at room temperature for several weeks. Analytical thin-layer chromatography and HPLC showed it to be homogeneous. The IR spectrum (Fig.6) measured in chloroform solution exhibited absorptions at 3680 and 3450 cm\(^{-1}\), indicative of hydroxyl functions, and showed strong bands at 1730 (C=O ester), 1710 and 1690 (C=O), and 1605 (aromatic) cm\(^{-1}\).

The UV spectrum (Fig. 7) was pH dependent, indicating the presence of an ionisable group. In methanolic hydrochloric acid, absorption maxima were observed at 408 (\(\epsilon\) 11,500), 391 (\(\epsilon\) 11,200) and 289 nm (\(\epsilon\) 35,000), whereas on treatment with aqueous sodium hydroxide, shifts of the UV absorption maxima to 440 (\(\epsilon\) 12,100) and 255 (\(\epsilon\) 29,500) nm were observed. The optical rotation \([\alpha]_{D}^{22} = +51^\circ\) (c = 1. dioxane) showed that the molecule was chiral. High resolution mass measurement of the putative molecular ion, m/z 486.1162, established a molecular formula of C\(_{24}\)H\(_{22}\)O\(_{11}\) for tetracenomycin X.
Figure 6: IR Spectrum (in CHCl₃) of Tetracenomycin X (36)

![IR Spectrum of Tetracenomycin X](image)

Figure 7: UV Spectrum of Tetracenomycin X (36)

![UV Spectrum of Tetracenomycin X](image)
2.3.2 Comparison with Tetracenomycin C

The above features, in particular the UV spectrum, suggested a close similarity of the new compound to tetracenomycin C (1), \( \text{C}_{23} \text{H}_{20} \text{O}_{11} \), an antibiotic isolated from *Streptomyces glaucescens* by Zeeck and coworkers,\(^1\)\(^4\) the history of which was reviewed in Chapter 1. Tetracenomycin C (1) showed an UV absorption spectrum with maxima at 403 (\( \epsilon \) 13,000), 386 (\( \epsilon \) 12,500) and 287 (\( \epsilon \) 47,600) nm in methanolic hydrochloric acid, but the absorption maxima shifted to 436 (\( \epsilon \) 13,000), 282 (\( \epsilon \) 19,200, sh) and 255 (\( \epsilon \) 33,800) nm in methanolic sodium hydroxide. In the IR spectrum (potassium bromide disc), absorption bands were observed at 1745 (C=O ester), 1724 (C=O), 1684 (C=O) and 1613 (C=O with H-bonding) cm\(^{-1}\).

As mentioned previously, at the time the present work commenced, the D-ring orientation and the stereochemistry of the three chiral centres (C-4, C-4a and C-12a) of tetracenomycin C had not been determined (refer section 1.5). Hence, there were two possible structures, (1) and (1a) (see Chapter 1, Fig.5), each of which had unknown stereochemistry, for tetracenomycin C with the former structure being favoured on biogenetic grounds. Similarly, tetracenomycin X also has two possible structures, (36) and (36a) [with respect to the D-ring only (refer Fig.11)]. However, it should be noted that for convenience in assigning spectral features, only the ultimately deduced structures will be written for tetracenomycins C and X, and their derivatives in the discussion on spectroscopic and chemical work carried out throughout this chapter.

Direct comparison of the \(^1\)H and \(^{13}\)C NMR spectra of the new antibiotic, and mass spectra of the diacetyl derivative with those of tetracenomycin C (1) and the diacetyl derivative (10) established tetracenomycin X as an \( \alpha \)-methyl derivative of tetracenomycin C (1). A detailed discussion of the spectroscopic data is presented in the following sections.
The $^1H$ NMR spectrum of the new antibiotic, tetracenomycin X measured at 200 MHz in deuteriochloroform is shown in Fig. 8. Signals were observed at $\delta$ 13.92 (singlet, phenolic hydroxyl, exchanged on addition of $D_2O$), $\delta$ 7.95 and 7.16 (both singlets, two aromatic protons), $\delta$ 5.55 (doublet with $J$=1.5 Hz, olefinic proton), $\delta$ 4.79 (doublet of doublets with $J$=10 and 1.5 Hz, methine proton), $\delta$ 4.41 (broad singlet, hydroxyl, exchanged on addition of $D_2O$), $\delta$ 4.0 (singlet, two methoxyl groups), $\delta$ 3.79 and 3.64 (both singlets, two methoxyl groups) and $\delta$ 2.87 (singlet, C-methyl, and doublet with $J$=10 Hz, hydroxyl, exchanged on addition of $D_2O$) ppm.

Apart from the extra O-methyl signal at $\delta$ 3.64 ppm, the $^1H$ NMR spectrum of the new antibiotic was very similar to that of tetracenomycin C (1) (refer Table 3), and thus established the close structural relationship between the two compounds.

Figure 8: 200 MHz $^1H$ NMR Spectrum (in CDCl$_3$) of Tetracenomycin X (36) ($\delta$ in ppm)
Table 3: Comparative $^1$H NMR Data (in CDCl$_3$) of Tetracenomycins C (1) and X (36).

<table>
<thead>
<tr>
<th>Assignment+</th>
<th>Tetracenomycin C (1) ($\delta$ in ppm, 100 MHz)</th>
<th>Tetracenomycin X (36) ($\delta$ in ppm, 200 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-H (d, 1H, $J$=1.5 Hz)</td>
<td>5.70</td>
<td>5.55</td>
</tr>
<tr>
<td>3-OCH$_3$ (s, 3H)</td>
<td>3.86</td>
<td>3.79</td>
</tr>
<tr>
<td>4-H (dd, 1H, $J$=10 Hz, $J$=1.5 Hz)</td>
<td>4.90</td>
<td>4.79</td>
</tr>
<tr>
<td>4-OH (d, 1H, $J$=10 Hz)</td>
<td>2.92*</td>
<td>2.87*</td>
</tr>
<tr>
<td>4a-OH (bs, 1H)</td>
<td>4.30*</td>
<td>4.41*</td>
</tr>
<tr>
<td>6-H (s, 1H)</td>
<td>7.98</td>
<td>7.95</td>
</tr>
<tr>
<td>7-H (s, 1H)</td>
<td>7.17</td>
<td>7.16</td>
</tr>
<tr>
<td>8-OCH$_3$ (s, 3H)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>9-COOCH$_3$ (s, 3H)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>10-CH$_3$ (s, 3H)</td>
<td>2.85</td>
<td>2.87</td>
</tr>
<tr>
<td>11-OH (s, 1H)</td>
<td>13.88*</td>
<td>13.92*</td>
</tr>
<tr>
<td>12a-OH (s, 1H)</td>
<td>4.86*</td>
<td>--.--</td>
</tr>
<tr>
<td>12a-OCH$_3$ (s, 3H)</td>
<td>--.--</td>
<td>3.64</td>
</tr>
</tbody>
</table>

* Exchanged on addition of D$_2$O.
+ Assignments (except for 12a-OCH$_3$) are those reported for tetracenomycin C (1), and are reasonably extended to tetracenomycin X (36).

$^{13}$C NMR Spectrum

The 50 MHz natural abundance $^{13}$C NMR spectrum of tetracenomycin X in deuteriochloroform is shown in Fig. 9. All the carbon atoms gave discrete resonances under proton-noise decoupled conditions except for two carbons (C-6 and C-11a at $\delta$ 121 ppm) which were equivalent. The assignments of the $^{13}$C NMR spectrum of the new antibiotic, tetracenomycin X (refer Table 4) were made by comparison with reported data for tetracenomycin C (1), by $^{13}$C-$^1$H long range selective decoupling experiments, and from the results of the biosynthetic experiment (section 2.4).
The $^{13}\text{C}$ NMR spectrum and assignments of tetracenomycin C (1) have been reported by Zeeck and coworkers.\textsuperscript{1-4} Their assignments were made by the use of $^{13}\text{C}$ increment calculations and $^{13}\text{C}-^1\text{H}$ long range selective proton decoupling experiments.

Apart from an additional $\alpha$-methyl signal at $\delta$ 56.2 ppm, the $^{13}\text{C}$ NMR spectrum of the new compound is similar to that of tetracenomycin C (1), thus further establishing the close structural relationship between tetracenomycins C and X.

Figure 9: 50 MHz $^{13}\text{C}$ NMR Spectrum (in CDCl$_3$) of Tetracenomycin X (36) ($\delta$ in ppm)
Table 4: $^{13}$C NMR data of Tetracenomycins C (1) and X (36) (δ in ppm).

<table>
<thead>
<tr>
<th>Assignments</th>
<th>Tetracenomycin C (1) (DMSO-d$_6$, 25 MHz)</th>
<th>Tetracenomycin X (36) (CDCl$_3$) (50 MHz)</th>
<th>Tetracenomycin X (36) (DMSO-d$_6$) (50 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>190.8</td>
<td>190.7</td>
<td>191.6</td>
</tr>
<tr>
<td>C-2</td>
<td>99.6</td>
<td>101.7</td>
<td>100.8</td>
</tr>
<tr>
<td>C-3</td>
<td>167.2</td>
<td>167.8</td>
<td>167.5</td>
</tr>
<tr>
<td>C-4</td>
<td>69.3</td>
<td>69.7</td>
<td>69.6</td>
</tr>
<tr>
<td>C-4a</td>
<td>83.4</td>
<td>83.8</td>
<td>85.5</td>
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<tr>
<td>C-5</td>
<td>193.6</td>
<td>193.1</td>
<td>193.5</td>
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<tr>
<td>C-5a</td>
<td>140.1</td>
<td>140.5</td>
<td>140.6</td>
</tr>
<tr>
<td>C-6</td>
<td>118.8</td>
<td>121.1</td>
<td>120.2</td>
</tr>
<tr>
<td>C-6a</td>
<td>128.4</td>
<td>129.3</td>
<td>128.7</td>
</tr>
<tr>
<td>C-7</td>
<td>108.2</td>
<td>109.0</td>
<td>109.5</td>
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<tr>
<td>C-8</td>
<td>157.0</td>
<td>158.1</td>
<td>157.5</td>
</tr>
<tr>
<td>C-9</td>
<td>109.0</td>
<td>107.4</td>
<td>108.6</td>
</tr>
<tr>
<td>C-10</td>
<td>136.6</td>
<td>138.9</td>
<td>137.2</td>
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<td>C-10a</td>
<td>128.3</td>
<td>126.7</td>
<td>128.0</td>
</tr>
<tr>
<td>C-11</td>
<td>165.7</td>
<td>167.0</td>
<td>166.0</td>
</tr>
<tr>
<td>C-11a</td>
<td>118.8</td>
<td>121.1</td>
<td>120.3</td>
</tr>
<tr>
<td>C-12</td>
<td>197.4</td>
<td>195.6</td>
<td>196.7</td>
</tr>
<tr>
<td>C-12a</td>
<td>84.9</td>
<td>87.0</td>
<td>87.7</td>
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<tr>
<td>10-CH$_3$</td>
<td>20.7</td>
<td>21.0</td>
<td>20.9</td>
</tr>
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<td>3-OCH$_3$</td>
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<td>56.8#</td>
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<td>56.9#</td>
<td>57.0#</td>
</tr>
<tr>
<td>9-OCH$_3$</td>
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<td>52.7</td>
<td>52.9</td>
</tr>
<tr>
<td>12-OCH$_3$</td>
<td>----</td>
<td>56.2#</td>
<td>55.8#</td>
</tr>
<tr>
<td>Ester C=O</td>
<td>174.4</td>
<td>171.0</td>
<td>173.5</td>
</tr>
</tbody>
</table>

# Interchangeable.
Preparation of Tetracenomycin X Diacetate and Analysis of Its Mass Spectrum

Details of the mass spectrum of tetracenomycin C diacetate (10), in which only the phenolic and secondary hydroxyls are acetylated, but not of the parent compound tetracenomycin C (1), were reported by Siebers. Acetylation of tetracenomycin X under similar conditions to those described by Siebers for the synthesis of tetracenomycin C diacetate (10) also gave a diacetate. Analysis of the spectral data of this product showed that the same phenolic and secondary hydroxyl functions were esterified as with tetracenomycin C diacetate (10), leading to the structure (44) for the new diacetate. Thus, significant downfield shifts (Δδ 1.18 and 0.48 ppm) of the C-4 and C-6 proton resonances were observed, indicating that the C-4 and C-11 hydroxyl groups had been acetylated. [This indicated that one of the tertiary hydroxyls carried the additional methyl group (refer section 2.4.2)].

\[
\begin{align*}
\text{(10)} & \quad R=H \\
\text{(44)} & \quad R=\text{Me}
\end{align*}
\]

It was also clear on analysis of the \(^1\)H NMR spectrum of tetracenomycin X diacetate (44), that the same C-8 phenolic hydroxyl carried a methyl ether as in tetracenomycin C (1), and not the C-11 phenolic hydroxyl. If the C-11 phenolic hydroxyl was methylated, then a downfield shift of the C-7 proton signal would have been observed on acetylation of the C-8 phenolic hydroxyl.

The mass spectrum of tetracenomycin X diacetate (44) was then compared with that of the diacetate (10) of tetracenomycin C. The mass spectra of these diacetates

---

i. Downfield shifts (Δδ 0.3-0.4 ppm) are observed for the ortho and para protons on acetylation of a phenol.\(^{15}\)
showed several prominent ions which differed by 14 mass units, whilst others were common to both spectra.

In the mass spectrum of tetracenomycin C diacette (10) (see Fig.2 in Chapter 1 and Scheme 4a), a molecular ion at m/z 556 \((\text{C}_{27}\text{H}_{24}\text{O}_{13})\) was observed, together with loss of a methoxyl group (mass 31) and characteristic losses of ketene (mass 42) and acetic acid (mass 60), giving peaks at m/z 525 \((\text{C}_{26}\text{H}_{21}\text{O}_{12})\), 514 \((\text{C}_{25}\text{H}_{22}\text{O}_{12})\) and 454 \((\text{C}_{23}\text{H}_{18}\text{O}_{10})\) respectively, in the higher mass region. Framework fragmentation of the molecule (10) gave the ions at m/z 401 \((\text{C}_{27}\text{H}_{17}\text{O}_{9})\) and m/z 156 \((\text{C}_{25}\text{H}_{22}\text{O}_{9})\), which on loss of ketene, gave the base peak at m/z 359 \((\text{C}_{18}\text{H}_{15}\text{O}_{8})\) and the ion at m/z 114 \((\text{C}_{5}\text{H}_{6}\text{O}_{3})\).

In comparison, the molecular ion of the diacetyl derivative (44) of tetracenomycin X (at m/z 570, CIMS m/z 571, associated with the molecular formula \(\text{C}_{28}\text{H}_{26}\text{O}_{13}\)) was 14 mass units higher than that of tetracenomycin C diacette (10). Similar losses of methoxyl (mass 31), ketene (mass 42) and acetic acid (mass 60), as observed for the EI mass spectrum of diacettate (10), were also observed for the mass spectrum of the diacetyl derivative of the new antibiotic (44) (refer Scheme 4a). Fragmentation of the diacettate (44) and loss of ketene gave the base peak at m/z 373 \((\text{C}_{19}\text{H}_{17}\text{O}_{8})\) which was again 14 mass units higher than that of (10), and the ion at m/z 114 \((\text{C}_{5}\text{H}_{6}\text{O}_{3})\) which remained unchanged [and clearly indicated that the extra \(\delta\)-methyl group was present in the ABC ring portion of the molecule (refer section 2.4.2)]. This characteristic retro-diene cleavage giving the base peak m/z 373 \((\text{C}_{19}\text{H}_{16}\text{O}_{8})\) and the ion at m/z 114 \((\text{C}_{5}\text{H}_{6}\text{O}_{3})\) was also observed in the mass spectra of tetracenomycin X (36) (Fig.10 and Scheme 4b) and other derivatives. These assignments have been verified by high resolution mass measurements.

In conclusion, comparison of the \(^1\text{H}\) and \(^{13}\text{C}\) NMR. and mass spectra clearly established the new antibiotic, tetracenomycin X. as an \(\delta\)-methyl derivative of tetracenomycin C (1). These spectral data also provided some evidence for the location of the additional methyl ether which will be discussed in section 2.4.2.
Scheme 4a

\[
\begin{align*}
\text{Me} & \quad \text{OAc} \\
\text{Me} & \quad \text{OAc} \\
\text{Me} & \quad \text{OMe} \\
\text{Me} & \quad \text{OMe} \\
\end{align*}
\]

(10) $R=\text{H}$ $C_{27}H_{24}O_{13}$ (m/z 556)
(44) $R=\text{Me}$ $C_{26}H_{26}O_{13}$ (m/z 570)

- $\text{OMe}$
- $\text{CH}_3\text{CO}_2\text{H}$
- $\text{CH}_2\text{CO}$

(10) $R=\text{H}$ $C_{26}H_{21}O_{12}$ (m/z 525)
(44) $R=\text{Me}$ $C_{27}H_{23}O_{12}$ (m/z 539)

(10) $R=\text{H}$ $C_{25}H_{22}O_{12}$ (m/z 514)
(44) $R=\text{Me}$ $C_{26}H_{24}O_{12}$ (m/z 528)

- $\text{C}_3\text{H}_6\text{O}_4$
- $\text{CH}_2\text{CO}$

(10) $R=\text{H}$ $C_{26}H_{17}O_{9}$ (m/z 401)
(44) $R=\text{Me}$ $C_{21}H_{19}O_{9}$ (m/z 415)

$C_7H_8O_4$ (m/z 156)

$C_5H_6O_3$ (m/z 114)
Scheme 4b

\[ C_{24}H_{22}O_{11} \text{ (m/z 486)} \]  

2.4 THE BIOSYNTHESIS AND STRUCTURE DETERMINATION OF TETRACENOMYCIN X

In this section is described the results of a biosynthetic study of tetracenomycin X using labelled acetate and the structure determination of this compound using a combination of biosynthetic, spectroscopic and chemical techniques. There were two main problems associated with the structure determination of tetracenomycin X, apart from stereochemical aspects which will be discussed in section 2.4.3. Firstly, as the D-ring orientation of tetracenomycin C (1) had not been established (vide supra), we needed to determine the orientation of the D-ring of tetracenomycin X. and of the tetracenomycins in general. Secondly, we needed to establish the position of the additional \( \sigma \)-methyl of the new compound.
2.4.1 Establishment of the Biosynthetic Origin and the D-ring Orientation of Tetracenomycin X

Prior to the present work, the biosynthetic origin of the tetracenomycins and the elloramycins had not been established. There were also two possible orientations of the D-ring for both tetracenomycins C and X as shown in Fig. 5 (Chapter 1) and Fig. 11 respectively. It should be noted that no inference has been made at this stage concerning the location of the additional methyl group in tetracenomycin X at one or other of the tertiary hydroxyl groups.

Using the current concepts of biosynthesis, one could envisage the formation of these structures from head-to-tail condensation of ten acetate units via the polyketide pathway as shown in Fig. 11. Both tetracenomycins C and X carry the oxygen derived from acetate carbonyl groups at C-1 and C-3, with respect to the non-junction carbons of the D-ring (Fig. 11). Hence, structures (1) and (36) respectively, are preferred for tetracenomycins C and X on biosynthetic grounds, as their formation involve the introduction of only one oxygen on the D-ring (at C-4), whereas in structures (1a) and (36a), the introduction of two oxygens (at C-2 and C-4), and the removal of another (at C-3) are required.

**Figure 11**

- acetate unit
- original carbonyl oxygen of acetate unit
- may or may not be retained from original acetate unit
In order to verify the proposed biogenetic origin of tetracenomycin X (and by analogy, of the tetracenomycins and elloramycins in general), a biosynthetic study using a $^{13}$C labelled precursor was conducted. The results of this biosynthetic experiment could also be used to determine the D-ring orientation of tetracenomycin X and of the other tetracenomycins. Incorporation of a $^{13}$C labelled precursor in the molecule would result in enhancement of specific carbons of the $^{13}$C NMR spectrum of the derived antibiotic. The orientation of the D-ring could then be determined from the labelling pattern.

**Labelling Studies**

Biosynthetic studies using $^{13}$C labelled precursors$^{18a,b}$ are attractive since the analysis of the $^{13}$C NMR spectrum of the labelled metabolite allows the establishment of the labelling pattern without recourse to the extensive chemical degradation required for $^{14}$C studies. In principle, incorporation of $^{13}$C labelled precursor in a compound increases the $^{13}$C content of particular sites. This incorporation is observed as increases in the intensities of the signals in the $^{13}$C NMR spectrum of the enriched metabolite. Generally, a two-fold increase in $^{13}$C abundance is required to be certain that enrichment has occurred because of inherent errors in measuring $^{13}$C intensities. The 50 MHz $^{13}$C proton-noise decoupled NMR spectra of tetracenomycin X in both deuteriochloroform (Fig.9) and deuteriodimethyl sulfoxide have been recorded, and the data and assignments are shown in Table 4.

**Choice of Precursor**

Since the expected mode of biogenesis fundamentally involves condensation of acetate units, either singly (either $[1-^{13}$C] or $[2-^{13}$C]) or doubly ($[1.2-^{13}$C$_2$]) labelled acetate would be possible precursors. Incorporation of labelled acetate, observed as enriched signals in the $^{13}$C NMR spectrum of the derived compound, would verify the biosynthetic origin of tetracenomycin X. However, the interest in this experiment was also directed towards determining the D-ring orientation of the new antibiotic, and
consequently, the precursor was chosen to achieve both objectives. The suitability of the various $^{13}\text{C}$-labelled precursors will now be considered.

Incorporation of [1,2-$^{13}\text{C}_2$]acetate into tetracenomycin X would give rise to the respective labelling patterns for the structures (36) and (36a) as shown in Fig. 12. Although the labelling patterns for the A-, B- and C-rings of both structures (36) and (36a) are the same, the labelling patterns for the D-rings are different (Fig. 12). However, at this stage of the work, the C-4a and C-12a carbon resonances had not been assigned. Establishment of the D-ring orientation of tetracenomycin X by analysis of the labelling pattern of the derived compound would, therefore, be difficult. A further problem could arise from possible difficulty in resolving the appropriate $^{13}\text{C}-^{13}\text{C}$ spin couplings. Consequently, this precursor was not considered suitable.

Figure 12
If $[1^{-13}C]$acetate were used as a precursor, only every second carbon of the resulting tetracenomycin X would be labelled. The labelling pattern for the respective structures, (36) and (36a), is shown in Fig. 13. As with the previous case, the labelling patterns for the D-rings are inherently different even though the labelling patterns for the A-, B- and C-rings of both structures (36) and (36a) are the same. Thus, if (36) is the correct structure, one would observe enrichment in the $\text{C}=\text{O}$ and $\text{C}=\text{OMe}$ resonances of the D-ring in the $^{13}$C NMR spectrum. On the other hand, if (36a) is the correct structure, the $\text{CH}=\text{and CHOH}$ signals would be enhanced. The difference in the enrichment pattern would allow clear assignment of the orientation of the D-ring.

Figure 13

In principle, $[2^{-13}C]$acetate would be as suitable a precursor as $[1^{-13}C]$acetate for our purposes. Its incorporation into tetracenomycin X would lead to the labelling pattern as shown in Fig. 14. Again, there is a clear difference in the enrichment pattern of the D-ring allowing the assignment of the correct structure. However, this precursor was not used since a high degree of randomization (redistribution of label) has been observed for experiments using $[2^{-13}C]$acetate.$^{19}$
Hence, [1-$^{13}$C]acetate was chosen as the precursor for our purposes since its incorporation into tetracenomycin X would not only verify the biogenesis of the new antibiotic, and by analogy that of the tetracenomycins and ellaroramycins, but it would also allow the establishment of the orientation of the D-ring.

Results and Discussion

Conditions for the suitable production of tetracenomycin X (12 mg/L) were first established. Preliminary experiments on cultures of Nocardia mediterranea (strain NT 19) grown in shaken baffled flasks in the modified production medium containing glucose (20 g/L) and Bovril (20 g/L) showed that the production of tetracenomycin X commenced 35 hours after inoculation and reached a maximum after 65 hours (refer Fig.15). Therefore, each of 35 flasks was pulse-fed with 90 atom % $^{13}$C sodium [1-$^{13}$C]acetate (total of 0.5 g) as a sterile aqueous solution after an initial growth period of 40 hours. The culture was harvested after 66 hours, and the labelled antibiotic was extracted and purified as previously described to give a total of 42 mg of pure compound.
The $^{13}$C NMR spectrum of the labelled compound, compared with that of an unlabelled sample, showed ten enriched signals. The spectrum showed a 4-fold enrichment for C-10 due to the acetate starter effect, and approximately 3-fold enhancements for nine other carbons as designated in Table 5. This accords with the expectation for a compound derived from incorporation of [1-\textsuperscript{13}C]acetate in a head-to-tail fashion via the polyketide route as shown in Fig.13 (see also Scheme 2, Chapter 1). These results therefore verify the expected biosynthetic origin of tetracenomycin X, and thus of the tetracenomycins and elloramycins.

In particular, the $\textsuperscript{1}C=O$ and $\textsuperscript{3}C$-OMe carbons of the D-ring were strongly enhanced, while the $\textsuperscript{1}CH=$ and $\textsuperscript{3}CHOH$ signals remained at natural abundance. These results therefore established that the D-ring orientation of tetracenomycin X as shown by structure (36), and not (36a), in Fig.13 was correct. Accordingly, the D-ring orientation was established for the tetracenomycin C (1) (and for the tetracenomycins in general).
Table 5: 50 MHz $^{13}$C NMR Data (in CDCl$_3$) of $^{13}$C Labelled Tetracenomycin X (36).

<table>
<thead>
<tr>
<th>Assignments</th>
<th>δ in ppm</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>190.7</td>
<td>3-fold</td>
</tr>
<tr>
<td>C-2</td>
<td>101.7</td>
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</tr>
<tr>
<td>C-3</td>
<td>167.8</td>
<td>3-fold</td>
</tr>
<tr>
<td>C-4</td>
<td>69.7</td>
<td>0</td>
</tr>
<tr>
<td>C-4a</td>
<td>83.8</td>
<td>3-fold</td>
</tr>
<tr>
<td>C-5</td>
<td>193.1</td>
<td>0</td>
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<td>C-5a</td>
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<td>C-11a</td>
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<td>C-12a</td>
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<tr>
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<td>56.9#</td>
<td>0</td>
</tr>
<tr>
<td>9-OCH$_3$</td>
<td>52.7</td>
<td>0</td>
</tr>
<tr>
<td>12-OCH$_3$</td>
<td>56.2#</td>
<td>0</td>
</tr>
<tr>
<td>Ester C=O</td>
<td>171.0</td>
<td>3-fold</td>
</tr>
</tbody>
</table>

# Interchangeable.
2.4.2 O-Methyl Group Location

In principle, there were four possible positions for the O-methyl group, i.e. on C-4, C-4a, C-11 or C-12a. Two of these four possibilities, C-4 and C-11 hydroxyls, were eliminated by chemical and spectroscopic analysis of tetracenomycin X.

Firstly, the pH dependence of the UV spectrum of the new antibiotic indicated the presence of an ionizable group which was determined to be a phenolic hydroxyl (on C-11) by its colour reaction with ferric chloride on TLC plates, and by its chemical shift at δ 13.98 ppm in the $^1$H NMR spectrum. Secondly, the observed multiplicity of the C-4 proton resonance (doublet of doublets) indicated that it is coupled to two other protons, in particular, the secondary alcohol at δ 2.87 ppm ($J = 10$ Hz) (refer Table 3). The above observations were confirmed by analysis of the $^1$H NMR spectrum of tetracenomycin X diacetate (44) (synthesised as previously described), in which the secondary and phenolic hydroxyls were acetylated (vide supra section 2.3.2).

In addition, a framework fragmentation in the EI mass spectrum of this diacetate (44) gave a base peak at m/z 373 ($C_{19}H_{17}O_8$) which was 14 mass units higher than the base peak of tetracenomycin C diacetate (10), and an ion at m/z 114 ($C_5H_6O_3$) which remained unchanged (refer Scheme 4a). This clearly showed that the O-methyl group was located on the ABC-ring portion of the molecule and not on the C-4 hydroxyl function.

Therefore, there remained only two positions (C-4a and C-12a) for the location of the additional O-methyl group, and having established the D-ring orientation, we could write two possible structures (36) and (36b), both of which have unknown stereochemistry, for tetracenomycin X. A decision between these structures could then be made using a biosynthetic approach. In principle, if (36) is the correct structure, then the carbon bearing the angular hydroxyl group would be enhanced in the $^{13}$C NMR spectrum of the labelled tetracenomycin X, whereas if (36b) is correct.
then the carbon bearing the angular α-methyl group would be enhanced. However, at the time this work was undertaken, although previously the signals at δ 83.4 and 84.9 ppm\(^{ii}\) in the \(^{13}\text{C}\) NMR spectrum of tetracenomycin C (1) had been assigned to C-4a and C-12a by Siebers,\(^{3}\) the author did not distinguish between these two signals.

Utilizing biosynthetically labelled tetracenomycin X, we were able to assign the signal at δ 83.8 ppm to C-4a, and the signal at δ 87 ppm to C-12a. However, several different techniques were attempted before the position of the α-methyl group in this homologue of tetracenomycin C (1) was established unequivocally.

\(^{13}\text{C Acetylation Shift Experiment}\)

It is known\(^{22}\) that acetylation of secondary hydroxyl groups causes both downfield α-shifts (Δδ 1.5 - 4 ppm) and upfield β-shifts (Δδ 1 - 5 ppm) in the \(^{13}\text{C}\) NMR spectra of the carbons α and β to the hydroxyl functions. Similar β-shifts are observed on acetylation of tertiary alcohols, but the α-carbon is shifted to lower field by approximately 10 ppm. In principle, we can utilize these α- and β-carbon shifts to locate the angular hydroxyl, and accordingly, the α-methyl group of tetracenomycin X. Therefore, the initial strategy was to synthesise tetracenomycin X triacetate (45). The assignments for the tertiary hydroxyl and α-methyl groups could then be established by analysis of the \(^{13}\text{C}\) NMR spectrum of the triacetyl derivative.

\(^{ii}\) The corresponding signals occur at δ 83.8 and 87 ppm in tetracenomycin X (36).
Hence, tetracenomycin X was treated with acetic anhydride and pyridine according to the reaction conditions described by Siebers\textsuperscript{3} for the synthesis of tetracenomycin C tetraacetate (12). However, this reaction with tetracenomycin X gave only a mixture of mono- and diacetates in 34\% and 51\% yield respectively. The structure (46) for the monoacetate was established by analysis of its spectroscopic data. In the $^1$H NMR spectrum, only one acetate signal (at $\delta 2.09$ ppm) was observed, indicating that the compound was a monoacetyl derivative of tetracenomycin X. A significant downfield shift ($\Delta \delta 1.33$ ppm) to $\delta 6.12$ ppm. observed for the C-4 proton, as expected on acetylation of the C-4 hydroxyl function, assigned this compound as the 4-O-acetyl tetracenomycin X (46). This assignment was further substantiated by analysis of the mass spectrum. Retro-diene cleavage of the molecule in the mass spectrum gave the base peak at m/z 373 ($C_{19}H_{17}O_8$) and the ion at m/z 156 ($C_7H_8O_4$) which, on loss of ketene, gave the ion at m/z 114 ($C_5H_6O_3$), indicating that the C-4 hydroxyl function was acetylated. The structure assignment of the diacetate (44) has previously been discussed.

Reaction of tetracenomycin X with acetic anhydride and one equivalent of DMAP,\textsuperscript{23a,b} on the other hand, gave monoacetate (46) and a different diacetyl derivative in 41\% and 45\% yields respectively. It was clear from the $^1$H NMR data of the new diacetate that both the secondary and tertiary hydroxyl functions were acetylated, and not the phenolic hydroxyl. A significant shift ($\Delta \delta 1.33$ ppm) was observed for the C-4 proton signal as expected for acetylation of the secondary hydroxyl group, whereas the C-6 proton resonance remained unchanged compared to the starting material. The C-11 phenolic hydroxyl signal was also observed at $\delta 14.0$ ppm in the $^1$H NMR spectrum. With an excess of acetic anhydride and two
equivalents of DMAP, mainly this diacetate (81%) and traces of a triacetate were obtained.

Nevertheless, this diacetyl derivative [the structure (47) was subsequently established for this compound] was as suitable a candidate as the triacetate (45) for the acetylation shift experiment since we were only concerned with the carbons at the α and β positions to the C-4 and C-4a hydroxyl groups. The symbols α and β, and α₁ and β₁, respectively, denote the carbons in these positions relative to C-4 and C-4a. In principle, a net upfield or downfield shift of C-4, a net downfield shift of C-4a and a net α-shift upfield of C-12a would be expected for structure (47). On the other hand, downfield α-shifts of C-4 and C-12a and upfield β-shifts of C-1, C-3, C-4a and C-12 would be expected in the ¹³C NMR spectrum if the o-methyl group was located on C-4a as shown by structure (47a).

On analysis of the ¹³C NMR spectrum, however, the position of the o-methyl group could not be unequivocally established. Although the shift pattern (summarized in Table 6) indicated that the o-methyl group is on C-12a, shifts of similar magnitude, possibly due to concentration effects, were also observed for some carbons which should not be affected by acetylation of the hydroxyl groups.
Table 6

<table>
<thead>
<tr>
<th>Assignment</th>
<th>$\Delta \delta$ (ppm) #</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-4</td>
<td>-3.96</td>
</tr>
<tr>
<td>C-3</td>
<td>-0.7</td>
</tr>
<tr>
<td>C-4a</td>
<td>+1.64</td>
</tr>
<tr>
<td>C-5</td>
<td>-4.0</td>
</tr>
<tr>
<td>C-11</td>
<td>-3.75</td>
</tr>
</tbody>
</table>

# $\Delta \delta = \delta$ (diacetate) - $\delta$ (tetracenomycin X)

$^{13}$C Isotope Shift Experiment

We then turned our attention to the use of deuterium isotope shifts in $^{13}$C NMR spectroscopy for the determination of the hydroxyl-bearing carbons. Deuteration causes, with few exceptions, upfield shifts of the OD-bearing carbon. The $\beta$-effect is due to deuteration of a directly bonded hydroxyl group, and is in the range of 0.11-0.15 ppm, whereas the $\gamma$-effect is due to deuteration of an hydroxyl group on a vicinal carbon, and is in the range of 0.03-0.06 ppm. It might be difficult to identify unambiguously the carbons based on these small shifts ($\Delta \delta$ 0.1-0.03 ppm) because shifts of similar magnitude are observed due to concentration effects. However, the presence of isotopic doublets or multiplets for the $\beta$- and $\gamma$-carbons in a 1:1 mixture of the normal and deuterated compound is clear evidence of these hydroxy-bearing carbons.

Accordingly, in a 1:1 mixture of deuterated and non-deuterated tetracenomycin X, isotopic multiplets of the type $\beta \gamma$ (Fig.16) would be expected for C-4 and C-4a if the $\alpha$-methyl group is located on C-12a, whereas isotopic doublets of the type $\beta$ (Fig.17) would be expected for both C-4 and C-12a if C-4a is the $\alpha$-methyl bearing-carbon. The carbon resonances at $\delta$ 83.8 and 87.0 ppm have previously been assigned to C-4a and C-12a respectively using the results from the biosynthetic experiment. Hence, we would only need to observe isotopic doublets of the type $\beta$ for either of the carbons resonances in order to locate the angular $\alpha$-methyl group. In both cases, isotopic doublets (type $\beta$) would be expected for C-11.
Thus, a calculated amount of deuterium oxide (D\textsubscript{2}O) was added to a 5\% w/v solution of tetracenomycin X in d\textsubscript{6}-DMSO,\textsuperscript{iii} and the solution was treated with anhydrous calcium sulphate to remove residual water. The \textsuperscript{13}C NMR spectrum was then recorded at 50 MHz at room temperature. Analysis of the results, summarised in Table 7, revealed that the angular \(\alpha\)-methyl group is located at C-12a.

### Table 7

<table>
<thead>
<tr>
<th>Assignments ((\delta) in ppm)</th>
<th>Type</th>
<th>Isotopic shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-4 (69.0)</td>
<td>(\gamma\ \beta_1)</td>
<td>(\Delta\beta_1 = 2) Hz, (\Delta \gamma = ) line broadening</td>
</tr>
<tr>
<td>C-4a (83.8)</td>
<td>(\beta \ \gamma_1)</td>
<td>(\Delta\beta = 3) Hz, (\Delta \gamma_1 = 1.5) Hz</td>
</tr>
<tr>
<td>C-12a (87.0)</td>
<td>(\gamma)</td>
<td>(\Delta \gamma = ) line broadening</td>
</tr>
<tr>
<td>C-11 (167.0)</td>
<td>(\beta)</td>
<td>(\Delta\beta = 2) Hz</td>
</tr>
</tbody>
</table>

\textsuperscript{iii.} Generally, the hydrogen exchange rate of an hydroxyl group in DMSO solutions is sufficiently slow to allow direct observation of shifts of such magnitude.
Proton Decoupled $^{13}\text{C}$ NMR Spectroscopy

It was envisaged that the position of the $\alpha$-methyl group could also be assigned by analysis of the specific proton-decoupled $^{13}\text{C}$ NMR spectrum of tetracenomycin X. In the fully-coupled spectrum of tetracenomycin X, signals at $83.8$ and $87.0$ ppm both occur as multiplets due to long range coupling (refer Fig. 18) which can be removed by selective decoupling of neighbouring substituents, in particular the $\alpha$-methyl group at $83.64$ ppm.

Indeed, irradiation of this $\alpha$-methyl group in the $^1\text{H}$ NMR spectrum converted the approximately 8-lined multiplet of the carbon at $87.0$ ppm (C-12a) to a doublet ($J=3$ Hz), whereas no significant simplification of the multiplet at $83.8$ ppm was observed. Thus, this result indicated that C-12a carries the $\alpha$-methyl group. On the other hand, simplification of the multiplet at $83.8$ ppm was observed on irradiation of the C-4a hydroxyl function.

Figure 18: 50 MHz $^1\text{H}$ Decoupled $^{13}\text{C}$ NMR Spectrum (in CDCl$_3$) of C-4a ($83.8$ ppm) and C-12a ($87.0$ ppm)

Therefore, we can conclude from the results of both the isotopic shift and the proton decoupling experiment that C-12a ($87.0$ ppm) is the $\alpha$-methyl bearing carbon.
2.4.3 Determination of Stereochemistry

The optical rotation $\left[\alpha\right]_{D}^{22} = +51^\circ$ indicated that tetracenomycin X was chiral. The chiral centres are at C-4, C-4a and C-12a. The most direct method for the determination of the accurate relative atomic arrangement in a molecule is undoubtedly X-ray analysis. It has been successfully employed in the structure determination of a large number of biologically active compounds.

Application of this method for the determination of the configuration of tetracenomycin X was envisaged, but not investigated as the chances of rationalising the X-ray crystal data seemed slim at the time the present work was undertaken. An attempt had previously been made by Siebers$^3$ to determine the relative configuration of tetracenomycin C (1) via X-ray structure analysis, but had been unsuccessful.$^iv$

Other approaches were therefore used to define first the relative configuration, and then the absolute configuration at C-4, C-4a and C-12a.

**Determination of the Relative and Absolute Stereochemistry at C-4 and C-4a**

Nuclear Overhauser enhancement spectroscopy$^{26}$ is a powerful technique for elucidating relative configuration and has been applied to structural problems in many natural products. If an irradiating field is applied to a nucleus X, a n.O.e (nuclear Overhauser effect) enhancement in the intensity of the absorption signal of a spatially proximate nucleus Y can be observed. Increased energy transfer between X and Y promotes the relaxation of Y, thus increasing the population of the lower energy level of Y and enhancing the intensity of the Y signal.

Hence, a n.O.e. enhancement in the intensity of the C-4 hydroxyl signal of tetracenomycin X observed upon irradiation of the C-4a hydroxyl, and vice versa, would indicate a cis stereochemistry. [Likewise, the presence of a n.O.e. enhancement in the intensity of either the C-4 or C-4a hydroxyl on irradiation of C-12a 0-methyl resonance would indicate that the C-12a 0-methyl group is cis to these two substituents.

$^iv$ Zeeck and coworkers later solved the X-ray structure$^8$, and also determined the absolute configuration$^8$ of tetracenomycin C (1) using the method of Helmchen.$^{10}$
(vide infra). However, there was no detectable n.O.e. enhancement in the intensity of the C-4 and C-4a hydroxyl and in the C-12a o-methyl resonances upon irradiation of any these signals.

It is possible that no n.O.e. enhancements were observed due to rapid exchange between the two hydroxyl (C-4 and C-4a) groups. In principle, it may be possible to slow this exchange down by cooling. No detectable enhancements, however, were observed even at -30°C in dry dichloromethane.

The determination of the relative stereochemistry of the vicinal diol system (C-4 and C-4a hydroxyls) by chemical means was then investigated. The cis stereochemistry of the C-4 and C-4a hydroxyls could be confirmed by synthesising a 5-membered cyclic carbonate derivative of tetracenomycin X. Trans stereochemistry of the C-4 and C-4a hydroxyl groups would not permit the formation of a cyclic carbonate derivative, nor could a cyclic carbonate be formed between one of these hydroxyls and the C-11 phenolic hydroxyl function. The carbonyl stretching frequencies of 5-membered cyclic carbonates (1800-1820 cm\(^{-1}\))\(^{v}\) in infrared spectroscopy have been well documented in the literature.\(^{27}\)

Accordingly, treatment of tetracenomycin X with pyridine and 20% phosgene in toluene\(^{28}\) gave a carbonate in 70% yield. The structure (48) was determined for this compound by analysis of its spectroscopic data. In particular, the infrared spectrum showed a carbonyl stretching frequency at 1840 cm\(^{-1}\), clearly indicative of a 5-membered cyclic carbonate, thus establishing the cis stereochemistry of the C-4 and C-4a hydroxyl groups, and simultaneously confirming the location of the angular o-methyl at C-12a (vide supra).

\[
\text{(48)}
\]

\(^{v}\) The carbonyl stretching frequencies of 5-membered cyclic carbonates (1800-1820 cm\(^{-1}\)) are much higher than those observed for 6-membered cyclic carbonates (1760-1780 cm\(^{-1}\)).\(^{27}\)
The absolute stereochemistry of the chiral centres at C-4, C-4a and C-12a of tetracenomycin X was then determined by the method of Trost and coworkers. They reported the use of o-methylmandelate esters for the establishment of the absolute stereochemistry of secondary alcohols. Their method, based on the model proposed by Dale and Mosher for correlating NMR shifts and absolute stereochemistry, utilizes an "extended Newman projection" in which the intervening ester linkage is omitted, and the o-methyl group eclipses the hydrogen of the secondary hydroxyl centre. The substituent which eclipses the phenyl ring in such a projection is then always upfield with respect to that of the starting material, presumably as a result of the shielding it experiences due to the phenyl ring.

In the case of the (R)-o-methylmandelate ester of tetracenomycin X (49), the absolute configuration at C-4 would be (R) if an upfield shift is observed for the C-4a hydroxyl signal in the 1H NMR spectrum. On the other hand, the C-4 centre would have an (S) configuration if an upfield shift is observed for the C-3 methoxyl resonance. The opposite applies for the (S)-o-methylmandelate ester (50), i.e. the absolute configuration at C-4 would be (R) if the C-3 methoxyl group is shielded and the 1H NMR signal shifts upfield, whereas if the C-4a hydroxyl resonance is shifted upfield, then C-4 would have an (S) configuration (refer Figs. 19 and 20).

Thus, both the (R)-ester (49) and the (S)-ester (50) were prepared by the selective esterification of the C-4 hydroxyl of tetracenomycin X with the respective (R)- and (S)-o-methylmandelic acids, DCC and DMAP in dichloromethane. The desired (R)- and (S)-esters, (49) and (50), were obtained in 75% yield with greater than 99% enantiomeric excess. Analysis of the 1H NMR spectrum of the (R)-ester (49) showed that the C-4a hydroxyl resonance was shifted upfield by $\Delta \delta 0.47$ ppm with respect to that of the starting material. Analogously, the C-3 methoxyl signal was shifted to higher field by $\Delta \delta 0.42$ ppm in the case of the (S)-ester (50). These shifts of the hydroxyl and methoxyl groups to higher field are to be expected if the absolute configuration of the C-4 hydroxyl is (R) (Fig. 19 and 20). We can thus conclude that the C-4 centre has (R) configuration.

The cis stereochemistry of the C-4 and C-4a hydroxyl groups was previously determined, and using the method of Trost and coworkers, we have determined the
absolute configuration at C-4 to be \((R)\). Therefore, accordingly, the absolute configuration at C-4a is also \((R)\).

**Determination of the Relative and Absolute Stereochemistry at C-12a**

In the previous section, attempts to determine whether the stereochemistry at C-4, C-4a and C-12a was \textit{cis} using nuclear Overhauser enhancement measurements were unsuccessful. Although the negative results from the experiment suggested a \textit{trans} relationship between the C-12a methoxyl group and the C-4 and C-4a hydroxyls, we were again unable to confirm this suggestion using nuclear Overhauser enhancement spectroscopy. No n.O.e. enhancement was observed for the C-4 proton resonance on irradiation of the C-12a methoxyl group of tetracenomycin X and \textit{vice versa}. We then turned our attention to the use of chiroptical methods for the determination of the configuration at C-12a.

Compounds which are optically active are capable of rotating the plane of polarised light by unequal velocity of transmission (optical rotatory dispersion) and by unequal absorption (circular dichroism) of left and right circularly polarised light.\(^{31}\) These phenomena are associated with the electronic transitions occurring in the chiral environment of the chromophore. Compounds with absorptions above 200 nm show Cotton effects which are usually very large rotations. This anomalous dispersion is characterised by “peaks” and “troughs” in the optical rotatory dispersion (ORD) spectrum and “maxima” and “minima” in the circular dichroism (CD) curve. The absolute configuration of compounds of unknown chirality can be determined by comparison of their ORD and CD curves with those of known model compounds.

At this stage, however, Zeeck and coworkers\(^{6-8}\) reported the isolation and structure of elloramycin (24), a new antibiotic produced by \textit{Streptomyces olivaceus} strain Tü 2353 (refer Chapter 1). Included in the paper were the spectral data for the tetramethyl derivative (32) of the aglycone, elloramycinone (30) [and of tetracenomycin C (1)], in which the C-4 and C-4a hydroxyls and the C-12a methoxyl group have \textit{cis} stereochemistry.
Accordingly, the relative stereochemistry at C-12a of tetracenomycin X could be determined by synthesising the trimethyl ether derivative and comparing its spectroscopic data with those of the tetramethyl derivatives (32) of elloramycinone and tetracenomycin C. If their spectral data are identical, then tetracenomycin X will have the same relative configuration at C-12a (and also at C-4 and C-4a).

Initial efforts to synthesise tetracenomycin X trimethyl ether were met with difficulties. Treatment of tetracenomycin X with commercial silver oxide in methyl iodide, after 60 hours, gave a mixture of mono- and dimethylated compounds, which was then subjected to further methylation to give the trimethyl ether (32) in very low yield (5%).

With other reagents such as barium oxide in methyl iodide or methyl triflate, only a monomethylated compound, together with starting material were obtained. The monomethyl derivative was assigned the structure (51) on the basis of its spectroscopic data. The $^1$H NMR spectrum of the monomethyl ether (51) showed an additional signal at $\delta$ 3.40 ppm and the C-4 proton resonance, which was observed as a doublet of doublets in the $^1$H NMR spectrum of the starting material, occurred as a doublet ($J = 1.5$ Hz), indicating that the C-4 hydroxyl was methylated. In addition, framework fragmentation of the molecule in the mass spectrum gave the base peak at $m/z$ 373 ($C_{19}H_{17}O_8$) which remained unchanged, and the ion at $m/z$ 128 ($C_6H_8O_3$) which was 14 mass units higher than that of the corresponding D-ring fragmentation of the starting material, thus confirming that the C-4 hydroxyl was methylated.

Treatment of tetracenomycin X with freshly prepared silver oxide in methyl iodide under ultrasound for 60 hours, however, furnished a trimethyl ether in 19% yield after purification. Indeed, the spectroscopic data (UV, $^1$H and $^{13}$C NMR spectra) of this trimethyl ether of tetracenomycin X, were identical with those reported for permethylethers (32) of elloramycinone and tetracenomycin C.

From this result, we can conclude that the trimethyl ether of tetracenomycin X has the same relative stereochemistry as that of the tetramethyl ethers of tetracenomycin C and elloramycinone. Thus, the C-12a O-methyl group of tetracenomycin X has cis stereochemistry with respect to the C-4 and C-4a hydroxyl.
functions. Having previously determined, using the method of Trost et al.,\textsuperscript{29} that absolute configurations at C-4 and C-4a are \((R)\), accordingly, the absolute stereochemistry at C-12a is also \((R)\).

Therefore, on the basis of all the above information, the structure (36) was established for tetracenomycin X.

In addition, the absolute stereochemistry which has been established for tetracenomycin X (36) is the same as that determined by Rohr\textsuperscript{8} for tetracenomycin C (1) and elloramycinone (30). This correspondence of stereochemistry was then confirmed by comparison of CD spectral data of tetracenomycin X trimethyl ether (32) with those reported for the tetramethyl ethers of tetracenomycin C and elloramycinone. Tetracenomycin X trimethyl ether (32) exhibited the same multiple Cotton effects, although with slightly lower intensities, as those observed for tetracenomycin C and elloramycinone tetramethyl ethers (32).
2.4.4 Miscellaneous

Absolute Stereochemistry of Tetracenomycin C

In the dissertation reporting the isolation and structure of elloramycin (24), Rohr also mentioned the establishment of the absolute configuration of the C-4, and accordingly, the C-4a and C-12a hydroxyl functions of tetracenomycin C (1) using the method of Helmchen. This method is similar to that discussed above for tetracenomycin X (36). In their case, the 4-0-2'-phenylbutyryl esters of tetracenomycin C (1) with (R) and (R)(S)-2-phenylbutyric acid were synthesised (refer Fig.21), and their $^1$H NMR spectra analysed.

Figure 21

![Chemical Structures](image)
Upfield shifts were observed for the C-6 and C-7 proton resonances ($\Delta \delta$ 0.25 and 0.07 ppm respectively) in $^1$H NMR spectrum of the ($R$) ester (52). On the other hand, in the $^1$H NMR spectrum of the diastereomeric mixture of the esters, the C-3 methoxyl resonance of the ($S$) ester (53) was shifted upfield by $\Delta \delta$ 0.21 ppm. From these results, they determined the absolute configuration at C-4, and accordingly C-4a and C-12a, to be all ($R$). These paramagnetic shifts of the aromatic protons, 6-H ($\Delta \delta$ 0.25 ppm) and 7-H ($\Delta \delta$ 0.07 ppm) reported in their paper, are also observed in the ($R$)-o-methyl mandelate ester (49) of tetracenomycin X ($\Delta \delta$ 0.34 and 0.09 ppm, respectively).

_Semi-Synthetic Tetracenomycin X_

It is interesting to note that tetracenomycin X (36) has been synthesised from elloramycin (24) by Rohr. Thus, the sugar moiety of elloramycin (24) was hydrolysed using trifluoroacetic acid. Treatment of the aglycone, elloramycinone (30), with trimethylchlorosilane and pyridine resulted in selective etherification of the

**Scheme 5**
C-11 and C-4 hydroxyls to give the trimethylsilyl derivative (54). Methylation of the C-8 hydroxyl, followed by cleavage of the trimethylsilyl protecting group gave the target compound (36) (Scheme 5).

2.5 BIOLOGICAL ACTIVITY

In the disc diffusion assay, the sensitivity of various organisms towards tetracenomycin X (36) was tested. The results showed that the biological activity spectrum of tetracenomycin X (36) is similar to that of tetracenomycin C (1) and elloramycin (24). Both tetracenomycin C (1) and elloramycin (24) are narrow spectrum antibiotics, showing activity against various Streptomyces species, but are

Table 8: The Biological Activity of Tetracenomycin X (36)

<table>
<thead>
<tr>
<th>Tested Organism</th>
<th>Concentration (µg/mL)</th>
<th>Inhibitory zone (x cm) #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>15.5</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>31.0</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>62.0</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>124.0</td>
<td>--, --</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>15.5</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>31.0</td>
<td>--, --</td>
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<tr>
<td></td>
<td>62.0</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>124.0</td>
<td>--, --</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>15.5</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
<td>31.0</td>
<td>--, --</td>
</tr>
<tr>
<td></td>
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<td>--, --</td>
</tr>
<tr>
<td></td>
<td>124.0</td>
<td>--, --</td>
</tr>
<tr>
<td>Streptomyces aureofaciens</td>
<td>15.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>31.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>62.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>124.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Streptomyces fragilis</td>
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<td>1.15</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>62.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>124.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

# Excluding disc diameter.
* No inhibition observed.
inactive against Gram-negative bacteria. Similarly, the new antibiotic, tetracenomycin X (36), is active against Gram-positive bacteria such as *Streptomycetes* but is inactive against Gram-negative bacteria and yeasts (refer Table 8). The biological activity of the semi-synthetic tetracenomycin X (36) (i.e. elloramycinone-8-methyl ether) has also been investigated by Rohr (refer section 1.3.3 in Chapter 1).

2.6 CONCLUSION

The present work has defined the structure of the new antibiotic isolated from *Nocardia mediterranea*, strain NT 19 from the Commonwealth Serum Laboratories, Melbourne, to be the 12a-o-methyl analogue (36) of tetracenomycin C.

Prior to the present work, the biogenesis of the tetracenomycin and elloramycin antibiotics had not been established. In this chapter, the biosynthetic origin of the new antibiotic, tetracenomycin X (36) (and by analogy, of the tetracenomycins and elloramycins), was determined, using [1-13C]acetate, to result from head to tail condensation of acetate units.

The D-ring orientation of tetracenomycin X, and of the tetracenomycins in general, was defined, via biosynthetic means, as corresponding to structure (36) and not (36a). When the present work commenced, the orientation of the D-ring of the tetracenomycin antibiotics, in particular tetracenomycin C (1), had not been proven. The angular o-methyl group of tetracenomycin X (36) was subsequently located at C-12a using spectroscopic techniques.

The relative and absolute stereochemistry of tetracenomycin X were also determined in this work. The three chiral centres, C-4, C-4a and C-12a, were determined to have *cis* stereochemistry using a combination of spectroscopic and chemical techniques. Finally, the absolute stereochemistry of the centres were determined to be all (R) by synthesising the (R)- and (S)-o-methylmandelate esters of tetracenomycin X, (49) and (50) respectively, and analysis of their 1H NMR data.

The biological activity of the new antibiotic, tetracenomycin X (36), against a range of microorganisms was found to be similar to that of tetracenomycin C (1).
Tetracenomycin X (36) is active against Gram-positive bacteria such as *Streptomyces*, but is inactive against Gram-negative bacteria and yeasts.
CHAPTER 3  

INTRODUCTION

Biological oxidation processes may occur either by removal of electrons or hydrogen, or by addition of oxygen to a substrate. The oxidative reactions of a variety of organic substrates by molecular oxygen are of interest. These reactions are catalysed by enzymes, the oxygenases. Two subclasses of oxygenases may be defined: dioxygenases which introduce both atoms of an oxygen molecule into a substrate, and mixed function oxygenases (monooxygenases and hydroxylases) which transfer only one atom of oxygen to a substrate.

3.1 Dioxygenases

The dioxygenases generally contain iron, which is either directly linked with the enzyme protein or exists in ferroporphyrinic IX (ferritin). The metal activates the oxygen–enzyme complex which then adds to a substrate with elimination of a peroxide. The enzyme–metal complex reforms on transfer of the oxygen to the substrate. In reactions involving dioxygenases, cyclic peroxides are possible intermediates and frequently, carbon–carbon double bonds are oxidatively cleaved, e.g., in the oxidation of catechol (83) to vanillic acid (56). In certain cases there is no cleavage of the C-C bond of the cyclic peroxide, e.g., in the biooxidations of menaquinones \( \text{B}_1 \) (57) and \( \text{B}_2 \) (58) and dihydroquinate \( \text{A}_2 \) (59). The 5-membered cyclic peroxide (58) is

\[
\begin{align*}
\text{(83)} & \xrightarrow{\text{dioxygenase}} \text{(56)} \\
\text{(57)} & \xrightarrow{\text{dioxygenase}} \text{(58)} \\
\text{(58)} & \xrightarrow{\text{dioxygenase}} \text{(59)}
\end{align*}
\]
CHAPTER 3: TETRACENOMYCIN X - THE BIOGENETIC ORIGIN OF THE OXYGEN ATOMS

3.1 INTRODUCTION

Biological oxidation processes may occur either by removal of electrons or hydrogen, or by addition of oxygen to a substrate. The oxidative reactions of a variety of organic substrates by molecular oxygen are of interest. These reactions are catalysed by enzymes, the oxygenases. Two subclasses of oxygenases may be defined: dioxygenases which introduce both atoms of an oxygen molecule into a substrate, and mixed function oxygenases (monoxygenases and hydroxylases) which transfer only one atom of oxygen to a substrate.

3.1.1 Dioxygenases

The dioxygenases generally contain iron which is either directly linked with the enzyme protein or exists as ferroprotoporphyrin IX (haem). The metal activates the oxygen-enzyme complex which then adds to a substrate with elimination of a proton. The enzyme-metal complex reforms on transfer of the oxygen to the substrate. In reactions involving dioxygenases, cyclic peroxides are possible intermediates and, frequently, carbon-carbon double bonds are oxidatively cleaved, e.g. in the conversion of catechol (55) to cis,cis-muconic acid (56). In certain cases there is no cleavage of the C-C bond of the cyclic peroxide, e.g., in the biosyntheses of prostaglandins E₁ (59) and I₂ (60) and thromboxane A₂ (61). The 5-membered cyclic peroxide (58) is

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\end{array}
\]

O₂

dioxygenase

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{C} = \text{O} \\
\text{O} \\
\end{array}
\]

(55)
(56)
possibly formed by stereospecific addition of an oxygen molecule at C-9 and C-11 of arachidonic acid (57), followed by addition of another oxygen atom at C-15 with concomittant cyclisation (Scheme 6).

Scheme 6

![Scheme 6 Diagram]

The biological hydroxylation of some benzenoid systems [e.g. (62)] is another example where the cleavage of C-C bonds does not occur on addition of oxygen.\(^{3,4a,b}\) In this case, a four-membered cyclic peroxide intermediate (63), formed by addition of an oxygen molecule to a carbon-carbon double bond of benzene, has been postulated. The mechanism for the reduction of the peroxide (63) to the vicinal cis diol system (64) is, however, unknown (Scheme 7).

Scheme 7

![Scheme 7 Diagram]
3.1.2 Mixed Function Oxygenases

In reactions involving mixed function oxygenases, only one of the oxygen atoms is introduced into a substrate; the other is reduced to water.\(^{39a}\) Several types of mixed function oxidations can be distinguished on the basis of substrate specificity. A number of these reactions involve oxidizing amines and thio compounds to hydroxy and oxide derivatives while in others, hydrogen atoms (on tetragonal carbon atoms) are stereospecifically replaced by hydroxyl groups.

In the case of carbon-carbon double bonds, epoxides are generally the first reaction products which may then be transformed by various reactions to, e.g., dihydroxy (65) and monohydroxy (66) derivatives or thioethers (67) (Scheme 8).\(^{39a}\)

\[\text{Scheme 8}\]

3.1.3 Present Work

The biogenetic origin of tetracenomycin X (36) (and by inference that of the tetracenomycin and elloramycin antibiotics) from ten acetate units via the polyketide pathway was established in the previous chapter.

This part of the thesis describes investigations of the biogenetic origin of the oxygens in tetracenomycin X (36), using \[^{18}O_2\] oxygen incorporation.
3.2 POSSIBLE SITES OF 18-OXYGEN INCORPORATION IN TETRACENOMYCIN X

3.2.1 Incorporation of Four Oxygen Atoms

A biosynthetic scheme involving the polyketide pathway was previously proposed for the tetracenomycin and elloramycin antibiotics (see Scheme 2, Chapter 1 and also Scheme 9). The formation of tetracenomycin X (36c) was suggested to involve the putative fully aromatic decaketide intermediate (40), and consequently, all but four oxygens would have been derived from acetate units (Scheme 9). There would, therefore, exist four sites which require the subsequent introduction of oxygen, i.e. at C-4, C-4a [the original acetate oxygen having been lost], C-5 and C-12a.

Scheme 9

![Scheme 9 diagram]

Of particular interest is the formation of the vicinal cis diol system at C-4a and C-12a. The question of whether two of these incorporated oxygen atoms are derived from one molecule of oxygen arises. If this cis diol system is derived from one molecule of oxygen, the double hydroxylation process would possibly have involved a four-membered cyclic peroxide formed by oxidation of the decaketide intermediate (40), and would have parallels in the biological hydroxylation of some benzenoid systems (vide supra).

A biosynthetic pathway for the double hydroxylation process is outlined in Scheme 10. Stereospecific addition of an oxygen molecule, activated by, for example,
a dioxygenase enzyme, at C-4a or C-12a of the decaketide (40) would give the cyclic peroxide (68). The hydroxylated compound (69) may arise by cleavage of the peroxide bond. Hydroxylation at C-4 and oxidation at C-5, followed by methylation would afford tetracenomycin X (36c). Alternatively, the aromatic precursor may first be oxidized to the quinone intermediate (42) which could be converted to the hydroxy derivative (71) via peroxide (70) as previously described. Hydroxylation at C-4 followed by methylation would give tetracenomycin X (36c). It is unlikely that the double hydroxylation step involves epoxidation of an aromatic or quinone precursor.

Scheme 10

followed by epoxide hydrolysis as such a route would usually give a \textit{trans} diol system unless a subsequent inversion process is involved.
There are numerous literature examples of natural products which possess similar vicinal cis diol systems at ring junctions, typified by the angucycline antibiotics: the sakyomincs [e.g. sakyomicin A (72)], the saquayamycins [e.g. saquayamycin A (73)], the vineomycins [e.g. vineomycin A1 (74)], and most recently reported, the urdamycin antibiotics [e.g. urdamycin D (75)].

The aglycones of these angucycline antibiotics are known to be derived from acetate units via the polyketide pathway as shown in Scheme 11. Presumably, the biogenetic origin of the oxygens of these antibiotics, in particular, of the vicinal cis diol system at C-4a and C-12b, is similar to that of tetracenomycin X (36).

Scheme 11

\[
\begin{align*}
\text{MeCO}_2H & \quad \rightarrow \quad \rightarrow \\
& \quad \rightarrow
\end{align*}
\]

R=glycosides
R\(^1\)=OH or H
acetate unit
3.2.2 Incorporation of Three Oxygen Atoms

An alternative biosynthetic pathway for the formation of tetracenomycin X (36) is shown in Scheme 12. This pathway involves head-to-tail condensation of ten acetate units, followed by intramolecular cyclisation to form the decaketide intermediate (76). In this partially aromatised (with respect to the A- and B-rings) intermediate (76), the original acetate carbonyl oxygen is retained at C-4a. Subsequent hydroxylation at C-4 and C-12a, oxidation at C-5 and methylation would give tetracenomycin X (36d) (Scheme 12). Consequently, all but three of the oxygen atoms in tetracenomycin X (36d) would have been derived from acetate units. Hence, there would be three possible sites for \([^{18}O_2]\) oxygen incorporation, i.e. at C-4, C-5 and C-12a.

As with the case of four oxygens incorporated, the question arises as to whether two of the labelled oxygens, i.e. at C-4 and C-12a or at C-5 and C-12a, come from one molecule of oxygen. For example, formation of the 1,3-dioxygenated system at C-5 and C-12a could involve the formation of a five-membered cyclic peroxide (77) [such as the type involved in the biosyntheses of prostaglandins E₁ (59) and I₂ (60) and thromboxane A₂ (61)] by stereospecific addition of oxygen to decaketide (76) (Scheme 13). The hydroxylated derivative (78) could then arise by cleavage of the peroxide bond. Oxidation at C-4 (for a dioxygenated system at C-5 and C-12a) or at C-5 (for a dioxygenated system at C-4 and C-12a) and methylation, would furnish...
tetracenomycin X (36d). It is interesting to note that the sakyomicin, saquayamycin, vineomycin and urdamycin antibiotics also possess similar oxygen functions at C-4a (which may have been retained from the original acetate unit) and at C-12 and C-12b (a 1,3-dioxygenated system which may have been derived from one molecule of oxygen).

**Scheme 13**

![Scheme 13](image)

3.3 ESTABLISHMENT OF THE BIOGENETIC ORIGIN OF THE OXYGEN ATOMS IN TETRACENOMYCIN X

A biosynthetic study using $^{18}$O$_2$-oxygen was conducted with two aims. Firstly, we needed to establish the biogenetic origin of the oxygen atoms of tetracenomycin X (36) (in particular, those at C-4, C-4a, C-5 and C-12a), and secondly, to determine whether two of these oxygens come from the same molecule of oxygen.

3.3.1 Preliminary Work

Considerable experimentation$^{20}$ was necessary to establish a suitable fermentation procedure under a controlled oxygen atmosphere. It was established that production of tetracenomycin X (36) commenced after an initial growth period of 35 hours (see also Fig.15, Chapter 2) in normal shaken culture. However, subsequent production
was inhibited severely when the fermentation was conducted in a closed system due partially to a substantial reduction in the pH of the culture broth. This problem was overcome by adding barium carbonate to the culture broth at the time of controlled oxygen addition. The tetracenomycin X (36) production could also have been affected by the restriction in cultural aeration leading to the microorganism becoming anaerobic. Thus, the gas mixture in the closed system was circulated directly through the fermentation medium and a flask containing concentrated aqueous barium hydroxide (to trap expired carbon dioxide) by means of a peristaltic pump.

Preliminary experiments also showed that approximately 500 mL of oxygen per flask (each containing 100 mL of culture broth) were consumed during the tetracenomycin X (36) production phase (10-12 hours) in which a maximum yield of 1 mg/100 mL of the antibiotic was obtained. The yield of tetracenomycin X (36) was slightly reduced compared to that obtained from fermentation in an open system (1.2 mg/100 mL). Attempts to select more productive strains of *Nocardia mediterranea* were unsuccessful.

3.3.2 Methods of Analysis

*Analysis by* $^{13}$C NMR Spectroscopy

We initially considered the use of $^{13}$C NMR spectroscopy to determine the sites of $^{18}$O enrichment by exploiting the resulting isotope shift in resonances of $^{13}$C nuclei, a technique introduced independently by Vederas$^{40}$ and Van Etten.$^{41}$ It has been shown that isotope substitution with $^{18}$O induces an upfield shift ($\Delta\delta$ 0.05-0.01 ppm) in the $^{13}$C NMR positions of directly attached carbons, and in some cases, $\beta$-carbons.

A significant quantity of $[^{18}$O$_2]$oxygen (2.5 L) would, however, be required to obtain sufficient labelled compound (approximately 5 mg) for a reasonable signal-to-noise ratio in the $^{13}$C NMR spectrum because of the low yield of the antibiotic (36) and the high oxygen consumption by the *Nocardia* strain.
In addition, analysis by $^{13}$C NMR spectroscopy would not allow us to determine if two of the incorporated oxygens were derived from the same molecule of oxygen. In view of this limitation and of the high cost involved, analysis by mass spectrometry (in conjunction with chemical transformations) was pursued.

**Analysis by Mass Spectrometry**

Since we planned to determine the $^{18}$O labelling regiochemistry of tetracenomycin X (36) using mass spectrometry, it was necessary to analyse the mass spectral fragmentation pattern of the antibiotic. Some assignments of mass spectral data of tetracenomycin X diacetate (44), discussed in Chapter 2, reasonably extend to tetracenomycin X (36).

In the EIMS of tetracenomycin X (36) (Fig. 10, Chapter 2), a molecular ion at m/z 486 ($C_{24}H_{22}O_{11}$), together with ions at m/z 468 and m/z 455, which arose by loss of water and a methoxyl group respectively, were observed. Losses of formaldehyde (mass 30), giving ions at m/z 438 (i.e. m/z 468-30) and m/z 425 (i.e. m/z 455-30) were also observed in the higher mass region. The prominent fragmentation in the EIMS of tetracenomycin X (36) is the retrodiene cleavage, giving the base peak at m/z 373 ($C_{19}H_{17}O_8$) and the ion at m/z 114 ($C_5H_6O_3$) (see Scheme 4b, Chapter 2). Loss of methanol (mass 32) from the base peak (m/z 373), giving an ion at m/z 341, was also observed.

The molecular ion cluster in the mass spectrum would establish the total number of labelled oxygens incorporated in tetracenomycin X (36). The ions from the retrodiene cleavage would indicate the number of $^{18}$O atoms in the ABC-ring and D-ring fragments. However, it may not be possible to establish unequivocally the sites of enrichment in the ABC-ring fragment as we were unable to assign structures to any of the other ions. Hence, some chemical transformations (which will be discussed in section 3.3.3) would be required in conjunction with mass spectral analysis to aid in determining the regiochemistry of $^{18}$O enrichment.

The second objective of this biosynthetic experiment, namely to determine if two of the labelled oxygens are derived from one molecule of oxygen, could be achieved
by mass spectral analysis of labelled tetracenomycin produced in a known $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen mixture. The rationale for this experiment is as follows. If, for example, a metabolite contains three positions known to be derived from aerial oxygen, and is produced in a system containing a mixture of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen, the probability that the positions will be labelled by $^{16}\text{O}_3$, $^{16}\text{O}_2^{18}\text{O}$, $^{16}\text{O}^{18}\text{O}_2$ and $^{18}\text{O}_3$, is a function of the relative amounts of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen present. The probabilities may be considered in two ways.

For the situation where all three positions are labelled by different molecules of oxygen at random and denoting X and Y as the mole fractions of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen, the expected ion abundance ratios of the molecular ion cluster, *i.e.*, $M^{+}$ (m/z 486), $M^{+}+2$ (m/z 488), $M^{+}+4$ (m/z 490) and $M^{+}+6$ (m/z 492) ions can be represented by $X^3$, $3X^2Y$, $3XY^2$ and $Y^3$ respectively. If two of these oxygens are incorporated on the ABC-ring moiety (which includes the ring junction substituents) and one on the D-ring fragment, then the expected ion abundance ratios of the base peak ion cluster (m/z 373, 375 and 377) and the isotopic ions at m/z 114 and 116 can be represented by $X^2$, $2XY$, $Y^2$, X and Y respectively. Thus, if the *Nocardia* strain was fed with, for example, a 30:70% mixture of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen, the expected isotopic distributions for the molecular and base peak ion clusters, and ions at m/z 114 and 116, as shown in Table 9a, would be observed in the mass spectrum of the labelled compound.

Alternatively, for the case where three positions are labelled, and two specific oxygens come from the same molecule of oxygen, incorporation of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen is no longer randomised. For example, if the oxygen atoms at C-5 and C-12a are derived from one molecule of oxygen, the expected ion abundance ratios of the molecular ion cluster, and the ions at m/z 114 and 116 can be represented by $X^2$, $XY$, $X$, $XY$, $Y^2$, X and Y. For the base peak ion cluster, on the other hand, the ion abundance ratios of m/z 373 and 377 can be represented by X and Y, while the ion abundance ratio of m/z 375 should be zero. Table 9b shows the expected isotopic distributions of the various ion clusters for labelled tetracenomycin X produced in a 30:70% mixture of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen. Similarly, if the oxygen atoms at C-4 and C-12a are derived from one molecule of oxygen, the expected ion abundance
ratios of the molecular ion cluster, and the ions at m/z 114 and 116 can be represented by $X^2$, $XY$, $XY$, $Y^2$, $X$ and $Y$. The expected ion abundance ratios of the base peak ion cluster, on the other hand, can be represented by $X^2$, $2XY$ and $Y^2$. We have assumed in all the above calculations, and also in the following calculations, that no unlabelled tetracenomycin X (36) was produced before feeding the oxygen mixture. In addition, the isotopic effects of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen are expected to be insignificant and, thus, have not been taken into consideration.


<table>
<thead>
<tr>
<th></th>
<th>(i) Random Incorporation</th>
<th>(ii) Specific Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z</td>
<td>Isotopic Distribution (%)</td>
<td>m/z Isotopic Distribution (%)</td>
</tr>
<tr>
<td>114</td>
<td>30.0</td>
<td>114</td>
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<tr>
<td>116</td>
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<tr>
<td>375</td>
<td>42.0</td>
<td>375</td>
</tr>
<tr>
<td>377</td>
<td>49.0</td>
<td>377</td>
</tr>
<tr>
<td>486</td>
<td>2.7</td>
<td>486</td>
</tr>
<tr>
<td>488</td>
<td>18.9</td>
<td>488</td>
</tr>
<tr>
<td>490</td>
<td>44.1</td>
<td>490</td>
</tr>
<tr>
<td>492</td>
<td>34.3</td>
<td>492</td>
</tr>
</tbody>
</table>

a Normalised (%) with respect to each ion cluster.

For the second situation where four oxygens are incorporated in a metabolite, and denoting $X$ and $Y$ to be the mole fractions of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$-oxygen, the expected ion abundance ratios can be represented by:

(i) $X^4, 4X^3Y, 6X^2Y^2, 4XY^3, Y^4$ (molecular ion cluster), $X^3, 3X^2Y, 3XY^2, Y^3$ (base peak ion cluster), and $X$ and $Y$ (m/z 114 and 116) respectively, where all four oxygens are derived from different molecules of oxygen, i.e. by random incorporation.

(ii) $X^3, 2X^2Y, (XY^2 + X^2Y), 2XY^2, Y^3$ (molecular ion cluster), $X^2, XY, XY, Y^2$ (base peak ion cluster) and $X$ and $Y$ (m/z 114 and 116), where two oxygen atoms at C-4a and C-12a come from one molecule of oxygen, i.e. by specific incorporation. The
expected isotopic distributions of the respective ion clusters for tetracenomycin X produced in a 30:70\% mixture of \([^{16}O_2]\)- and \([^{18}O_2]\)oxygen are shown in Table 10a and b.

There is therefore a clear difference in the isotopic distribution of the respective ion clusters where three or four positions are randomly or specifically labelled in tetracenomycin X (36), and this difference would determine whether two of the labelled oxygens are derived from the same molecule of oxygen.

**Table 10 : Incorporation of Four Oxygens - Expected Isotopic Distribution for a 30 : 70\% Mixture of \([^{16}O_2]\)-and \([^{18}O_2]\)oxygen.**

<table>
<thead>
<tr>
<th></th>
<th>(i) Random Incorporation</th>
<th>(ii) Specific Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z</td>
<td>Isotopic Distribution (%)(^a)</td>
<td>m/z</td>
</tr>
<tr>
<td>114</td>
<td>30.0</td>
<td>114</td>
</tr>
<tr>
<td>116</td>
<td>70.0</td>
<td>116</td>
</tr>
<tr>
<td>373</td>
<td>2.7</td>
<td>373</td>
</tr>
<tr>
<td>375</td>
<td>18.9</td>
<td>375</td>
</tr>
<tr>
<td>377</td>
<td>44.1</td>
<td>377</td>
</tr>
<tr>
<td>379</td>
<td>34.3</td>
<td>379</td>
</tr>
<tr>
<td>486</td>
<td>0.8</td>
<td>486</td>
</tr>
<tr>
<td>488</td>
<td>7.6</td>
<td>488</td>
</tr>
<tr>
<td>490</td>
<td>26.4</td>
<td>490</td>
</tr>
<tr>
<td>492</td>
<td>41.2</td>
<td>492</td>
</tr>
<tr>
<td>494</td>
<td>24.0</td>
<td>494</td>
</tr>
</tbody>
</table>

\(^a\) Normalised (\%) with respect to each ion cluster.

### 3.3.3 Results and Discussion

*Nocardia mediterranea* (strain NT 19) in two baffled flasks, each containing 100 mL of production medium was cultured on a rotary shaker for 37 hours. Barium carbonate was then added and the two culture flasks were connected to a gas handling manifold. Fermentation was then continued in a sealed system under an artificial atmosphere comprising nitrogen and a mixture of \([^{16}O_2]\)- and \([^{18}O_2]\)oxygen for a further 10 hours, during which time approximately 1 L of the \([^{16}O_2]\)- and
\[ ^{18}\text{O}_2 \] oxygen mixture was consumed. Samples of the system’s atmosphere were taken from the gas manifold for EIMS analysis. The mole percentage of \([ ^{16}\text{O}_2 ]\) and \([ ^{18}\text{O}_2 ]\) oxygen were determined to be 28% and 72% 1.5 hours after the fermentation was continued under a sealed system and 26% and 74% 12 hours later. The culture broth was then centrifuged and the supernatant filtered. Extraction and purification of the aqueous filtrate, as previously described (refer Chapter 2), afforded 1.3 mg of labelled tetracenomycin X. A further 0.5 mg of labelled compound was obtained from the cell mass.

In the EI mass spectra of the labelled compound (from the aqueous fraction and the cell mass), several clusters of ions were observed. In the mass region of the molecular ion (m/z 486), the labelled tetracenomycin X showed second, third and fourth molecular ions carrying one, two and three \(^{18}\text{O}\) atoms at m/z 488, 490 and 492 respectively. A similar cluster of ions was observed for the \((M^+ \cdot H_2O)\) fragment at m/z 468, 470, 472 and 474. Framework fragmentation gave the base peak ion (ABC-ring moiety) at m/z 373 together with base peaks carrying one and two \(^{18}\text{O}\) atoms at m/z 375 and 377, and a D-ring fragment ion at m/z 114, together with an \(^{18}\text{O}\) isotope peak at m/z 116.

Several conclusions were drawn from the above mass spectral analysis. Firstly, the molecular ion cluster showed that three \(^{18}\text{O}\) atoms were incorporated in tetracenomycin X, a conclusion substantiated by the \((M^+ \cdot H_2O)\) ion cluster and the ion clusters from retrodiene cleavage of the labelled compound. Secondly, the framework fragmentation established that one of the sites of \([^{18}\text{O}_2]\) oxygen incorporation was on the D-ring of tetracenomycin X, i.e. at C-4, while the other two sites were on the ABC-ring moiety, presumably at C-5 and C-12a and implying that the original acetate carbonyl oxygen at C-4a had been retained (vide supra section 3.2.2).

In order to establish the location of the two labelled sites in the ABC-ring fragment, a portion of the labelled tetracenomycin X was converted to the known desmethoxyanhydro derivative (79) using the method of Siebers for the related reaction on tetracenomycin C (1). Aromatisation of the labelled tetracenomycin X results in the elimination of the ring junction substituents at C-4a and C-12a. If two
18O atoms were retained in the aromatised derivative, we could conclude that only one of the bridgehead substituents (presumably the C-12a O-methyl group) was labelled, and that a total of three labelled oxygens were incorporated in tetracenomycin X (36) at C-4, C-5 and C-12a.

Hence, the enriched tetracenomycin X and a catalytic amount of p-toluenesulphonic acid in xylene were heated under reflux (Scheme 14). Purification afforded a desmethoxyanhydro derivative which exhibited UV and EI (M+ 438) mass spectra identical to those reported for the corresponding aromatised derivative from tetracenomycin C (1). The EIMS also showed molecular ions carrying one and two oxygens at m/z 440 and 442, thus establishing that two 18O atoms were retained in the aromatised compound (79). This result also confirmed that only three oxygens in tetracenomycin X, i.e. at C-4, C-5 and C-12a, were labelled, and thus eliminated the possibility of four oxygens being introduced initially (at C-4, C-4a, C-5 and C-12a) by metabolic oxidation, with one (most likely the C-5 carbonyl) being subsequently lost by exchange with the fermentation medium or during extractive work up. If the latter was true, then only one of the 18O atoms would have been retained in the desmethoxyanhydro derivative (i.e at C-4).

Therefore, the 18O labelling regiochemistry, as shown by structure (36d), was established for labelled tetracenomycin X.

Scheme 14

![Scheme 14](image)

* labelled oxygens

Finally, we can conclude, from the base peak ion cluster (m/z 373, 375 and 377) in the EIMS of labelled tetracenomycin X (36d), that both oxygens at C-5 and C-12a...
were derived from different molecules of oxygen. The presence of the isotopic base peak at m/z 375 would be unlikely if two of the labelled oxygens came from the same molecule of oxygen. However, we could not determine if the oxygen atoms at C-4 and C-12a were derived from one or two molecules of oxygen as the ratios of the molecular ion cluster did not correspond to expected values calculated for the two alternatives (i.e. both oxygen atoms from the same or from different molecules of oxygen) for incorporation of a 28:72% (or 26:74%) mixture of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygeng. This anomaly may be due to poor mixing of the gases at the initial stages when the fermentation was continued under a sealed system.

Using the mass spectral intensities of the base peak ion cluster of the EIMS of labelled tetracenomycin X (36d) isolated from the aqueous fraction, the averaged percentage of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygeng utilised by the microorganism was calculated to be 41:59%. The expected isotopic distributions of the ions at m/z 114 and 116 were then calculated using the established percentages of the gas mixture. The observed isotopic distribution of the ion cluster at m/z 114 and 116 was found to be in good agreement with the expected ratios (see Table 11). A model set of calculations is shown in Appendix (i).

Similar analysis of the mass spectrum of labelled tetracenomycin X (36d) isolated from the cell mass gave concordant results. The averaged percentage of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygeng utilised by the microorganism was calculated to be 43:57%. The expected isotopic distributions of the ions at m/z 114 and 116 were then calculated using the established percentages of the gas mixture. The expected and observed isotopic distribution of the ions at m/z 114 and 116 were in agreement (see Table 12). Calculations using these mass spectral intensities (from EIMS of labelled tetracenomycin X isolated from the aqueous fraction and the cell mass) also showed that 41% of the unlabelled tetracenomycin X (36) was already present at the time the atmosphere was changed.

The comparisons for the expected and observed ratios of the molecular and (M+H2O) ion clusters in both cases, however, were poor, indicating the difficulty in obtaining accurate mass spectral intensity measurements of weak ions in EIMS.
3.4 CONCLUDING REMARKS

We have determined, using a mixture of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygen, that a total of three oxygens in tetracenomycin X (36) were derived by metabolic oxidation. The $^{18}\text{O}$ labelling regiochemistry was established to be at C-4, C-5 and C-12a using a combination of mass spectrometry and a chemical transformation. The C-5 and C-12a oxygen atoms were also established to have come from different molecules of oxygen by analysis of the isotopic distribution of the base peak ion cluster. However, we were unable to determine whether the C-4 and C-12a oxygen atoms were derived from the same molecule of oxygen.

The averaged percentage of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygen (42:58%) utilised by the microorganism and the amount of unlabelled tetracenomycin X (36) (41%) already present at the time of controlled oxygen addition were calculated using the mass spectral intensities of the base peak ion cluster. The observed isotopic distributions of the ion cluster at m/z 114 and 116 in the EI mass spectra (of labelled tetracenomycin X isolated from the aqueous fraction and the cell mass) were in accord with the expected ratios calculated for a 42:58% mixture of $^{16}\text{O}_2$- and $^{18}\text{O}_2$-oxygen.

The above biosynthetic experiment has also provided some insight into the biosynthesis of tetracenomycin X (36) and possibly of the angucycline antibiotics. Incorporation of three $^{18}\text{O}$ atoms into tetracenomycin X (36) provided strong evidence that the oxygens at C-1, C-3, C-4a, C-8, C-9 ester function, C-11 and C-12 were derived from acetate, and that the biosynthetic pathway involved the putative intermediate (76) in which the original acetate carbonyl oxygen at C-4a was retained. This postulate could be verified by incorporation of [1-$^{13}\text{C}$, $^{18}\text{O}_2$]acetate into tetracenomycin X (36) and analysis of the labelled compound by $^{13}\text{C}$ NMR spectroscopy. Similarly, the oxygens at C-1, C-3, C-4a, C-7 and C-8 (where present) of the aglycones of these angucycline antibiotics are also likely to have been derived from acetate whereas the oxygens at C-12 and C-12b are possibly introduced by metabolic oxidation. The biogenetic origin of the oxygens in this group of antibiotics remains to be confirmed.
CHAPTER 4

1. Melting points were determined with a Reichert hot-stage apparatus. Melting points (MP) and boiling points (BP) are uncorrected.

2. Infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrophotometer in chloroform solution (0.5 mm cells), unless otherwise stated. The following abbreviations were adopted: b (broad) ; s (strong) ; m (medium) ; w (weak). Assignment of the absorptions were obtained if appropriate. The 1600-1800 cm⁻¹ region of the IR spectra was calibrated by the absorption of polystyrene film at 1601 cm⁻¹.

3. Ultraviolet (UV) spectra were recorded on a Varian Cary 90 UV-Visible Spectrophotometer in methanol solution, unless otherwise indicated. The wavelengths of absorption maxima (λmax) were reported in ethanol:water followed by the extinction coefficients (ε) (λmax). UV spectra were calibrated by the absorption of a bromophenol blue solution filter at 600.9 nm.

4. ¹H NMR spectra were recorded on several instruments operating at the following frequencies: (a) 200 MHz (JEOL 200 SX-2006); (b) 300 MHz (Varian XL-300); and (c) 300 MHz (Varian XL-600). ¹H NMR data in the Experimental section refer to spectra recorded at 300 MHz using instrument (b) unless otherwise stated. Samples were run in deuteriochloroform (99.8% D) using tetramethylsilane as an internal standard (δ 0.0 ppm). Data were presented in the following order: chemical shift (ppm) relative to tetramethylsilane; multiplicity; intensity as the number of protons; coupling constant (J) in Hz; assignment (if appropriate). The following abbreviations were adopted: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplets, dt (doublet of triplets), Exchanging protons were identified by their disappearance upon addition of deuterium oxide. Homonuclear decoupling experiments were performed on instrument (b).

5. ¹³C NMR spectra were recorded on instrument (b) operating at 75.4 MHz and ¹³C NMR data in the Experimental section refer to spectra recorded on this instrument unless stated otherwise. Samples were run in deuteriochloroform unless otherwise indicated using tetramethylsilane as an internal standard (δ 0.0 ppm). Data were presented in the following order: chemical shift (ppm) relative to tetramethylsilane; and assignments where appropriate.

6. Low resolution electron impact mass spectra (LCIMS) were recorded at 70 eV on a Varian 4400 spectrometer. Data were presented in the following order: mass/relative intensity as a percentage of the base peak. Chemical ionization mass spectra (CIMS) were run with ethane as the reagent gas, unless stated otherwise, and
General Topics

1. Melting points were determined with a Reichert hot-stage apparatus. Melting points (MP) and boiling points (BP) are uncorrected.

2. Infrared (IR) spectra were recorded on a Perkin-Elmer 683 Infrared Spectrophotometer in chloroform solution (0.5 mm cells), unless otherwise stated. The following abbreviations were adopted: b (broad); s (sharp); sh (shoulder); w (weak). Assignment of the absorptions were attempted if appropriate. The 1800-1600 cm\(^{-1}\) region of the IR spectra was calibrated by the absorption of polystyrene film at 1601 cm\(^{-1}\).

3. Ultraviolet (UV) spectra were recorded on a Varian DMS 90 UV-Visible Spectrophotometer in methanol solution, unless otherwise indicated. The wavelengths of absorption maxima (\(\lambda_{\text{max}}\)) were reported in nanometres followed by the extinction coefficients (\(e\)). All UV spectra were calibrated by the absorption of a holmium filter at 360.9 nm.

4. \(^1\)H NMR spectra were recorded on several instruments operating at the following frequencies: (a) 200 MHz (JEOL JNM FX-200), (b) 200 MHz (Varian XL200E) and (c) 300 MHz (Varian XL300). \(^1\)H NMR data in the Experimental sections refer to spectra recorded at 200 MHz using instrument (a) unless otherwise stated. Samples were run in deuteriochloroform (99.8% D) using tetramethylsilane as an internal standard (\(\delta 0.0\) ppm). Data were presented in the following order: chemical shift (ppm) relative to tetramethylsilane; multiplicity; intensity as the number of protons; coupling constant (J) in Hz; assignment (if appropriate). The following abbreviations were adopted: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublets). Exchangeable protons were identified by their disappearance upon addition of deuterium oxide. Most heteronuclear decoupling experiments were performed on instrument (a).

5. \(^13\)C NMR spectra were recorded on instrument (a) operating at 50.10 MHz and \(^13\)C NMR data in the Experimental Section refer to spectra recorded on this instrument unless stated otherwise. Samples were run in deuteriochloroform, unless otherwise indicated, using tetramethylsilane as an internal standard (\(\delta 0.0\) ppm). Data were presented in the following order: chemical shift (ppm) relative to tetramethylsilane and assignments where appropriate.

6. Low resolution electron impact mass spectra (EIMS) were recorded at 70 e.v. on either (a) AEI MS-902 or (b) VG-Micromass 7070E double focussing mass spectrometers. Data were presented in the following order: m/z value; relative intensity as a percentage of the base peak. Chemical ionisation mass spectra (CIMS) were run with ammonia as the reagent gas, unless stated otherwise, and
recorded on instrument (b). High resolution mass spectra (HRMS) were recorded on either instrument (a) or (b) using heptacosafluorotributylamine or perfluorokerosene as a reference.

7. Microanalyses were conducted by the Australian National University Analytical Services Unit, Canberra.

8. Analytical thin layer chromatography (tlc) was performed on aluminium plates precoated with Merck Kieselgel 60 F_{254} or Merck aluminium 60 F_{254} neutral (type E). Column chromatography was carried out by one of the following:
   (i) flash chromatography using Merck Kieselgel 60 (40-60 µm) or Merck aluminium oxide 90 active neutral (60-200 µm) as the adsorbent.
   (ii) medium pressure liquid chromatography (MPLC) using Merck LiChroprep RP-8 (40-63 µm).
   (iii) high performance liquid chromatography (HPLC) using a Waters 8C_{18} 10 µm Radial-pak cartridge or a Waters C_{18} µ Bondapak reverse phase stainless steel column (3.9 mm x 30 cm). For both (ii) and (iii), compounds were detected in the eluates using a Waters R403 differential refractometer.

9. Solvents were purified and dried using standard procedures.

10. Ethereal diazomethane was prepared from N-methyl-Nitroso-p-toluenesulfonamide (Diazald) according to standard procedures and then dried over potassium hydroxide pellets.

11. Reaction temperatures refer to external or bath temperatures, unless otherwise indicated. Temperatures of -78°C and 0°C refer to dry ice/acetone and ice-water baths respectively.
Notes On Nomenclature

The Chemical Abstracts nomenclature\(^{42}\) for the following compound is as shown below. However, the trivial name has been adopted in the Introduction, Results and Discussion and the Experimental sections.

\[ \text{Me} (36) \]

\[ 4-(R), 4a-(R), 12a-(R)-1,4,4a,8,12,12a-Hexahydro-4,4a,11-trihydroxy-3,8,12a-trimethoxy-9-methoxycarbonyl-10-methyl-1,5,12-trioxo-naphthacene. \]
Isolation of Tetracenomycin X (36)

The inoculum of the *Norcardia Mediterranea* species (strain NT 19) was first prepared in a minimal medium* and then in a complex medium* with the pH adjusted to 6.5. The antibiotic, tetracenomycin X (36), was produced by growth in a modified production medium consisting of 50% glucose and 50% Bovril (20 g / L) in distilled water in baffle conical flasks (100 mL per flask). The flasks were incubated at 28°C on an open shaker at 200 r.p.m.. After fermentation for 70 h., the aqueous culture (7 L) was acidified to pH 3 with aqueous hydrochloric acid (2M), centrifuged and filtered. The mycelium was extracted several times with acetone and the filtrate was extracted three times with ethyl acetate. The acetone and ethyl acetate extracts were combined, dried over sodium sulphate and evaporated. The resulting residue was then partitioned between chloroform (100 mL) and 2% aqueous sodium bicarbonate solution (30 mL). The bicarbonate-washed chloroform solution was evaporated and chromatographed three times on Sephadex LH-20 (eluted with chloroform). Further purification was achieved by reverse phase HPLC (60% methanol in water) to afford 84 mg of tetracenomycin X (36).

* The minimal and complex media were prepared as described by Birner et al.,\(^\text{13}\)

**Physical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>152-154°C</td>
</tr>
<tr>
<td>([\alpha]_D^{22})</td>
<td>+51° (c = 1, dioxane)</td>
</tr>
<tr>
<td>IR</td>
<td>3680 (s, free O-H), 3450 (br, H-bonded O-H), 1730 (s), 1710 (s), 1690 (s), 1605 (s) cm(^{-1}).</td>
</tr>
<tr>
<td>UV</td>
<td>MeOH 408 (ε 11,250), 389 (ε 11,000), 290 (ε 33,000) nm.</td>
</tr>
<tr>
<td></td>
<td>MeOH / HCl 408 (ε 11,500), 391 (ε 11,200), 289 (ε 35,000) nm.</td>
</tr>
<tr>
<td></td>
<td>MeOH / NaOH 440 (ε 12,100), 425 (sh), 255 (ε 29,500) nm.</td>
</tr>
<tr>
<td>(^1)H NMR</td>
<td>CDC(_3) 13.92 (s, 1H, exh., 11-OH), 7.95 (s, 1H, 6-H), 7.16 (s, 1H, 7-H), 5.55 (d, 1H, J = 1.0 Hz, 2-H), 4.79 (dd, 1H, J = 1.0 and 9.8 Hz, 4-H), 4.41 (s, 1H, exh., 4a-OH), 4.0 (s, 6H, 9-CO(_2)CH(_3) and 8-OCH(_3)), 3.79 (s, 3H, 3-OCH(_3)), 3.64 (s, 3H, 12a-OCH(_3)), 2.87 (s, 4H, exh., 4-OH and 10-CH(_3)) ppm.</td>
</tr>
<tr>
<td></td>
<td>d(_6)-acetone 13.97 (s, 1H, exh., 11-OH), 7.97 (s, 1H, 6-H), 7.63 (s, 1H, 7-H), 5.47 (d, 1H, J = 1.0 Hz, 2-H), 5.12 (s, 1H, exh., 4a-OH), 4.95 (dd, 1H, J = 1.0 and 9.8 Hz, 4-H), 4.81 (d, 1H, exh., J = 9.8 Hz, 4-OH), 4.02 (s, 3-H, 9-CO(_2)CH(_3)), 3.92 (s, 3H, 8-OCH(_3)), 3.77 (s, 3H, 3-OCH(_3)), 3.55 (s, 3H, 12a-OCH(_3)), 2.83 (s, 3H, 10-CH(_3)) ppm.</td>
</tr>
<tr>
<td>(^1)3C NMR</td>
<td>CDC(_3) 195.58 (s, C-12), 193.07 (s, C-5), 190.70 (s, C-1), 171.02 (s, CO(_2)Me), 167.78 (s, C-3), 166.96 (s, C-11), 158.14 (d, (^3)J = 4.4 Hz, C-8), 140.54 (s, C-5a), 138.87 (q, (^3)J = 5.9 Hz, C-10), 129.32 (d, (^3)J = 5.0 Hz, C-9).</td>
</tr>
</tbody>
</table>
C-6a), 126.67 (s, C-10a), 121.15 (bs, C-11a), 121.15 (dd, J = 167.2 Hz, XJ = 5.9 Hz, C-6), 109.00 (s, C-7), 107.37 (dd, J = 166.8 Hz, XJ = 4.4 Hz, C-9), 101.70 (d, J = 162.6 Hz, C-2), 86.99 (m, C-12a), 83.83 (m, C-4a), 69.67 (dd, J = 143.6 Hz, XJ = 4.0 Hz, C-4), 56.88, 56.56 and 56.24 (all q, J = 146.0 Hz, 3 x OCH3), 52.74 (q, J = 146.0 Hz, CO2CH3), 21.00 (q, J = 130.2 Hz, 10-CH3) ppm.

d6-DMSO 196.73 (s, C-12), 193.54 (s, C-5), 191.55 (s, C-1), 173.52 (s, CO2Me), 167.53 (s, C-3), 165.98 (s, C-11), 157.52 (d, XJ = 4.4 Hz, C-8), 140.63 (s, C-5a), 137.17 (q, XJ = 5.9 Hz, C-10), 128.68 (d, XJ = 5.0 Hz, C-6a), 128.00 (s, C-10a), 120.29 (bs, C-11a), 120.15 (dd, J = 167.2 Hz, XJ = 5.9 Hz, C-6), 109.50 (s, C-7), 108.61 (dd, J = 166.8 Hz, XJ = 4.4 Hz, C-9), 100.80 (d, J = 162.6 Hz, C-2), 87.68 (m, C-12a), 85.47 (m, C-4a), 69.56 (dd, J = 143.6 Hz, XJ = 4.0 Hz, C-4), 56.96, 56.82 and 55.76 (all q, J = 146.0 Hz, 3 x OCH3), 52.92 (q, J = 146.0 Hz, 9-CO2CH3), 20.88 (q, J = 130.2 Hz, 10-CH3) ppm.

EIMS 486 (M+, C24H22O11, 7%), 468 (1), 455 (4), 373 (C19H16O8, 100), 357 (18), 341 (13), 114 (C5H6O3, 13).

HRMS C24H22O11 requires 486.1162 found 486.1160.
C19H17O8 requires 373.0923 found 373.0921.
C5H6O3 requires 114.0317 found 114.0316.

Production of 13C Labelled Tetracenomycin X (36)

90 Atom % 13C sodium [1-13C]acetate (0.5 g) was added in four portions as a sterile aqueous solution to each of the 35 flasks (100 mL per flask) after an initial growth period of 40.5 h. (refer to Figure 15). After a total incubation period of 66 h., the flask contents were combined and the mycelium was separated by centrifugation and filtration. The 13C labelled antibiotic (42 mg) was extracted and purified as previously described.

13C NMR CDCl3 198.6 (C-12, 3-fold enrichment), 193.1 (C-5), 190.7 (C-1, 3-fold), 171.0 (9-CO2Me, 3-fold), 167.8 (C-3, 3-fold), 166.9 (C-11, 3-fold), 158.1 (C-8, 3-fold), 138.8 (C-10, 4-fold), 129.3 (C-6a, 3-fold), 126.7 (C-10a), 121.1 (C-6), 109.0 (C-7), 107.4 (C-9), 101.7 (C-2), 87.0 (C-12a), 83.8 (C-4a, 3-fold), 69.7 (C-4, 3-fold), 56.9, 56.5 and 56.2 (all OCH3), 52.7 (9-CO2CH3), 21.0 (10-CH3) ppm.
Feeding Program for the Production of Labelled Tetracenomycin X (36)

<table>
<thead>
<tr>
<th>Fermentation time (h.)</th>
<th>Tetracenomycin X estimates of liquor (mg / L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>35.0</td>
<td>0.1</td>
</tr>
<tr>
<td>39.0</td>
<td>0.3</td>
</tr>
<tr>
<td>40.5</td>
<td>-    1st feed (1 mL / flask)</td>
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<tr>
<td>41.0</td>
<td>1.5</td>
</tr>
<tr>
<td>43.0</td>
<td>1.8</td>
</tr>
<tr>
<td>44.5</td>
<td>-    2nd feed (1 mL / flask)</td>
</tr>
<tr>
<td>47.0</td>
<td>2.8</td>
</tr>
<tr>
<td>48.5</td>
<td>-    3rd feed (2 mL / flask)</td>
</tr>
<tr>
<td>52.5</td>
<td>-    4th feed (1 mL / flask)</td>
</tr>
<tr>
<td>66.0</td>
<td>7.1</td>
</tr>
</tbody>
</table>

* These estimates were obtained by comparison of the thin layer chromatograms of the aqueous culture with chromatograms of standard solutions of pure tetracenomycin X (36).

Tetracenomycin X, 4,11-diacetate (44)

Acetic anhydride (70 µL, 25 µmol) was added to a stirred solution of tetracenomycin X (36) (2 mg, 4.1 µmol) in pyridine (150 µL) at room temperature. The reaction was monitored by tlc (10% methanol in chloroform). After 2 h., the reaction mixture was quenched with water and extracted with dichloromethane (3 x 5 mL). The extracts were dried over sodium sulphate and evaporated to dryness. The residue was then purified using Sephadex LH-20 chromatography (eluted with chloroform) to give the diacetate (44) in 81% yield (1.9 mg).

UV:
- MeOH: 403 (sh, 10000), 388 (ε 9200), 290 (ε 36500) nm.
- MeOH / HCl: 388 (ε 3300), 290 (ε 36000) nm.
- MeOH / NaOH: 440 (ε 5200), 282 (ε 17000), 242 (ε 26000) nm.

1H NMR:
- CDCl₃: 8.43 (s, 1H, 6-H), 7.26 (s, 1H, 7-H), 5.98 (bs, 1H, 4-H), 5.61 (d, 1H, J = 1.5 Hz, 2-H), 4.20 (s, 1H, exch., 4a-OH), 3.99 and 3.97 (both s, each 3H, 9-CO₂CH₃ and 8-OCH₃), 3.74 (s, 3H, 3-OCH₃), 3.59 (s, 3H, 12a-OCH₃), 2.73 (s, 3H, 10-CH₃), 2.49 (s, 3H, H₃CCO₂), 2.09 (s, 3H, H₃CCO₂) ppm.

EIMS:
- 570 (M⁺, C₂₈H₂₆O₁₅, 1%), 486 (2), 468 (5), 452 (5), 438 (19), 422 (29),
A solution of tetracenomycin X (36) (3 mg, 6.2 µmol) in a mixture of acetic anhydride and pyridine (50 µL : 250 µL) was stirred overnight at room temperature. The reaction mixture was then poured into ice, left standing for 4 h. and extracted with chloroform (3 x 10 mL). The chloroform extracts were dried over sodium sulphate and concentrated. Purification of the residue using Sephadex LH-20 chromatography (eluted with chloroform) gave the monoacetate (46) (33.7%, 1.1 mg) and the diacetate (44) (51.1%, 1.8 mg).

**Tetracenomycin X, 4-acetate (46)**

**UV**
MeOH 410 (sh), 390, 290 nm.

**$^1$H NMR**
CDCl$_3$ 13.89 (s, 1H, exch., 11-OH), 7.89 (s, 1H, 6-H), 7.17 (s, 1H, 7-H), 6.12 (d, 1H, J = 2.0 Hz, 4-H), 5.62 (d, 1H, J = 1.5 Hz, 2-H), 4.30 (s, 1H, exch., 4a-OH), 3.98 (s, 6H, 9-C$_2$H$_5$ and 8-OCH$_3$), 3.75 (s, 3H, 3-OCH$_3$), 3.63 (s, 3H, 12a-OCH$_3$), 2.88 (s, 3H, 10-CH$_3$), 2.09 (s, 3H, H$_3$CO$_2$) ppm.

**EIMS**
528 (M$^+$, C$_{26}$H$_{24}$O$_{11}$, 1%), 510 (C$_{26}$H$_{22}$O$_{11}$, 1), 497 (C$_{25}$H$_{21}$O$_{11}$, <1), 468 (C$_{24}$H$_{20}$O$_{10}$, 2), 438 (C$_{23}$H$_{18}$O$_{9}$, 22), 422 (C$_{23}$H$_{18}$O$_{8}$, 24), 373 (C$_{21}$H$_{19}$O$_{9}$, 3), 114 (C$_5$H$_6$O$_3$, 24), 43 (100).

**HRMS**
C$_{26}$H$_{24}$O$_{12}$ requires 528.1268 found 528.1263.

**Tetracenomycin X, 4,4a-diacetate (47)**

Acetic anhydride (30 µL, 0.25 mmol) and 4-dimethylaminopyridine (5 mg, 41 µmol) were added slowly to a cooled (0°C), stirred solution of tetracenomycin X (36) (20 mg, 41 µmol) in dichloromethane (1 mL). The reaction mixture was then allowed to warm up to room temperature. After 15 h., the reaction mixture was diluted with dichloromethane (5 mL) and washed with 1N hydrochloric acid (1 mL), 2% sodium bicarbonate solution (1 mL) and saturated sodium chloride solution (1 mL).

The organic phase was then dried over sodium sulphate and evaporated. Purification using gravity column chromatography yielded 40.7% (1 mg) of the monoacetate (46) and 44.9% (1.1 mg) of the diacetate (47).
UV
MeOH  403 (ε 10200), 386 (ε 9800), 290 (ε 38400) nm.
MeOH / HCl  403 (ε 10200), 386 (ε 9750), 290 (ε 38400) nm.
MeOH / NaOH  443 (ε 9700), 398 (sh), 320 (sh, ε 7950), 267 (ε 34000) nm.

1H NMR
C6D6  14.17 (s, 1H, exch., 11-OH), 7.67 (s, 1H, 6-H), 7.01 (s, 1H, 7-H),
6.73 (s, 1H, 4-H), 5.51 (s, 1H, 2-H), 3.97 and 3.94 (both s, each 3H,
9-CO2CH3 and 8-OCH3), 3.86 (s, 3H, 3-OCH3), 3.80 (s, 3H, 12a-OCH3),
2.87 (s, 3H, 10-CH3), 2.21 (s, 3H, H3CCO2), 1.88 (s, 3H, H3CCO2) ppm.

13C NMR
C6D6  196.69 (C-12), 189.07 (C-5), 188.92 (C-1), 172.86 (ester C=O),
169.77, 168.31 and 168.01 (C-3 and 2 x H3CCO2), 167.82 (C-11), 157.44 (C-8),
140.30 (C-5a), 138.44 (C-10), 129.79 (C-6a), 128.94 (C-10a), 121.00
and 120.07 (C-6 and C-11a), 108.30 (C-7), 106.75 (C-9), 102.29 (C-2),
85.50 (C-4a), 83.25 (C-12a), 65.76 (C-4), 57.20, 56.85 and 56.09 (all
OCH3), 52.62 (9-CO2CH3), 21.00, 20.71 and 20.42 (2 x H3CCO2 and
10-CH3) ppm.

EIMS
570 (M+, C28H26O13, 1%), 539 (C27H23O12, 3), 528 (C26H24O12, 8),
469 (C24H21O10, 13), 452 (C24H20O9, 32), 438 (C23H18O9, 25), 422
(C23H18O8, 54), 373 (C19H17O8, 100), 114 (C5H6O3, 47).

CIMS (NH3)
571 (M+1, 1%), 529 (5), 453 (51), 423 (100).

HRMS
C28H26O13 requires 570.1373 found 570.1368.

Deuterium-Isotopic Shift Experiment

A sample of tetracenomycin X (36) (5 mg) was exchanged several times with
d4-methanol, dried thoroughly and combined with an unexchanged sample (5 mg) of the
antibiotic (36). A solution (ca. 5% w/v) of this 1:1 mixture of deuterio and non-deuterio
tetracenomycin X (36) in d6 DMSO was prepared, treated with calcium sulphate
(non-indicating Drierite) to remove residual water, and filtered. The 13C NMR spectrum
was recorded at 50 MHz at 22°C on a Varian XL 200E instrument (refer Table 7).

Selective 1H-13C NMR Decoupling Experiment

The 1H and fully coupled 13C NMR spectra of tetracenomycin X (36) were first
recorded in deuteriochloroform at 200 MHz and 50 MHz, respectively on a Varian XL
200E instrument. Various signals in the 1H NMR spectrum were then selectively irradiated
and the coupled 13C NMR spectra recorded. Irradiation of the signal at δ 4.41 ppm
(4a-OH) caused simplification of the multiplet at δ 83.83 ppm (C-4a), whereas the
approximately 8-lined multiplet at $\delta$ 87.0 ppm (C-12a) collapsed to a doublet ($J=3$ Hz) on irradiation of the proton signal at $\delta$ 3.64 ppm (12a-OMe).

**NOE Spectroscopy**

NOE difference spectra were measured at 200 MHz on a Varian XL200E. Samples were ca. 0.02M in CDCl$_3$ and were degassed with argon before recording spectra. Four experiments were run with three experiments irradiating proton resonances (the C-4 and C-4a hydroxyls and C-12a O-methyl) with the decoupler set to where there were no proton resonances. The last experiment provided a 'control' spectrum. The decoupler power level was set below saturation level in order to enhance frequency selectivity (generally at 40 dB below 2 W). 1000 Transients were accumulated for each experiment with 5 sec. of preirradiation prior to each transient. Phase cycling and quadrature detection were employed in order to accumulate a difference spectrum. The FID's were processed by subtracting each in turn from the control. Each resulting FID was Fourier transformed with equal line broadening (1.5 Hz) and % NOE enhancements were calculated as the integral of the enhanced proton(s), divided by the corresponding integral in the controlled spectrum and multiplied by 100. No significant NOE enhancements were, however, observed in the intensity of the C-4 and C-4a hydroxyl and in the C-12a O-methyl resonance upon irradiation of any of these signals at 22ºC. The above experiments were repeated in CD$_2$Cl$_2$ at -30ºC. However, no significant NOE enhancements were observed.

**Tetracenomycin X, 4,4a-carbonate (48)**

A cooled solution of 20% phosgene in toluene (30.5 µL, 62 µmol) was added to a cooled, stirred solution of tetracenomycin X (36) (15 mg, 31 µmol) and dry pyridine (9.8 µL, 0.12 mmol) in dry dichloromethane (0.5 mL). The reaction mixture was then allowed to warm up to room temperature. After 2 h., the reaction mixture was diluted with dichloromethane (5 mL) and washed with water (3 x 1 mL). The aqueous washings were re-extracted with dichloromethane (5 mL) and the extracts were combined, dried and concentrated. The residue was purified twice using Sephadex LH-20 chromatography (eluted with chloroform) to afford the cyclic carbonate (48) in 65% yield (10.3 mg).

- **MP** 240 - 243ºC.
- **IR** 3585 (free O-H), 1845 (s, 5-membered cyclic carbonate C=O), 1730 (s), 1710 (sh), 1685 (s), 1602 cm$^{-1}$.
- **UV** CHCl$_3$ 419 (ε 12,920), 395 (ε 11,950), 292 (ε 42,860) nm.
MeOH 435 (ε 7,530), 411 (ε 10,070), 395 (ε 9,800), 292 (ε 25,500) nm.
MeOH / HCl 415 (ε 10,540), 395 (ε 9,800), 293 (ε 23,130) nm.
MeOH / NaOH 442, 267 nm (dec.).

^1H NMR CDCl₃ 14.11 (s, 1H, exch., 11-OH), 8.03 (s, 1H, 6-H), 7.19 (s, 1H, 7-H), 5.71 (s, 1H, 4-H), 5.21 (bs, 1H, 2-H), 4.00 (s, 6H, 9-CO₂CH₃ and 8-OCH₃), 3.82 (s, 3H, 3-OCH₃), 3.70 (s, 3H, 12a-OCH₃), 2.87 (s, 3H, 10-CH₃) ppm.

^13C NMR CDCl₃ 192.96 (C-12), 187.91 (C-5), 185.34 (C-1), 167.93, 167.47, 164.69, 158.49 (C-8), 151.13, 140.55 (C-5a), 139.17 (C-10), 129.80 (C-6a), 125.68 (C-10a), 121.35 (C-11a), 122.29 (C-6), 108.20 (C-7), 107.60 (C-9), 104.49 (C-2), 84.44 (C-12a*), 83.92 (C-4a*), 74.03 (C-4), 57.38, 56.83 and 56.39 (3 x OCH₃), 52.83 (9-CO₂CH₃), 21.13 (10-CH₃) ppm.

EIMS 512 (M+, C₂₅H₂₀O₁₂, <1%), 468 (7), 452 (15), 438 (52), 422 (C₂₃H₁₈O₈, 100), 407 (45), 399 (12), 391 (27), 377 (12), 361 (36), 333 (21), 44 (95).

CIMS (NH₃) 530 (M⁺+18, 7%), 513 (M⁺+1, 3), 469 (5), 439 (100), 423 (45).

HRMS C₂₃H₂₂O₁₁ requires 422.1002 found 422.1003.

* Interchangeable

4-(R), 4a-(R), 12a-(R)-1,4,4a,8,12,12a-Hexahydro-4a,11-dihydroxy-3,8,12a-trimethoxy-9-methoxycarbonyl-4-[2-(R)-2-methoxy-2-phenylacetyl]-10-methyl-1,5,12-trioxonaphthacene (49).

N,N-Dimethylaminopyridine (2.6 mg, 21 µmol) was added to a solution of tetracenomycin X (36) (5.1 mg 10.5 µmol), followed by (R)-O-methylmandelic acid (3.5 mg, 21 µmol) and dicyclohexylcarbodiimide (2.3 mg, 21 µmol) in dichloromethane (1 mL) at room temperature. After 10 min., the dicyclohexylurea was filtered off and washed with a small amount of cold dichloromethane. The filtrate was evaporated to dryness. Purification was achieved by preparative tlc (silica, 20 x 20 cm plate, 5% methanol in chloroform) to provide 85% (11.3 mg) of the ester (49).

IR 3680, 3610, 3500, 1760, 1735, 1715, 1700, 1620, 1601 cm⁻¹.
UV MeOH 443 (ε 2900), 413 (ε 13200), 393 (ε 12000), 291 (ε 41200), 240 (ε 28200) nm.
MeOH / HCl 413 (ε 13500), 393 (ε 12800), 291 (ε 45000), 240 (ε 27700) nm.
MeOH / NaOH 440 (ε 14500), 320 (sh, ε 5700), 285 (sh, ε 21500), 254 (ε 37300) nm.
$^1$H NMR CDCl$_3$ 13.87 (s, 1H, exch., 11-OH), 7.61 (s, 1H, 6-H), 7.44 (m, 5H, Ar.H of mandelate ester), 7.10 (s, 1H, 7-H), 6.06 (d, 1H, J = 1.5 Hz, 4-H), 5.60 (d, 1H, J = 1.5 Hz, 2-H), 4.82 (s, 1H, CH of mandelate ester), 4.00 (s, 3H, 8-OCH$_3$), 3.98 (s, 3H, 9-CO$_2$CH$_3$), 3.94 (s, 1H, exch., 4a-OH), 3.70 (s, 3H, 3-OCH$_3$), 3.60 (s, 3H, 12a-OCH$_3$), 3.38 (s, 3H, OCH$_3$ of mandelate ester), 2.86 (s, 3H, 10-CH$_3$) ppm.

$^{13}$C NMR CDCl$_3$ 195.0 (C-12), 192.4 (C-5), 190.1 (C-1), 169.6 (9-CO$_2$Me), 167.8 (C-3), 167.5 (C-11), 167.1 (C=O of mandelate ester), 158.1 (C-8), 140.6 (C-5a), 138.9 (C-10), 135.1 (Ar.C of mandelate ester), 129.3 (C-6a), 128.8, 128.4 and 127.3 (all Ar.C's of mandelate ester), 126.3 (C-10a), 121.5 (C-6 and C-11a), 108.9 (C-7), 107.6 (C-9), 102.6 (C-2), 86.7 (C-12a), 82.8 (C-4a), 81.7 (CH of mandelate ester), 69.2 (C-4), 57.4 (OCH$_3$ of mandelate ester), 56.8 (3-OCH$_3$), 56.6 (12a-OCH$_3$), 56.2 (8-OCH$_3$), 52.7 (9-CO$_2$CH$_3$), 21.0 (10-CH$_3$) ppm.

EIMS 603 (C$_{32}$H$_{27}$O$_{12}$, 1%), 468 (1), 452 (3), 438 (13), 422 (4), 409 (3), 395 (3), 372 (4), 357 (5), 121 (C$_7$H$_9$O, 100), 113 (2), 77 (25).

CIMS (NH$_3$)

HRMS 652 (C$_{33}$H$_{30}$O$_{12}$ M$^+$+18, 1%), 635 (M$^+$+1, 100), 469 (14), 453 (19), 423 (15), 373 (11), 184 (61), 121 (45).

C$_{32}$H$_{27}$O$_{12}$ requires 603.1503 found 603.1503.

C$_8$H$_9$O requires 121.0653 found 121.0653.

The above experiment was repeated using (S)-O-methylmandelic acid to give the (S)-ester (50), after purification, in 80.5% yield (10.8 mg).

$^1$H NMR CDCl$_3$ 13.88 (s, 1H, exch., 11-OH), 7.87 (s, 1H, 6-H), 7.35 (m, 5H, Ar.H of mandelate ester), 7.16 (s, 1H, 7-H), 6.07 (d, 1H, J = 1.5 Hz, 4-H), 5.50 (d, 1H, J = 1.5 Hz, 2-H), 4.84 (s, 1H, CH of mandelate ester), 4.25 (s, 1H, exch., 4a-OH), 4.00 (s, 3H, 8-OCH$_3$), 3.98 (s, 3H, 9-CO$_2$CH$_3$), 3.62 (s, 3H, 12a-OCH$_3$), 3.37 (s, 6H, 3-OCH$_3$ and OCH$_3$ of mandelate ester), 2.86 (s, 3H, 10-CH$_3$) ppm.

$^{13}$C NMR CDCl$_3$ 194.9 (C-12), 191.7 (C-5), 190.0 (C-1), 169.7 (9-CO$_2$Me), 167.8 (C-3), 167.4 (C-11), 167.1 (C=O of mandelate ester), 158.1 (C-8), 140.6 (C-5a), 138.9 (C-10), 135.3 (Ar.C of mandelate ester), 129.3 (C-6a), 129.1, 128.7 and 127.3 (all Ar.C's of mandelate ester), 126.3 (C-10a), 121.1 (C-6 and C-11a), 108.8 (C-7), 107.5 (C-9), 102.9 (C-2), 86.6 (C-12a), 82.7
(C-4a), 81.9 (CH of mandelate ester), 69.4 (C-4), 57.2 (OCH3 of mandelate ester), 56.6 (3-OCH3), 56.4 (12a-OCH3), 56.0 (8-OCH3), 52.7 (9-CO2CH3), 21.0 (10-CH3) ppm.

d6-acetone 196.9, 192.8, 190.8, 170.6, 168.7, 168.0, 167.6, 159.0, 141.6, 138.6, 137.1, 130.3, 129.5, 129.2, 128.2, 128.1, 121.4, 121.3, 110.1, 108.9, 103.1, 88.1, 84.3, 83.1, 70.4, 69.6, 57.7, 56.9, 56.8, 54.4, 52.6, 21.1 ppm.

EIMS 603 (C32H27O12, 1%), 468 (2), 452 (3), 438 (8), 422 (5), 409 (3), 395 (2), 372 (4), 357 (5), 121 (C7H9O, 100), 77 (17).

CIMS (NH3)
652 (C33H30O12 M++18, 4%), 635 (M++1, 31), 469 (6), 453 (9), 439 (18), 423 (17), 184 (100), 121 (47).

HRMS C32H27O12 requires 603.1503 found 603.1503.
C8H9O requires 121.0653 found 121.0653.

** Attempted Preparation of Tetracenomycin X, 4,4a,11-trimethyl ether (32) **

(i) 2,6-Di-tert-butyl-4-methylpyridine (10.6 mg, 51.5 µmol) and methyl triflate (5.8 µL, 51.5 µmol) were added to a solution of tetracenomycin X (36) (5 mg, 10.3 µmol) in dry dichloromethane (1 mL). The mixture was heated under reflux under nitrogen for 24 h. The reaction mixture was then cooled, diluted with dichloromethane (5 mL), washed with 5% aqueous sodium bicarbonate solution (3 x 1 mL), 2M aqueous hydrochloric acid (3 x 1 mL) and then brine (1 mL). The organic fraction was dried over sodium sulphate and concentrated. Purification of the complex crude mixture by preparative tlc (silica, 20 x 20 cm plate, 2% methanol in chloroform) yielded 20% (1.0 mg) of the monomethyl ether (51) and 50% of unchanged starting material (36).

**Tetracenomycin X, 4-methyl ether (51) **

IR 3680, 3610, 3500, 1745, 1710, 1700 (sh), 1610 cm⁻¹.

UV 382, 347, 286.

^1H NMR CDCl₃ 13.93 (s, 1H, exch., 11-OH), 7.98 (s, 1H, 6-H), 7.18 (s, 1H, 7-H), 5.54 (d, 1H, J = 1.5 Hz, 2-H), 4.40 (d, 1H, J = 1.5 Hz, 4-H), 4.18 (s, 1H, exch., 4a-OH), 3.99 (s, 6H, 8-OCH₃ and 9-CO₂CH₃), 3.79 (s, 3H, 3-OCH₃), 3.64 (s, 3H, 12a-OCH₃), 3.40 (s, 3H, 4-OCH₃), 2.88 (s, 3H, 10-CH₃) ppm.

HRMS \( \text{C}_{25}\text{H}_{24}\text{O}_{11} \) requires 500.1319 found 500.1319.

(ii) An excess of freshly prepared diazomethane in ether was added to a stirred solution of tetracenomycin X (36) (5 mg, 10.3 \( \mu \)mol) and boron trifluoride etherate (4 \( \mu \)L, 30.9 \( \mu \)mol) in dry dichloromethane (500 \( \mu \)L). After 18 h., the reaction mixture was diluted with dichloromethane (5 mL), washed with water (2 \times 1 mL) and brine (1 mL), dried over sodium sulphate and then evaporated. Purification of the residue using preparative tlc (silica, 20 \times 20 \text{ cm plate}, 5\% methanol in chloroform) afforded 5\% (0.5 mg) of the monomethyl ether (51) and 80\% of the unchanged starting material (36).

(iii) Methyl iodide (4 mL) and barium oxide (4 mg) were added to a solution of tetracenomycin X (36) (5 mg, 10.3 \( \mu \)mol) and the reaction mixture was stirred at room temperature for 60 h. The precipitate was filtered, washed with a small amount of dichloromethane and the filtrate was evaporated to dryness. The residue was then purified twice by preparative tlc (silica, 20 \times 20 \text{ cm plate}, 2\% methanol in chloroform) to give 20\% (1 mg) of the monomethyl ether (51) and 60\% of unchanged starting material (36).

**Tetracenomycin X, 4,4a,11-trimethyl ether (32)**

A stirred solution of tetracenomycin X (36) (10 mg, 20.6 \( \mu \)mol) in methyl iodide (4 mL) was treated with commercial silver oxide (4 mg) at room temperature for 60 h. The silver salts were then separated by centrifugation and the supernatant was evaporated to dryness. A \(^1\text{H} \text{NMR}\) of the residue showed a mixture of starting material (36) and mono- and dimethylated compounds. This mixture was then subjected to further methylation for another 48 h. and worked up as previously described. Purification was achieved by preparative tlc (silica, 20 \times 20 \text{ cm plate}, 2\% methanol in chloroform) to afford 5\% of the trimethyl ether (32).

A mixture of tetracenomycin X (5 mg, 10.3 \( \mu \)mol) and freshly prepared silver oxide (5 mg) in methyl iodide (4 mL) was subjected to ultrasound for 50 h. in a water bath kept at 10\(^{\circ}\text{C}\). The silver salts were then separated by centrifugation and the supernatant was concentrated. Purification was achieved by preparative tlc (silica, 20 \times 20 \text{ cm plate}, 2\% methanol in chloroform) to afford 19\% (1 mg) of the trimethyl ether (32).

\begin{align*}
\text{IR} & : 1735 (s), 1715, 1690, 1660, 1609 (s) \text{ cm}^{-1} . \\
\text{UV} & : \text{MeOH} 392 (\epsilon 3200), 340 (\text{sh,} \epsilon 4400), 287 (\epsilon 31000) \text{ nm} . \\
\text{\(^1\text{H} \text{NMR}\)} & : \text{CDCl}_3 8.14 (s, 1\text{H,} 6-\text{H}), 7.07 (s, 1\text{H,} 7-\text{H}), 5.30 (s, 1\text{H,} 2-\text{H}), 4.86 (s, 1\text{H,} 4-\text{H}), 3.98 (s, 3\text{H,} 9-\text{CO}_2\text{CH}_3), 3.94 (s, 3\text{H,} 8-\text{OCH}_3), 3.90 (s, 3\text{H,} 11-\text{OCH}_3), 3.79 (s, 3\text{H,} 3-\text{OCH}_3), 3.74 (s, 3\text{H,} 12a-\text{OCH}_3), 3.64 (s, 3\text{H,} 12b-\text{OCH}_3) .
\end{align*}
Production of $^{18}$O Labelled Tetracenomycin X (36d)

The inoculum of *Nocardia mediterranea* (strain NT 19) was prepared as previously described and the *Nocardia* species in two baffled flasks, each containing 100 mL of production medium, 50% glucose (20 g/L) and 50% Bovril (20 g/L), was cultured on a rotary shaker for 37 h. Barium carbonate was then added and the two flasks were connected to a gas handling manifold. Fermentation was then continued in a sealed system under an artificial atmosphere containing nitrogen and a mixture of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$oxygen. The gas mixture in the closed system was circulated directly through the fermentation medium and a flask containing saturated barium hydroxide solution (to trap expired carbon dioxide) by means of a peristaltic pump. Samples of the system’s atmosphere were taken from the gas manifold for EIMS analysis. The mole percentages of $[^{16}\text{O}_2]$- and $[^{18}\text{O}_2]$oxygen were determined to be 28% and 74% 12 h. later. An approximate atmospheric pressure in the sealed system was maintained by addition of nitrogen. The culture broth was then centrifuged and the supernatant filtered. Extraction and purification of the aqueous filtrate, as previously described, afforded 1.3 mg* of labelled tetracenomycin X (36d). A further 0.5 mg* of labelled compound (36d) was obtained from the cell mass as described previously.

* estimated by UV spectroscopy using ε values calculated previously.

**Tetracenomycin X (36d) from Aqueous Fraction**

EIMS ** 486 (100%), 488 (51.6), 490 (55.9), 492 (34.8); 468 (100), 470 (51.8), 472 (53.2), 474 (28.9); 373 (100), 375 (54.5), 377 (38.7); 114 (100), 116 (51.3)

**Tetracenomycin X (36d) from Cell Mass**

EIMS ** 486 (100%), 488 (57.6), 490 (39.7), 492 (33.6); 468 (100), 470 (51.3), 472 (55.5), 474 (30.5); 373 (100), 477 (37.6); 114 (100), 116 (48.0).

** summed scan and each ion cluster is expressed as % with respect to the largest peak (100%).
Desmethoxy-anhydro-tetracenomycin X (79) *

A solution of tetracenomycin X (36) (0.5 mg, 1.0 µmol) and a catalytic amount of p-toluenesulphonic acid in xylene was heated with stirring under reflux for 5 h., during which the colour of the solution changed from yellow to orange-red. The reaction mixture was then cooled and evaporated to dryness. Purification was achieved by consecutive Sephadex LH 20 chromatography (chloroform) to afford 20% (0.1 mg)** of the desmethoxy-anhydro derivative (79).

UV (CHCl₃) 536 (Lit. ε 20600), 504 (ε 20100), 472 (sh, ε 11500), 352 (sh, ε 8100), 337 (ε 8600), 315 (ε 13100), 282 (ε 35400).

EIMS 438 (C₂₃H₁₃O₉, 17.0%), 423 (3.5), 407 (3), 395 (2), 377 (2.5), 349 (1.5), 57 (100).

HRMS C₂₃H₁₃O₉ requires 438.0951 found 438.0952.

* 1,4,11-Trihydroxy-3-8-climethoxy-9-methoxycarbonyl-10-methyl-naphthalene-5,12-quinone.

The above experiment was repeated with 0.2 mg (0.4 µmol) of labelled tetracenomycin X (36d). The labelled desmethoxy-anhydro derivative was obtained in 15% yield** after purification.

EIMS 438 (C₂₃H₁₃O₉, 2%), 440 (m+2, 1), 442 (m+4, <1), 423 (1.5), 407 (1), 409 (1), 381 (<1), 367 (<1), 353 (<1), 57 (100).

** The yield was calculated using literature ε values.³
Appendix (i)

a) Calculation of the mole percentages of $^{16}\text{O}_2$ and $^{18}\text{O}_2$.

Let the mole fractions of $^{16}\text{O}_2 : ^{18}\text{O}_2 = x : 1-x$.

For the base peak ion cluster (m/z 373, 375 and 377) containing two independent O atoms:

$$^{16}\text{O}_2 : ^{16}\text{O}^{18}\text{O} : ^{18}\text{O}_2$$

$$x^2 : 2x(1-x) : (1-x)^2$$

Using the mass spectral intensities of the EIMS of labelled tetracenomycin X (36d) isolated from the cell mass (see Table 12):

$$2x(1-x) = 56.35$$

$$37.64(2x) = 56.35(1-x)$$

$$x = 0.4281$$

$$=> % ^{16}\text{O}_2 : ^{18}\text{O}_2 = 42.8 : 57.2$$

The expected isotopic distribution of the ions at m/z 114 and 116 for 42.8 : 57.2% of $^{16}\text{O}_2$:

$[^{18}\text{O}_2]$ is:

<table>
<thead>
<tr>
<th>m/z</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>42.8</td>
</tr>
<tr>
<td>116</td>
<td>57.2</td>
</tr>
</tbody>
</table>

b) Calculation of the % of unlabelled tetracenomycin X (36) produced prior to controlled oxygen addition (using mass spectral intensities of the base peak ion cluster from EIMS).

Theoretical ratios of m/z 373, 375 and 377 => 0.183 : 0.49 : 0.327

Amount of unlabelled tetracenomycin X (36) formed = 100 - (100 x 0.183) x 100%

$$100 + 56.4 + 37.6 = 41\% of total tetracenomycin X isolated from the cell mass.$$

Ratios calibrated for unlabelled tetracenomycin X and expressed as % with respect to largest peak:

<table>
<thead>
<tr>
<th>m/z</th>
<th>Calibrated for 41% of unlabelled tetracenomycin X</th>
<th>Expressed as % w.r.t. largest peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>0.428(100-41) + 41 = 66.0</td>
<td>100</td>
</tr>
<tr>
<td>116</td>
<td>0.572(100-41) + 41 = 34</td>
<td>51.0</td>
</tr>
</tbody>
</table>
REFERENCES

12. This work was presented at the 5th National Royal Australian Chemical Institute Conference (Organic Chemistry Division) at the University of Adelaide, South Australia in May, 1986.
15. R. Rickards, Personal communication.


The microbial work was performed by Mrs. M. Anderson and Mrs. J. Rothschild.


CHAPTER 5

5.1 INTRODUCTION

Streptomycin (SD), the first member of the streptomycin antibiotics, was isolated from a fermentation of Streptomyces griseus in 1944. Since then, it has been independently isolated from several other microorganisms (refer Table 13). Preliminary tests revealed that streptomycin (SD) had significant antitumour and antibiotic activity, and hence prompted intensive research regarding its use as an antitumour drug. In the last two decades, studies have also been directed towards its laboratory synthesis and towards its mechanism of cytotoxicity.

Between 1970 and 1990, a streptomycin group of antibiotics was established by the inclusion of analogous compounds, hygromycin (81), spectinomycin (82), and demethylstreptomycin (83) (Table 13). In general, these compounds are referred to by their trivial names, although in the laboratory synthesis studies of streptomycin (SD), the systematic names have been used.

5.2 ISOLATION AND STRUCTURE

To date, four distinct members of the streptomycin group of antibiotics have been isolated from various Streptomyces species (Table 13). Degenerative methods, chemical transformations, chemistry of derivatives and the use of spectrometric techniques led to assignments of their structures.

5.2.1 The Structure of Streptomycin

Streptomycin (SD) was first isolated in 1949 from a culture of Streptomyces Antibiotica. The same compound has also been isolated independently from strep.
CHAPTER 5 : INTRODUCTION TO THE STREPTONIGRIN ANTIBIOTICS

5.1 INTRODUCTION

Streptonigrin (80), the first member of the streptonigrin antibiotics, was isolated from a fermentation of *Streptomyces flocculus* in 1959.\textsuperscript{43a} Since then, it has been independently isolated from several other microorganisms (refer Table 13).\textsuperscript{43b-d} Preliminary tests revealed that streptonigrin (80) had significant anticancer and antibiotic activity, and hence prompted intensive research regarding its use as an anticancer drug.\textsuperscript{45,66-68,71,72} In the last two decades, studies have also been directed towards its laboratory synthesis,\textsuperscript{46,74,81} its biosynthesis\textsuperscript{57-75} and towards its mechanism of cytotoxic action.\textsuperscript{43,54,56}

Between 1970 and 1987, a streptonigrin group of antibiotics was established by the inclusion of analogous compounds, lavendamycin (81),\textsuperscript{48,49} streptonigrone (82)\textsuperscript{44} and demethylstreptonigrin (83)\textsuperscript{51,52} (Table 13). In general, these compounds are referred to by their trivial names, although in the laboratory synthetic studies of streptonigrin (80), the systematic names have been used.

5.2 ISOLATION AND STRUCTURE

To date, four distinct members of the streptonigrin group of antibiotics have been isolated from various *Streptomyces* species (Table 13). Degradative studies, chemical transformations, formation of derivatives and the use of spectroscopic techniques led to assignments of their structures.

5.2.1 The Structure of Streptonigrin

Streptonigrin (80) was first isolated in 1959 from a culture of *Streptomyces flocculus*.\textsuperscript{43a} The same compound has also been isolated independently from strains
Table 13: The Streptonigrin Antibiotics

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptonigrin (80)</td>
<td><img src="image" alt="Streptonigrin Structure" /></td>
<td><em>Streptomyces flocculus</em></td>
</tr>
<tr>
<td></td>
<td><em>Actinomyces albus var. bruneomycini</em></td>
<td><em>S. rufochromogenes</em></td>
</tr>
<tr>
<td></td>
<td><em>S. echinatus</em></td>
<td>Unidentified <em>Streptomyces</em> species from China</td>
</tr>
<tr>
<td></td>
<td><em>Streptomyces lavendulae</em></td>
<td></td>
</tr>
<tr>
<td>Lavendamycin (81)</td>
<td><img src="image" alt="Lavendamycin Structure" /></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Streptomyces albus</em></td>
<td></td>
</tr>
<tr>
<td>Streptonigrone (82)</td>
<td><img src="image" alt="Streptonigrone Structure" /></td>
<td>Unidentified <em>Streptomyces</em> species from China</td>
</tr>
<tr>
<td>Demethylstreptonigrin (83)</td>
<td><img src="image" alt="Demethylstreptonigrin Structure" /></td>
<td><em>Streptomyces albus</em></td>
</tr>
<tr>
<td></td>
<td>Unidentified <em>Streptomyces</em> species from China</td>
<td></td>
</tr>
</tbody>
</table>
of *Actinomyces albus var. bruneomycini* 43b,c *S. rufochromogenes*, 43d *S. echinatus* 43d and, most recently, from an unidentified *Streptomyces* strain collected in China. 44 In 1963, the structure of streptonigrin (80) was deduced by Rao, Biemann and Woodward 45 using a combination of spectroscopic and degradative methods (Schemes 15-17a,b).

The exact elemental composition of streptonigrin (80) was not obtainable by standard combustion analysis. However, its composition was determined by mass spectrometric comparison of the hydroquinone derivative (84) (C$_{31}$H$_{36}$N$_{4}$O$_{8}$) and the deuteriohydroquinone derivative (85) (C$_{31}$H$_{18}$D$_{18}$N$_{4}$O$_{8}$), prepared by catalytic hydrogenation of streptonigrin (80) followed by exhaustive methylation with normal and heavy dimethyl sulphate, respectively (Scheme 15).

The structure determination of streptonigrin (80) primarily involved several oxidative degradation steps (refer Schemes 16-17a,b). The presence of a dimethoxyphenol was presupposed for streptonigrin (80) when the tribasic streptonigric acid (86) was obtained on treatment of (80) with alkaline hydrogen peroxide. The substitution pattern of the D-ring and the linkage of the D-ring to
the ABC-ring chromophore was then established by the following transformations. First, when $\alpha$-methyl streptonigric acid (88), obtained by alkaline hydrolysis of the ester (87), was treated with hot alkaline permanganate, the known 2,3,4-trimethoxybenzoic acid (89) was obtained. The position of the phenol group in the acid (86) was then determined by conversion of the trideuteriomethyl ether (90) [prepared from streptonigric acid (86)] to the undeuterated benzofuran (91) on treatment with nitric acid (Scheme 16).

Scheme 16
Treatment of streptonigrin (80) with alkaline permanganate gave the tetrabasic streptonigrinic acid. The structure (92) was established for this degradation product by a series of chemical transformations (Scheme 17a), and by $^1$H NMR studies. Treatment of the methyl ester (93) with nitric acid gave tetramethyldesamino streptonigrinate (94) which, on base hydrolysis, gave the corresponding acid (95). The 5-methyl-2,2'-bipyridyl (96), obtained by decarboxylation of the acid (95) over soda lime, was identical with an authentic sample, thus establishing the linkage position of the B- and C-rings (Scheme 17a). Streptonigrinic acid (92) was also oxidized to pyridine-2,3,6-tricarboxylic acid (97) using sodium hypochlorite. This acid (97), on thermal decarboxylation over soda lime, gave the pyridine diacid (98) which was identical with an authentic sample. $^1$H NMR studies of the tetramethyl ester (93) demonstrated the presence of two adjacent protons (dd, 1H each, J=8 Hz, δ 8.28 and 8.83 ppm), a primary amino group (s, 2H, δ 7.84 ppm, exchangeable with D$_2$O) and a methyl group attached to an aromatic ring (s, 3H, δ 2.58 ppm). Sequential treatment of the acid (92) with hydrogen and platinum dioxide then alkaline permanganate, and followed by distillation from soda lime, gave 3-amino-5-methylpyridine (99), therefore establishing the positions of the amino and methyl groups on the C-ring (Scheme 17a).

Initially, the existence of a quinone or quinonimine was presupposed for the A-ring of streptonigrin (80) on the basis of the oxidative degradations described (vide supra), and colour reactions with several test reagents. The A-ring was subsequently determined to be an aminoquinone rather than a hydroxyquinonimine since a derivative (100), (C$_{27}$H$_{26}$N$_4$O$_8$), containing only two new o-methyl groups was obtained, even on prolonged heating of streptonigrin (80) with dimethyl sulphate, acetone and potassium carbonate. The arrangement of the quinonoid A-ring was determined by the following chemical sequence (Scheme 17b). Treatment of the ester (100) with hydroxylamine followed by dithionite reduction gave the amino derivative (101). The amine (101) was then condensed with biacetyl giving the quinoxaline (102). Oxidation of this derivative (102) with permanganate in hot pyridine yielded the ester (103). Base hydrolysis of the ester (103) gave the corresponding
Scheme 17a

\[ \text{MnO}_4^- / ^\cdot\text{OH} \]

\[ R=\text{H} \]

\[ R=\text{Me} \]

\[ \text{HNO}_3 / \text{ether} \]

\[ R=\text{Me} \]

\[ R=\text{H} \]

\[ \text{NaOCl} \]

1. \( \text{H}_2 / \text{PtO}_2 \)
2. \( \text{KMnO}_4 / ^\cdot\text{OH} \)
3. \( \text{soda lime} \)

\[ \text{Soda lime} \]

\[ \text{RO}_2\text{C} \]

\[ \text{RO}_2\text{C} \]

\[ \text{OH}^- \]

\[ \text{Soda lime} \]

\[ \text{HO}_2\text{C} \]

\[ \text{HO}_2\text{C} \]

\[ \text{HO}_2\text{C} \]

\[ \text{HO}_2\text{C} \]

\[ \text{Me} \]

\[ \text{Me} \]

1. H\(_2\)/PtO\(_2\)
2. KMnO\(_4\)/\(^\cdot\)OH
3. soda lime

\[ \text{NaOCl} \]

\[ \text{RO}_2\text{C} \]

\[ \text{RO}_2\text{C} \]

\[ \text{OH}^- \]

\[ \text{Soda lime} \]

\[ \text{HO}_2\text{C} \]

\[ \text{HO}_2\text{C} \]

\[ \text{HO}_2\text{C} \]

\[ \text{Me} \]
tricarboxylic acid (104) which was thermally decarboxylated to the tetracyclic compound (105) (Scheme 17b). The $^1$H NMR spectrum of (105) was consistent with the arrangement of the aromatic protons as shown.

All the above observations supported the structure, as indicated by (80), for streptonigrin. The $^1$H and $^{13}$C NMR data have been recorded and are likewise in accord with the structure (80).

A single crystal X-ray diffraction analysis reported by Chiu and Lipscomb

Scheme 17b
confirmed the structural assignment for streptonigrin (80). They demonstrated that the A, B and C rings of streptonigrin (80) are nearly coplanar due to hydrogen bonding between one hydrogen of the C-ring amino group and the quinoline B-ring nitrogen. The D-ring, on the other hand, is almost perpendicular to the plane of the other three rings.

5.2.2 The Structure of Lavendamycin

The antibiotic, lavendamycin (81), was isolated as a dark red solid from Streptomyces lavendulae (strain C22030) by Gould et al.48a,b Its structure was established by comparison of its physical data with those of streptonigrin (80) and several other compounds, and by its laboratory synthesis.48,49

Lavendamycin (81) exhibited IR absorption bands characteristic of a carboxylic acid and a quinone (3600-2800, 1740, 1692, 1610 and 1590 cm⁻¹). Its UV spectrum, which showed three absorption maxima at 391, 246 and 234 nm, was similar to that of streptonigrin (80) (382 and 245 nm). Elemental analysis of the compound established an empirical formula of C_{22}H_{14}N_{4}O_{4} for lavendamycin (81).

A possible structure (81) was proposed for lavendamycin by analysis of the \(^1\)H, \(^{13}\)C NMR and mass spectral data. This assignment was confirmed by a \(^{13}\)C NMR study of lavendamycin (81) in comparison with several model compounds, (106), (107), (108) and (109).48a,b Comparison of the carbon resonances for C-5, C-6, C-7 and C-8 of lavendamycin (81) with the corresponding signals of the model compounds, (106) and (107), indicated that the amino group of lavendamycin (81) resided at C-7. Both the proton-noise decoupled and proton-coupled \(^{13}\)C NMR spectra of the quinolinequinone portion of the molecule (81) resembled those of model (106). Similarly, the assignment of the \(\beta\)-carboline portion of lavendamycin (81) was confirmed by comparison with the model compound (108). Despite the facts that model (108) lacked the methyl substituent at C-3' and that the quinolinequinone chromophore at C-6' in lavendamycin is replaced by a phenyl group in the model (108), the \(^{13}\)C NMR spectrum of the \(\beta\)-carboline portion of lavendamycin (81) closely resembled that of the model (108). The structure of the
compound (81) was further confirmed by its subsequent synthesis\(^{49}\) (\textit{vide infra} section 5.3.2).

\begin{align*}
\text{(106)} &\quad \text{(107)} &\quad \text{(108)} \quad \text{R=H} &\quad \text{(109)} \quad \text{R=NO}_2
\end{align*}

5.2.3 The Structure of Streptonigrone

Fermentation of an unidentified \textit{Streptomyces} species (IA-CAS isolate no. 114) collected in the Fujian province in China and extraction of the culture filtrate gave the known antibiotic streptonigrin (80), together with a new, apparently related metabolite, streptonigrone (82).\(^{44}\) Comparison of the physical properties of the two compounds, (80) and (82), defined the structure of streptonigrone as the 2' -pyridone (82) corresponding to the 2'-pyridinecarboxylic acid (80) of streptonigrin.

Streptonigrone (82) is insoluble in aqueous sodium bicarbonate unlike the carboxylic acid, streptonigrin (80). High resolution mass measurement of the molecular ion of streptonigrone (82) established a molecular formula, \(C_{24}H_{22}N_4O_7\), which differs from that of streptonigrin (80), \(C_{25}H_{22}N_4O_8\), by the elements CO.

Comparison of the \(^1\)H NMR spectra of the two compounds, (80) and (82), showed that streptonigrone (82) retained all the carbon-bound protons of streptonigrin (80). It established that the A- and D-rings of streptonigrin (82) are unaltered in streptonigrone (82). The B-ring also remained intact although small shifts of the C-3 and C-4 protons indicated some change in their environment in streptonigrone (82). On the other hand, a significant upfield shift (\(\delta 0.48 \text{ ppm}\)) of the methyl group at C-3' of the C-ring to \(\delta 2.02 \text{ ppm}\) suggested structure (82) for streptonigrone. This assignment was confirmed by comparison with the corresponding resonance in the 3-methyl-2-pyridone (\(\delta 2.02 \text{ ppm}\) for the methyl resonance) model system (110).\(^{50}\)
5.2.4 The Structure of Demethylstreptonigrin

This antibiotic, established as 10'-o-demethyl analogue (83) of streptonigrin, was isolated from *Streptomyces albus* (strain MG883-12F2) by a Japanese group. It has also been isolated from an unidentified *Streptomyces* species together with the antibiotics, streptonigrin (80) and streptonigrone (82) by Rickards and coworkers.

The UV spectrum of this compound (83) exhibited absorption maxima at 385 and 245 nm. The structure of this antibiotic was deduced by comparison of its $^1$H and $^{13}$C NMR data with those of streptonigrin (80). With the aid of $^{13}$C - $^1$H long range selective proton decoupling (LSPD) experiments, the missing methyl group was determined to be that of the C-10' methoxyl in streptonigrin (80).

5.3 CHIROPTICAL PROPERTIES

The crystal structure of streptonigrin (80), as mentioned previously, has been solved. It was shown that the A-, B-, and C-rings are coplanar while the D-ring is skewed. The space group was determined to be the enantiomorphous P2$_1$2$_1$2$_1$, indicating that each individual crystal contained only one hand of the skewed streptonigrin (80) molecule, and therefore that this compound is optically active. Streptonigrin (80) is the first example of a phenylpyridine system in a natural product exhibiting atropisomerism, although several skewed biphenyls occur in nature. The chiroptical properties of streptonigrin (80) and its monoxime derivative (111) have been reported by Dohlakia and Gillard. In their paper, it was reported that the intensities of the CD bands of streptonigrin (80) ran parallel
with the extinction coefficients of the electronic absorption spectrum. This phenomenon is characteristic of molecules with inherently dissymmetric chromophores. In the CD spectrum, a shorter wavelength negative Cotton effect (corresponding to the biphenyl conjugated band at 245 nm) was observed. This result suggested that streptonigrin (80) has an $s$ configuration. 

No previous measurements of the chiroptical properties of streptonigrin (80) have been reported.

\[
\text{(111)}
\]

Recently, an attempt was made by Rickards and coworkers\(^{44}\) to measure the chiroptical properties of streptonigrone (82). However, the strong light absorption of this compound precluded optical rotation measurement. Nevertheless, it is probable that its inherently asymmetric skewed phenylpyridine chromophore may well have the same chirality as that of streptonigrin (80) (suggested to be $s$) in view of their co-occurrence in the Streptomyces species (see Chapter 7). It is also likely that demethylstreptonigrin (83) has the same chirality as the parent compound (80), whereas lavendamycin (81), which retains the $\beta$-carboline framework (\textit{vide infra} section 5.5), is expected to be achiral.

5.4 SYNTHETIC STUDIES

In the past 15 years, several research groups have investigated various synthetic approaches to streptonigrin (80) and/or its analogues, and lavendamycin (81). To date, three successful total syntheses of streptonigrin (80) and one of lavendamycin (81) have been achieved. The literature on the preliminary synthetic work for constructing the quinolinequinone AB-ring and the 4-phenylpyridine CD-ring
systems, and the first two total syntheses of streptonigrin (80) by Weinreb and coworkers\textsuperscript{54a,b} and Kende \textit{et al.}\textsuperscript{55} has been recently reviewed by Gould and Weinreb,\textsuperscript{46} and therefore does not need to be reiterated here. However, a brief summary of the most recent synthesis\textsuperscript{56} of streptonigrin (80) and of the synthesis of lavendamycin (81)\textsuperscript{49} will be given in the following sections.

5.4.1 The Synthesis of Streptonigrin

In the first two syntheses of streptonigrin (81), the CD-ring system was initially constructed, followed by the quinolinequinone moiety (i.e. D+C+AB ring construction). The third and most recent formal total synthesis, reported by Boger and Panek,\textsuperscript{56} involves a different methodology. Their strategy is based on the implementation of two consecutive inverse electron demand Diels-Alder reactions, i.e. 1,2,4,5-tetrazine + s-methyl thioimidate for the ABC-ring construction and 1,2,4-triazine + morpholine enamine for the CD-ring construction.

The preparation of the substrate for the first Diels-Alder reaction is summarized in Scheme 18. Treatment of 6-methoxyquinoline (112) with \textit{p}-toluenesulphonyl chloride and potassium cyanide afforded 2-cyano-6-methoxyquinoline (113) directly. The nitrile (114) was obtained on nitration of the quinoline (113). The conversion to the s-methyl thioimidate (115) was achieved in two steps by treatment of the nitrile (114) with hydrogen sulphide, followed by methyl iodide and aqueous sodium bicarbonate.

Construction of the ABC-ring system, giving the triazine (117), was accomplished by employing the inverse electron demand Diels-Alder reaction of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (116) with s-methyl thioimidate (115). Treatment of triazine (117) with the morpholine enamine of 2-((benzyloxy)-3,4-dimethoxypropiophenone (118) afforded the tetracyclic derivative (119). The conversion of this derivative (119) to the key tetracyclic intermediate (120), as described by Kende and coworkers\textsuperscript{55} in their earlier synthesis, completed the formal total synthesis of streptonigrin (80).
5.4.2 The Synthesis of Lavendamycin

The key step in the synthesis of lavendamycin (81) involved the condensation of an appropriate quinoline portion (123) with β-methyltryptophan (124).\textsuperscript{49} Synthesis of the C-7 halogenated quinoline (123) was achieved in several steps.
Selective nitration of the 8-methoxyquinaldic acid (121), followed by dealkylation of the resulting nitroquinaldic acid (122) and subsequent bromination of the phenol gave 7-bromo-8-hydroxy-5-nitroquinaldic acid (123) (Scheme 19).

Condensation of this compound (123) with β-methyltryptophan (41), followed by methylation of the crude product gave the methoxyquinoline amide (125) as a mixture of diastereomers. A Bischnler-Napieralski type ring closure of the amide (125) was effected using polyphosphoric acid and afforded the β-carboline (126).
Dithionite reduction of the β-carboline (126) proceeded smoothly giving the amine (127). The bromoquinone (128) was subsequently prepared by oxidation with potassium dichromate. Treatment of the bromoquinone (128) with sodium azide, followed by reduction of the azido group with sodium dithionite afforded lavendamycin methyl ester (129) which was identical with an authentic sample (Scheme 19).

5.5 BIOSYNTHESIS

The biosynthesis of streptonigrin (80),\textsuperscript{43a} extensively investigated by several research groups, is now known in considerable detail and will be reviewed in this section. However, there is little or no information on the biosyntheses of the other three members of this group of antibiotics, other than that which can be extrapolated from the streptonigrin data.

5.5.1 The Biosynthesis of Streptonigrin

Streptonigrin (81) can be viewed as being constructed \textit{via} a convergent pathway involving two units as shown by Fig.22.

\textbf{Figure 22}

![Figure 22](image)

Initially, Karpov and Romanov demonstrated\textsuperscript{57} that the four methyl groups of streptonigrin (80) were derived from methionine. This result was confirmed in
The same authors also established, using labelling studies with \([\beta-^{14}C]\)tryptophan (130a) and \([\beta-^{13}C]\)tryptophan (130b), that this amino acid (130) was the biological precursor of the 4-phenylpyridine CD-ring system of streptonigrin (80) (see Scheme 20a and b). Kuhn-Roth oxidation of the labelled streptonigrin (80) derived from \([\beta-^{14}C]\)tryptophan (130a), followed by Schmidt degradation located all of the radioactivity at C-3' of streptonigrin (80). Analysis by \(^{13}C\) NMR of streptonigrin (80) derived from \([\beta-^{13}C]\)tryptophan (130b) confirmed the above observation. In addition, it was shown that \([3-^{14}C]\)serine (134), a major precursor to the one carbon metabolite pool, labelled the methoxyl groups in streptonigrin (80) more heavily than the C-3' methyl group, indicating that C-methylation occurred at an earlier stage than O-methylation.

The proposed biogenesis of the 4-phenylpyridine CD-ring system of streptonigrin (80) is shown in Scheme 20a. Tryptophan (130) could first be methylated to \(\beta\)-methyltryptophan (124) and then condensed with a quinolinecarboxylic acid (or precursor). Intramolecular attack on an amide carbonyl by the nucleophilic \(\alpha\)-position of the indole, followed by aromatization of the resulting dihydropyridine (131) could afford the \(\beta\)-carboline (132). Finally, formation of the 4-phenylpyridine could be visualized as arising from cleavage of a hydroxylated \(\beta\)-carboline intermediate (133).

Isolation of \(\beta\)-methyltryptophan (124) from *Streptomyces floculus* and its reincorporation into streptonigrin (80) provided some evidence for the above proposal. It is also interesting to note that lavendamycin (81), an antibiotic isolated from *Streptomyces lavendulae*, retains the \(\beta\)-carboline framework of the proposed intermediate (133). However, it has not been established if lavendamycin (81) is a true intermediate in streptonigrin (80) biosynthesis or a shunt metabolite.
Scheme 20a

\[ \text{(130)} \quad \beta = {}^{14}\text{C} \]
\[ \text{(130a)} \quad \beta = {}^{13}\text{C} \]

or precursor
(numbering according to streptonigrin system)

Scheme 20b

\[ \text{(130c)} \]

or precursor
(numbering according to streptonigrin system)
Gould and Chang established the direction of the β-carboline ring cleavage by incorporation of [2-\(^{13}\)C, 1-\(^{15}\)N]tryptophan (130c) and examination of the \(^{13}\)C NMR spectrum of the derived streptonigrin (80a). The presence and location of the \(^{15}\)N label, determined from its spin-spin coupling to the enriched \(^{13}\)C at C-5' of the labelled streptonigrin (80a), established that the original N\(_1\)-C\(_7\), and not the N\(_1\)-C\(_2\) bond of tryptophan, was cleaved in streptonigrin biosynthesis (Scheme 20b). Two different possible mechanisms were presented for this ring cleavage, and will be discussed in Chapter 6.

Recent biosynthetic studies by Gould and Erickson provided evidence for the involvement of a modified shikimate pathway in the formation of the AB-quinoline moiety of streptonigrin (80), and hence the existence of a new pathway for quinoline biosynthesis. All the primary precursors of the antibiotic streptonigrin (80) have thus been identified.
Incorporation of [U-\textsuperscript{13}C\textsubscript{6}]glucose (62)\textsuperscript{20} resulted in specific labelling of the carbon atoms of streptonigrin (80). The size and location of each of the primary precursors were determined by \textsuperscript{13}C-\textsuperscript{13}C spin-coupling patterns and are shown in Schemes 21a and b. The labelling patterns of the C- and D- rings were fully consistent with the known derivation from \(\beta\)-methyltryptophan (124) (Scheme 21a).\textsuperscript{i}

The \(\text{C}_4 + \text{C}_2\) pattern of the A-ring was also consistent with its derivation from a modified shikimate pathway leading to the formation of a substituted anthranilic acid (137). As shown in Scheme 21b, the observed labelling pattern of the quinolinequinone moiety could be accounted for by a shikimate derived 4-aminoantrhanilic acid (137) condensing with a erythrose-4-phosphate unit (136a) with loss of the carboxyl group of the acid (137).

\textbf{Scheme 21b}

All three \(\text{C}_4\) biogenetic subunits of streptonigrin (80) were shown to have been derived from \textit{D}-erythrose, presumably \textit{via} erythrose-4-phosphate (136a), using \textsuperscript{14}C and \textsuperscript{13}C labelling studies.\textsuperscript{64} Incorporation of [1-\textsuperscript{13}C]-\textit{D}-erythrose (136b) gave

\textsuperscript{i.} The biosynthesis of tryptophan (130) \textit{via} the shikimate pathway has been established.\textsuperscript{63a,b}
labelled streptonigrin (80c) which showed enrichment in three carbon resonances, i.e. C-8, C-6' and C-9', in the $^{13}$C NMR spectrum (Scheme 21c).

Scheme 21c

![Scheme 21c](image)

The compounds, 4-aminoanthranilic acid (137), D-erythrose-4-phosphate (136) and $\beta$-methyltryptophan (124), have been suggested by Gould and Erickson$^{65}$ to be key precursors in the biosynthesis of streptonigrin (80). These compounds could be combined in sequences which would involve either a 7-aminoquinoline-2-carboxylic acid (138) (pathway A), or a $\alpha$-carboline (146) (pathway B) as a pivotal intermediate (Scheme 22). Preliminary studies$^{65}$ using [4-$^{15}$N]aminoanthranilic acid (137b) and deuterio-aminoquinoline (138b) respectively, and analysis of the derived labelled streptonigrin compounds, (80d) and (80e), by $^{15}$N and $^2$H NMR suggested that streptonigrin (80) was synthesised via pathway A (with R = H, Scheme 22). These studies also suggested that it was unlikely the hydroxylated compound (139) was an intermediate in the biosynthesis of streptonigrin (80). The $^{15}$N NMR spectrum of compound (80d) exhibited a single resonance at $\delta$ 73.6 ppm that was attributed to the C-7 amine nitrogen of streptonigrin (80), and a single resonance at $\delta$ 8.23 ppm was observed corresponding to a deuterium label at C-4 (Scheme 23).

In order to further substantiate the above results, studies using 7-amino-5-hydroxyquinoline-2-carboxylic acid (141) as a potential, later intermediate are currently being pursued.$^{65}$
Scheme 22

\[
\text{Pathway A}
\]

\[
\begin{align*}
\text{(137)} & \quad R=H \\
\text{(139)} & \quad R=OH
\end{align*}
\]

Scheme 23

\[
\text{Pathway B}
\]

\[
\begin{align*}
\text{(137b)} & \quad x=1, \ y=15 \\
\text{(80d)} & \quad x=2, \ y=14
\end{align*}
\]
5.6 BIOLOGICAL ACTIVITY

The streptonigrin group of antibiotics displays a fairly diverse spectrum of activity, including activity against bacteria, viruses and tumour cells. The most well-known of this group of compounds, streptonigrin (80) has been intensively investigated as a potential chemotherapeutic agent.

5.6.1 Biological Activity of Streptonigrin

Early preclinical trials showed streptonigrin (80) to have good antitumour activity in a variety of in vitro and in vivo systems. Streptonigrin (80) exhibited a broad spectrum of inhibition of various tumours, for example, carcinoma, sarcoma, Lewis lung carcinoma, Walker 256 carcinosarcoma, Ridgway osteogenic sarcoma, and certain types of lymphomas.

The clinical use of streptonigrin (80), however, is severely limited at present because of its high toxicity. This compound (80) is the most potent bone marrow depressant known. Additional side effects include depression, nausea, vomiting, diarrhoea and alopecia. Recent reports on the use of streptonigrin (80) in combination with other chemotherapeutic agents in clinical trials have been promising. In the United States, these combinations have included vincristine (142) and prednisone (143) for treating lymphosarcoma and reticula sarcoma. Similar combination chemotherapy has also been used in Europe, and has indicated possible benefits for treatment of primary malignant melanoma and non-Hodgkin lymphoma in children.

Evidence to date indicates that the principal target site of streptonigrin (80) is the nucleic acids, and that it exerts its antitumour action by extensive degradation of DNA, causing disruption of cell replication. Additionally, it has been reported that the antibiotic causes interference with the cell respiratory mechanism.
There is now general agreement that the DNA degradation requires the reduction of streptonigrin (80) to a semiquinone involving molecular oxygen, metal ions and free radicals.\(^{46,74}\) It has also been recognized that metal complexes constitute the antitumour-active form of the antibiotic.\(^{74}\) Detailed characterization of these complexes and elucidation of the reactions involved are currently under investigation.\(^{74}\)

The antibacterial activity of streptonigrin (80) was found to stimulate induction of phage production in lysogenic bacteria while inhibiting the net synthesis of bacterial DNA.\(^{75a,b}\) The mechanism of the bacteriocidal effect of streptonigrin (80) was investigated by White and coworkers.\(^{76a-e}\) They determined that bacterial lethality was not dependent on inhibition of DNA synthesis, but appears to be directly co-related with degradative damage to DNA.

Streptonigrin (80) has also been recognized to be a potent inhibitor of avian myeloblastosis virus (AMV) reverse transcriptase.\(^{77,78a-c}\) The \textit{in vitro} antiviral activity was also tested using an assay system consisting of human immuno-deficiency virus (HIV), a causative agent of acquired immune deficiency syndrome (AIDS) and a member of the human retrovirus family, and human T-lymphotrophic virus type 1 (HTLV-1)-carrying-MT-4 cells although the marked toxicity of streptonigrin (80) made it difficult for its antiviral activity to be evaluated.\(^{78a,c}\)
Structure-Activity Relationships

To date, a number of streptonigrin analogues have been synthesised.\(^{46,79-82}\) Until recently, the majority of these compounds have only been examined \textit{in vitro} during studies to determine the mechanism of action of streptonigrin \((80)\). There is now considerable evidence indicating that the 5,8-quinolinequinone moiety is the principle structural element responsible for the antineoplastic and antiviral activity of streptonigrin \((80)\).\(^{46,74,81}\) Other functional groups, such as the carboxyl group of the C-ring, appear to influence the degree of toxicity. For example, the methyl ester \((144)\) only has 1\% of the activity of the parent compound \((80)\) in tissue culture cells, 33\% of the activity against DNA polymerase of C-type RNA-virus, and shows no inhibition of AMV reverse transcriptase.\(^{79a,b}\)

Chemical modifications of streptonigrin \((80)\) to improve its chemotherapeutic properties have been intensively investigated.\(^{46,79,80}\) However, these modified derivatives [\textit{e.g.} \((144-147)\)]\(^{37}\) and \((148)\)\(^{46,80}\) were found to have considerably less activity than the parent compound \((80)\). As a result of the efforts aimed at the total synthesis of streptonigrin \((80)\), a new series of streptonigrin analogues recently became available. Of these derivatives, compound \((149)\)\(^{46,82}\) was found to have twice the activity of streptonigrin \((80)\) against \textit{Bacillus subtilis}, whereas compound \((150)\)\(^{49a,b}\) was inactive against KB cells.

![Chemical structures](image_url)

\((144)\) \(R=\text{OCH}_3\)
\((145)\) \(R=\text{NH}_2\)
\((146)\) \(R=\text{NHCH}_2\text{CH}_2\text{OH}\)
\((147)\) \(R=\text{NHCH}_2\text{CO}_2\text{H}\)
\((148)\)
These results suggested that, in addition to the 5,8-quinolinequinone fragment, the metal-binding site involving the functional groups of ring-C is necessary for significant biological activity. 74

5.6.2 Biological Activity of Other Streptonigrin Antibiotics

Lavendamycin

The antimicrobial activity of lavendamycin (81)\textsuperscript{48a,b,49-81} against a range of test organisms (for example, \textit{E. coli}, \textit{Staphylococcus aureus} and \textit{Proteus vulgaris}) was compared with that of streptonigrin (80). In general, their inhibition patterns were similar, with lavendamycin (81) being less potent than streptonigrin (80).

Lavendamycin (81) was also tested for its ability to induce bacteriophage production in the lysogenic strain of \textit{E. coli} W1709 (\lambda). The minimum inducing concentration of 0.003 \mu g/mL was comparable to that of streptonigrin (80) (0.008 \mu g/mL).

Lavendamycin was slightly active against P-388-J leukemia in mice, but had no inhibitory effect on standard P-388 or L1210 leukemias.\textsuperscript{48,49}

Streptonigrone

Streptonigrone (82) showed no antimicrobial activity in disc assays at 50 \mu g/mL against strains of \textit{Streptomyces aureofaciens}, \textit{S. fragilis}, \textit{B. subtilis}, \textit{E. coli} and \textit{Saccharomyces cerevisiae} on seeded agar plates.\textsuperscript{44} In contrast, streptonigrin (80), at this concentration, inhibited all the test organisms, indicating the importance of the carboxyl group for such activity.
Demethylstreptonigrin

Streptonigrin (80) is known to be active against Gram-positive and Gram-negative bacteria, but demethylstreptonigrin (83) has only weak antibacterial activity. The cytotoxic activities (50% inhibition concentrations) of streptonigrin (80) and demethylstreptonigrin (83) on P-388 leukemia cells \textit{in vitro} are 0.025 and 0.58 \(\mu\)g/mL respectively, whereas on P-388 resistant to adriamycin (151), the activities are 0.01 and 0.41 \(\mu\)g/mL respectively.\textsuperscript{51}

\begin{center}
\begin{tikzpicture}
\end{tikzpicture}
\end{center}

(151)

5.7 The Present Work

The present work has looked at two aspects of the biosynthesis of streptonigrin (80) and streptonigrone (82), and at an approach to the determination of their chiralities. The unusual 5-amino-4-phenylpyridine system of streptonigrin (80) is formed via the ring-opening of a \(\beta\)-carboline intermediate derived from tryptophan (130). Two possible mechanisms, both involving a hydrolytic step, for this \(\beta\)-carboline ring cleavage were investigated, and are discussed in section 6.2.

Streptonigrone, which has the 2-pyridone structure (82), co-occurs with, and is possibly biosynthetically derived from streptonigrin (80), the corresponding 2-pyridinecarboxylic acid. The attempted conversion of streptonigrin (80) to streptonigrone (80), investigated both as a biomimetic model and as a means of correlating the axial chirality of the two compounds, is described in section 6.3. An approach to determining the chirality of these two antibiotics, streptonigrin (80) and streptonigrone (82), is discussed in Chapter 7.
CHAPTER 6: ASPECTS OF THE BIOSYNTHESIS OF STREPTOMYCIN AND STREPTOMYCARONE

8.1 INTRODUCTION

The biosynthesis of streptomycin (88) has been widely studied\(^ {27-37}\) (see Chapter 5). In this chapter, various aspects, not yet understood, of the biosynthesis of streptomycin (88)\(^ {28-34}\) are examined. The first part (section 8.2) describes our investigations of two possible mechanisms for the \(\alpha\)-carboline ring cleavage required for the formation of the 4-phenylpyridine moiety in streptomycin (88) biosynthesis.

The second part (section 8.3) describes our investigations of the conversion of streptomycin (88) to streptomycarone (100) from most compounds, (85) and (86), had been isolated from an unidentified Streptomyces species collected by Chang.\(^ {39}\)

8.2 THE ATTEMPTED CLEAVAGE OF THE \(\alpha\)-CARBOLINE RING

Labeling studies have shown that pyrroline (126) is the biological precursor of the 4-phenylpyridine acid C-13 ring system of streptomycin (88) under Scheme 20a, Chapter 5. Biosynthetic experiments by Gould and Zhang\(^ {38-40}\) showed that the 4-phenylpyridine carboxylic acid moiety of streptomycin (88) was derived from cleavage of a \(\alpha\)-carboline intermediate (132). The cleavage of this \(\alpha\)-carboline ring cleavage has been discussed in Chapter 5, section 5.3.\(^ {19,40}\)

Two mechanisms were presented by Gould and his collaborators, which involved alternative modes of oxidation of an \(\alpha\)-hydroxy-\(\alpha\)-carboline system (133), followed by ring cleavage, and reduction of the resulting \(\alpha\)-pyrones, (134) and (135) (Scheme 24). In the first mechanism (pathway A), involving the formation of an aren oxide intermediate (132), the C-3' phenolic oxygen would be derived from atmospheric oxygen. In the second mechanism (pathway B), which involved oxidation of the \(\alpha\)-carboline moiety to a quinoneone intermediate (134), followed by ring cleavage, the C-3' oxygen would be derived from water.
CHAPTER 6 : ASPECTS OF THE BIOSYNTHESIS OF STREPTONIGRIN AND STREPTONIGRONE

6.1 INTRODUCTION

The biosynthesis of streptonigrin (80) has been widely studied57-75 (see Chapter 5). In this chapter, various aspects, not yet understood, of the biosynthesis of streptonigrin (80)43a-d are examined. The first part (section 6.2) describes our investigations of two possible mechanisms for the β-carboline ring cleavage required for the formation of the 4-phenylpyridine moiety in streptonigrin (80) biosynthesis.

The second part (section 6.3) describes our investigations of the conversion of streptonigrin (80) to streptonigrone (82).44 Both these compounds, (80) and (82), had been isolated from an unidentified Streptomyces species collected in China.44

6.2 THE ATTEMPTED CLEAVAGE OF THE β-CARBOLINE RING

Labelling studies have shown that tryptophan (130)58a,b is the biological precursor of the 4-phenylpicolinic acid C-D ring system of streptonigrin (80) (refer Scheme 20a, Chapter 5). Biosynthetic experiments by Gould and Chang58a,b showed that the 4-phenylpyridine carboxylic acid moiety of streptonigrin (80)43a-d was derived from cleavage of a β-carboline intermediate (133). The direction of this β-carboline ring cleavage has been discussed in Chapter 5 (section 5.5.1).60

Two mechanisms were presented by Gould and his collaborators60 which involved alternative modes of oxidation of an 8-hydroxy-β-carboline system (133), followed by ring cleavage, and reduction of the resulting α-quinones, (153) and (155) (Scheme 24). In the first mechanism (pathway A), involving the formation of an arene oxide intermediate (152), the C-8’ phenolic oxygen would be derived from atmospheric oxygen. In the second mechanism (pathway B), which involved oxidation of the β-carboline moiety to a quinoneimine intermediate (154), followed by ring cleavage, the C-8’ oxygen would be derived from water.
Scheme 24

A third mechanism, proposed by Rickards et al., for the β-carboline ring cleavage is formally an hydrolysis and has parallels in known ring opening reactions of some substituted indoles. As shown in Scheme 25, formation of the 4-phenylpyridine moiety of streptonigrin (80) can be visualised as involving covalent hydration of a β-carboline system (133) bearing electron-withdrawing substituents (carboxyl and quinolinequinone) adjacent to the electronegative nitrogen (N-1') of the pyridine ring. A 1,6-elimination from the covalent hydrate (156), possibly assisted by protonation of N-1', would result in cleavage of the C-N bond of the central ring generating the correctly substituted phenylpyridine moiety. By this mechanism, it is clear that the C-8' phenolic oxygen would be derived from water.

The present work examines the above mechanisms for the β-carboline ring cleavage reaction. Thus, the chemical feasibility of the mechanisms proposed by Rickards et al. (Scheme 25) and by Gould and coworkers (Scheme 24, pathway B), both of which involved a hydrolytic process, were investigated using model

---

i. This is the original N_{1}-C_{7b} bond of tryptophan.
compounds. The first objective was to attempt the ring-opening of the β-carboline system via hydrolytic cleavage (section 6.2.1). The second was to investigate the proposal of Gould and coworkers\textsuperscript{60} whereby the β-carboline moiety was initially oxidized to a quinoneimine (154), followed by hydrolytic cleavage to give the desired 4-phenylpyridine system (section 6.2.2).

Methyl β-carboline-3-carboxylate (159) was chosen as an appropriate model substrate and was prepared according to literature procedures\textsuperscript{84-86} (Scheme 26). The base-catalysed cyclization of tryptophan (130) with formaldehyde gave the tetrahydro-β-carboline-3-carboxylic acid (157).\textsuperscript{84a-c} The methyl ester (158) was then prepared in 80% yield by the method of Tada and coworkers.\textsuperscript{85} Dehydrogenation of the methyl ester (158) to the β-carboline (159) proved somewhat difficult. Variable yields (30-70%) were obtained on dehydrogenation of
compound (158) with sulphur in xylene, palladium on charcoal in refluxing toluene, or with lead tetraacetate in glacial acetic acid. However, dehydrogenation of (158) with DDQ in THF gave the β-carboline (159) in a consistent 75% yield.

6.2.1 Attempted Hydrolytic Ring Cleavage

An acid-catalysed ring-opening reaction of an indole has been reported by Rapoport and coworkers in their synthesis of apo-β-erythroidine (160).

![Scheme 27](image)

The mechanism could be visualised as involving β-protonation of indole (161), followed by nucleophilic attack of water on the resultant electron-deficient α-position of the iminium salt (162) (Scheme 27). The hydroxy intermediate (163) then underwent an acid-catalysed ring cleavage reaction giving the amino ketone (164).

In our case, however, treatment of the methyl ester (159) with hydrochloric acid and alcohol (ethanol and n-butanol) under reflux conditions gave...
unexpectedly the transesterified products, (165) and (166), but not the desired ring-opened compound (168). With prolonged heating in low concentrations of hydrochloric or sulphuric acid, only the carboxylic acid (167) was obtained; formation of phenol (168) was not observed. Complex mixtures, on the other hand, were obtained on heating with more concentrated solutions of sulphuric and hydrochloric acid (9N and 12N).

\[
\begin{align*}
\text{H}_2\text{O}/\text{H}^+/\Delta & \quad \downarrow \quad \text{H}^+/\Delta \\
\text{CO}_2\text{Me} & \quad \text{R} \quad \text{H} \\
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{H} \\
(159) & \quad (165) \quad R=\text{Et} \\
\text{CO}_2\text{H} & \quad \text{CO}_2\text{R} \\
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{H} \\
(167) & \quad (166) \quad R=\text{n-Bu} \\
\end{align*}
\]

Presumably, the driving force for the reaction in Rapoport's case is the relief of steric strain, which resulted in ring-opening. The \(\alpha\)-carboline system (159) is, however, not strained and therefore may not be subject to hydrolytic ring opening. Also, the loss of aromaticity of the benzenoid ring on hydration may be too high an energy barrier to circumvent, even with more forcing reaction conditions.

Introduction of an oxygen function on the benzenoid ring of the \(\beta\)-carboline (159) may alleviate the energy barrier problem associated with the hydration step and therefore induce ring opening. For example, with the 7-hydroxy (or methoxy)-\(\beta\)-carboline-3-carboxylic acid (169), the zwitterion (170) is resonance stabilized. This might facilitate a Michael-type addition involved in the "covalent hydration" step, and hence ring opening to give the 4-phenylpyridine (171) (Scheme 28). It is notable that such an oxygen function is present in streptonigrin (80) itself.

Prior to commencing the preparation of the 7-hydroxy derivative (169) for which there were no literature precedents, studies on the ring cleavage via the oxidation-hydrolysis route were undertaken.
6.2.2 Attempted Ring Cleavage via the Oxidation-Hydrolysis Route

Oxidation of the $\beta$-carboline (159) to the $\alpha$-quinoneimine (172), followed by a 1,2 hydration, and cleavage of the C-N bond as indicated would generate the phenylpyridine moiety (173) (refer Scheme 29). However, there were no
literature precedents for the direct oxidation of the \( \beta \)-carboline system (159) to the \( \sigma \)-quinoneimine. Oxidation the \( \beta \)-carboline (159) to the \( \sigma \)-quinoneimine (172) was attempted using Fremy’s salt [\( \text{ON(SO}_3\text{K)}_2 \)]\(^9\) but was unsuccessful with starting material being recovered.

While the presence of an oxygen function (\( \text{e.g.} \) OH) at C-8 of the benzenoid ring of the \( \beta \)-carboline (159) might facilitate this oxidation step, there are no known direct routes for the preparation of 8-hydroxy-\( \beta \)-carboline (175) although preparation of the 6-hydroxy derivative (174) has been reported. 6-Hydroxy-\( \beta \)-carboline (174) is not as appropriate a model as the 8-hydroxy derivative (175) in the context of streptonigrin (80) biosynthesis, but could be used to investigate the oxidation and ring-cleavage reactions. The 6-hydroxy compound could be oxidised to the \( \rho \)-quinoneimine (175) and then subjected to ring-opening conditions to give the phenylpyridine (176).

Accordingly, the 6-hydroxy derivative (174) was prepared by the method of Cain and coworkers\(^9\) from 5-hydroxy tryptophan (177) and its reactions with a variety of oxidizing agents investigated.
No reaction was observed when the \( \beta \)-carboline (174) was treated with palladium on charcoal in air,\(^92\) cerium(IV) on silica,\(^93\) nickel peroxide,\(^94\) silver(I) oxide\(^95\) in THF, or oxalyl chloride in dimethyl sulphoxide,\(^96\) while reaction with freshly prepared manganese dioxide\(^97\) or benzene selenic anhydride\(^98\) in THF gave only complex mixtures. Treatment of the \( \beta \)-carboline (174) with potassium ferricyanide and hydrogen peroxide\(^99\) in THF or with Fremy’s salt\(^90,100\) in acetonitrile and phosphate buffer (pH 6.8), on the other hand, gave a major product tentatively assigned, on the basis of the UV (broad absorption at 500 nm)\(^100\) and mass spectra (\( \text{M}^+\cdot256 \)) of the ethyl acetate extracts, as the \( \sigma \)-quinone (178) (Scheme 30). The formation of this \( \sigma \)-quinone is not surprising as there are reported examples of indole \( \sigma \)-quinones (180) obtained by oxidation of the corresponding 5-hydroxyindoles (179) with Fremy’s salt.\(^90,100\) Characterisation was, however, not possible as decomposition occurred on concentration of the extracts.

![Chemical structure](image)

\( (179) \quad \text{e.g.} \quad R=\text{CO}_2\text{Et} \quad R'=\text{Me} \)

\( (180) \)

When the 6-hydroxy-\( \beta \)-carboline (174) was treated with ten equivalents of freshly prepared lead(IV) oxide in THF,\(^101\) the resulting product adhered to the lead(IV) oxide. On washing with methanol, the methoxy derivative (181) was obtained, from a complex mixture, in 15% yield after purification (Scheme 30). Presumably, the highly reactive quinoneimine (78) intermediate reacted with methanol to give compound (181).

With this result, attention was thus focused on the 8-hydroxy analogue (182) as a model for the above reaction sequence [with lead(IV) oxide] in the context of streptonigrin (80) biosynthesis (refer Scheme 31). The 8-hydroxy derivative (182) could be oxidised to the quinoneimine (172) which, on treatment with methanol, may give methoxy compound (183) if nucleophilic attack by methanol occurs on C-7 of the quinoneimine (172). This methoxy compound (183) would have the
correct substitution pattern to give rise to the D-ring of streptonigrin (80) and could, in principle, be reoxidized to the quinoneimine (185). The C-N bond could then be hydrolysed to give phenylpyridine (186). It should be noted, however, that in streptonigrin (80) biosynthesis, the D-ring methoxyls are derived by hydroxylation and methylation, and not from methanol.

Scheme 31

Hence, we attempted to prepare this compound (182) by direct hydroxylation of the β-carboline ester (159). Treatment of the β-carboline (159) with Fenton’s reagent \((\text{H}_2\text{SO}_4/\text{H}_2\text{O}/\text{H}_2\text{O}_2/\text{FeSO}_4)^{89}\) afforded a mixture of hydroxylated β-
carbolines (5% yield), together with 60% of the starting material. These compounds were assigned the structures (174) and (182) on the basis of their spectroscopic data (vide infra).

\[ \text{(159)} \]
\[ \text{CO}_2\text{Me} \]
\[ \text{H} \]
\[ \text{N} \]
\[ \text{Fenton's Reagent} \]
\[ \text{R=6-OH} \]
\[ \text{R=8-OH} \]
\[ \text{(174)} \]
\[ \text{(182)} \]

On the other hand, treatment of the α-carboline (159) with Udenfriend's reagent (ascorbic acid/EDTA/FeSO}_4_/phosphate buffer\textsuperscript{102} in THF gave the tetrahydrofuranyl derivative (187) in 46% yield, together with 40% of recovered starting material. Formation of the THF adduct (187) was not surprising as THF is known to undergo radical reactions of this type. There are examples in the literature\textsuperscript{103} of homolytic α-oxyalkylation reactions of heteroaromatic bases with alcohols and ethers to give compounds such as (188) and (189). These alkylation reactions occur regioselectively at the positions α and γ to the heterocyclic nitrogens. Therefore, substitution at C-1 of the β-carboline (159) was in accord with expectation.

\[ \text{(159)} \]
\[ \text{CO}_2\text{Me} \]
\[ \text{H} \]
\[ \text{N} \]
\[ \text{Udenfriend's reagent} \]
\[ \text{THF} \]
\[ \text{α} \]
\[ \text{(187)} \]
\[ \text{(188)} \]
\[ \text{(189)} \]
When the reaction was repeated in acetonitrile, a mixture of two hydroxylated \( \beta \)-carboline-3-carboxylic acid methyl esters was obtained in 30% yield (in an approximately 1:1 ratio by \(^1\)H NMR spectroscopy) with 60% of the starting material being recovered. Attempts to separate the two hydroxylated species using HPLC were unsuccessful. We were, however, able to assign the structures (174) and (182) for these compounds by analysis of the spectroscopic data of the mixture. The EIMS of the mixture showed a molecular ion at \( m/z \) 242 (\( C_{13}H_{10}N_2O_3 \)) i.e. with one more oxygen than that of the starting material (159) (\( C_{13}H_{10}N_2O_2 \)). In the \(^1\)H NMR spectrum, two sets of signals were observed, one corresponding to the proton resonances of 6-hydroxy-\( \beta \)-carboline (174) (which was synthesised as previously described). The multiplicities of the other set of signals were consistent with the presence of an hydroxyl group at C-8, hence establishing the structure (182) for the second component.

The mixture of hydroxylated compounds was treated with lead(IV) oxide and the product residue washed with methanol. It was hoped that following reaction of the quinoneimines (175) and (172) with methanol, the resulting methoxy compounds (181) and (183) respectively, might be easily separable. The methoxy derivative (183) could be reoxidized to the quinoneimine (185) and subjected to ring-opening conditions. However, only the 5-methoxy adduct (181) was isolated in low yield from the complex reaction mixture.

At this stage of our work, Gould and coworkers\(^{104} \) reported evidence for an entirely oxidative \( \beta \)-carboline cleavage in streptonigrin (80) biosynthesis. Results from an \(^{18}\)O\(_2\) oxygen feeding experiment clearly indicate that the oxygen at C-8' of streptonigrin (80) was derived from molecular oxygen rather than from water. The \(^{13}\)C NMR spectrum of the labelled streptonigrin (80f) showed that the oxygen atoms of the three methoxyls, the C-5 quinone and, more importantly, the C-8' phenol, were derived from molecular oxygen.

Therefore, the oxygenation and cleavage of the intermediate \( \beta \)-carboline (133) leading to streptonigrin (80) were entirely oxidative processes. The proposal of Gould and coworkers\(^3 \) involving an arene oxide intermediate (152) would fulfill these requirements. While other oxidative mechanisms may be envisaged, any
processes requiring water as the source of the D-ring oxygens could be ruled out. Consequently, the "covalent hydration" route was not investigated further.

\[
\text{Me}^{18}\text{O} \quad \text{Me}^{18}\text{O} \quad \text{CO}_2\text{H} \\
\text{H}_2\text{N} \quad \text{H}_2\text{N} \\
\text{H}^{18}\text{O} \quad \text{Me}^{18}\text{O} \quad (80f)
\]

6.2.3 Conclusion

In this section, we investigated two possible mechanisms of the ring cleavage of the \(\beta\)-carboline system required for the formation of the 4-phenylpyridine moiety of streptonigrin (80). Both mechanisms investigated involved the formation of a "covalent hydrate" intermediate from the \(\beta\)-carboline moiety in which the resulting C-8' phenolic oxygen of streptonigrin (80) would be derived from water. In the first approach\(^44\) (Scheme 25), non-oxidative ring cleavage via acid-catalysed hydrolysis was investigated, with negative results were obtained. In the second investigation, the ring cleavage of the \(\beta\)-carboline system via the oxidation-hydration route was examined (Scheme 24, pathway B).\(^60\) Oxidation of the model, 6-hydroxy methyl \(\beta\)-carboline-3-carboxylate (174) with lead (IV) oxide, followed by treatment with methanol gave the 5-methoxy-6-hydroxy derivative (181) in moderate yield, presumably via an intermediate quinoneimine (175). An attempt to prepare the analogous 7-methoxy-8-hydroxy derivative (183) using the same route was unsuccessful.

While the present work was in progress, Gould and coworkers\(^104\) reported evidence for an entirely oxidative cleavage of the \(\beta\)-carboline moiety in streptonigrin biosynthesis. Thus, processes of the type we were examining, involving a hydrolytic step, were not involved in streptonigrin (80) biosynthesis. Our negative results thus, with hindsight, agreed with the true biosynthetic route, and further work in this area was discontinued.
6.3 THE ATTEMPTED CONVERSION OF STREPTONIGRIN TO STREPTONIGRONE

In this work, the biomimetic conversion of streptonigrin (80), to the related metabolite, streptonigrone (82) was examined. Both these compounds were isolated by Rickards and coworkers in 1985 from an unidentified Streptomyces species collected in the Fujian province in China, and they are clearly biogenetically related.

The most direct relationship would involve derivation of the 2-pyridone moiety of streptonigrone (82) from the 2-pyridinecarboxylic acid moiety of streptonigrin (80). However, it is also possible that streptonigrin (80) itself is not on the direct biosynthetic route to streptonigrone (82), but rather that the route to the two compounds diverge at a common, earlier precursor, e.g. at the β-carboline (133) stage (Scheme 20a, Chapter 5). In principle, the biosynthetic conversion of streptonigrin (80), or a precursor of streptonigrin (80), to streptonigrone (82) would involve additional decarboxylation and oxidation steps, or alternatively oxidation of the pyridine ring followed by decarboxylation. Of the two pathways, the latter is biosynthetically more interesting, and was investigated using model compounds.

A literature survey revealed several biomimetically feasible routes for the transformation of 2-pyridinecarboxylic acids to 2-pyridones. Approaches based on these routes will be discussed in the following sections.

6.3.1 The N-Oxide Route

The first approach involved the formation of 2-pyridinecarboxylic acid N-oxides [e.g., (191)] which are usually prepared by reaction of the respective 2-pyridinecarboxylic acids [e.g. (190)] with peroxyacetic acid. Treatment of these N-oxides with acetic anhydride and triethylamine, followed by base hydrolysis, has furnished the 2-pyridones [e.g. (194)] in moderate to high yields (Scheme 32). The mechanism of the rearrangement of substituted N-oxides in acetic anhydride has been studied. Mechanistically, the formation of the
2-pyridone (194) could proceed via the \(N\)-acetoxypyridinium intermediate (192). Decarboxylation and N-O fission, followed by hydrolysis of the acetate (193), would give pyridone (194). This reaction sequence (190 - 194)\(^{106}\) was successfully repeated in our laboratory.

Scheme 32

\[
\begin{align*}
\text{N-oxoacetic acid (190) reacted with hydrogen peroxide and acetic acid to produce the N-oxide (191).} \\
\text{The N-oxide (191) was then treated with acetic anhydride and triethylamine to yield the acetoxypyridinium intermediate (192).}
\end{align*}
\]

\[
\begin{align*}
\text{Scheme 33}
\end{align*}
\]

This conversion has also been investigated using the \(\beta\)-carboline-3-carboxylate system (159) (prepared as described previously in section 6.2) to gain a greater understanding of the chemistry of these type of reactions, and also as a model for the oxidative decarboxylation of a \(\beta\)-carboline precursor of streptonigrin (80). The successful conversion of \(\beta\)-carboline (159) to the indolo-\(\alpha\)-pyridone (201) will now be discussed.

It has been reported\(^{110}\) that peroxyacetic acid converts \(\alpha\)-carbolines \([e.g. (195)]\) quantitatively to their N-oxides \([e.g. (196)]\). However, treatment of either \(\beta\)-carboline-3-carboxylic acid (167) or its methyl ester (159) with acetic acid and hydrogen peroxide gave less than 50% yield of the N-oxides, (198) or (197) respectively, even after prolonged heating.
On the other hand, treatment of the methyl ester (159) with p-nitroperoxybenzoic acid afforded the N-oxide (197) in 90% yield. The ester N-oxide (197) was subsequently treated with potassium superoxide and 18-crown-6 ether in dry benzene\textsuperscript{111} to give the acid N-oxide (198) in 95% yield. Reaction of the N-oxide (198) with acetic anhydride and triethylamine\textsuperscript{106} gave a mixture of the acetate (199) and the diacetyl derivative (200) (in an approximately 1:1 ratio by \textsuperscript{1}H NMR spectroscopy) which was then subjected, without separation, to base-catalysed methanolysis to afford the target compound (201) in 58% yield over the two steps (Scheme 33).

Scheme 33

The author is aware of only one precedent, reported by Plieninger and coworkers,\textsuperscript{112} for the synthesis of this group of compounds. The indolo-\(\alpha\)-pyridones \textit{e.g.}(204)) were synthesised either by treating 2-acetyl-3-indolylacetic acid derivatives \textit{e.g.}(202)) with ammonia in methanol for several days or by heating 2-acyl-3-indolylacetamide derivatives \textit{e.g.}(203)) at high temperatures (Scheme 34).

Scheme 34
With a view to extending these studies to the antibiotic streptonigrin (80), synthesis of the N-oxide (205) was required. Treatment of this N-oxide (205) with acetic anhydride and triethylamine, followed by hydrolysis and deprotection should lead to streptonigrone (82). First we attempted to protect in one step, as acetyl derivatives, the C-7 and C-5′ amino and C-8′ phenolic groups because of their susceptibility to oxidation by peroxycarboxylic acids. The acetyl was an ideal protecting group as it could usually be introduced under mild reaction conditions and, in our case, should be easily removed in the hydrolysis step of the reaction sequence (see Scheme 32). Although there are two heterocyclic nitrogens in our substrate, it is likely that N-1 is deactivated by the quinone system and intramolecular hydrogen bonding to the C-5′ amine, hence favouring oxidation of the correct nitrogen (N-1′).

The desired "one-pot" protection step, however, was not a straightforward operation. Treatment of streptonigrin (80) with acetic anhydride and DMAP in ethyl acetate gave only a mixture of the acetate (206) and the mixed anhydride (207), which was easily hydrolysed with aqueous bicarbonate to afford the acetate (206) in 75% yield after purification.

That O- rather than N-acetylation had occurred was evidenced by chemical shifts in the 1H NMR spectrum of the acetate (206) compared to those of streptonigrin (80). It is known that downfield shifts are observed for the o and p (Δδ 0.3-0.4 ppm) and the m (Δδ 0.1-0.2 ppm) aromatic protons on acetylation of a phenol. In the case of monoacetate (206), both the 11′- (p, δ 7.01 ppm) and 12′-
(m, δ 6.89 ppm) proton resonances had been shifted downfield by Δδ 0.33 and 0.09 ppm respectively as expected upon acetylation of the C-8' hydroxyl. This result was not unexpected as both the C-7 and C-5' amino groups are substantially deactivated by delocalization of the electron pairs of the nitrogens. Treatment of the acetate (206) with more reactive acylating agents such as acetyl chloride, however, resulted only in decomposition of the substrate.

Nevertheless, it was decided to study the N-oxidation reaction using the O-acetyl derivative (206). To facilitate handling, the methyl ester (208) was first prepared in quantitative yield by esterification of acetate (206) with diazomethane, and subsequently treated with one mole equivalent of p-nitroperbenzoic acid in dichloromethane in the presence of aqueous sodium bicarbonate. Very little reaction was observed after several hours whereas a complex mixture of products was obtained with three mole equivalents of the oxidizing agent.

Presumably, oxidation of the amino groups or quinone double bond to possibly unstable products occurred in preference or in addition to oxidation of the pyridine nitrogen (N-1'). Hence, it was desirable at this stage to further attempt to acetylate the C-7 and C-5' amino groups. Prolonged heating (3 days) of the acetate (208) in acetic anhydride and ethyl acetate, however, gave mainly the N, O-diacyetyl derivative (209) and traces of a triacetyl derivative later shown to be the N, N, O-derivative

\[ \text{(80)} \]

\[ \text{NaHCO}_3 \quad (206) \quad \text{R} = \text{H} \]
\[ (207) \quad \text{R} = \text{Ac} \]
\[ (208) \quad \text{R} = \text{Me} \]

\[ \text{CH}_2\text{N}_2 \]

---

\[ \text{ii. The 11' and 12' proton resonances in the }^1\text{H NMR spectrum of streptonigrin (80) occur at } \delta \ 6.68 \text{ and } 6.174 \text{ ppm respectively.} \]
(210) (refer Scheme 35). The structural assignment of the diacetyl derivative was straightforward. In addition to the $O$-acetyl resonance at $\delta 2.0$ ppm, an $N$-acetyl was observed at $\delta 1.89$ ppm in the $^1H$ NMR spectrum. The presence of an unchanged C-7 amine resonance (at $\delta 5.17$ ppm) showed that acetylation had occurred selectively at the C-5' amino group. Reaction of compound (209) with a mixed anhydride prepared in situ by treatment of potassium acetate with methyl chloroformate in the presence of a catalytic amount of $N$-methylmorpholine,\textsuperscript{116} did not give further acetylation and the starting material was recovered.

The method reported by Kaneko and coworkers,\textsuperscript{117} (using sodium hydride and various acetylating agents) for the acetylation of deactivated amines in the syntheses of mitomycin C (211) analogues, offered a possible solution. It seemed feasible to utilise a similar procedure to acetylate the C-7 amino group (with the expectation of further acetylation of the C-5' amide group) of diacetyl derivative (209). Hence, diacetate (209) was treated with 2.2 mole equivalents of sodium hydride in DMF at room temperature. A dark green solution was obtained which, upon addition of 5 mole equivalents of acetyl chloride at $-20^\circ$ C. gave a triacetate in 55% yield after purification. Examination of the $^1H$ NMR spectrum assigned this compound as the triacetyl derivative (210), and not the isomeric acetate (213) (see Scheme 35). Clearly, acetylation occurred at the C-5' amide rather than the C-7 amine group. If the C-7 amino group had been acetylated giving the triacetol derivative (213), an additional N-acetyl resonance at $\delta 1.8 - 2.0$ ppm, the disappearance of the C-7 amine signal and possibly an upfield or downfield shift of the C-6 methoxyl signal would be observed. Instead, the presence of the C-7 amine resonance unchanged at $\delta 5.18$ ppm, and acetyl signals at $\delta 2.07, 2.24$ and 2.27 ppm confirmed the structure (210) for the triacetol derivative. Although the C-6 methoxyl resonance has not been assigned, the absence of significant shifts in any of the methoxyl signals provided additional evidence for the structure of the triacetol derivative (210).

Conceivably, the C-5' amide proton is more acidic than the C-7 amine protons and was therefore more reactive towards the hydride base. However, since 2.2 mole equivalents of sodium hydride and 5 mole equivalents of acetyl chloride were
used, it would be expected to obtain some of the tetraacetyl derivative (212) which would be as suitable as the desired triacetyl (213) for the N-oxidation step. Formation of the tetraacetyl derivative (213) was not observed in the reaction.

Scheme 35

In view of the difficulties encountered in the attempts to protect the various functional groups and to prepare the N-oxide derivative (205) via intermolecular oxidation with aromatic percarboxylic acids, the possible use of an internal peroxycarboxylic acid was investigated. Recently, Corey and coworkers\textsuperscript{118} reported the intramolecular epoxidation of arachidonic acid (57) using an internal peroxycarboxylic acid (214) (see Scheme 36).
As the availability of streptonigrin (80) was limited, model studies with 2-pyridinecarboxylic acid (190) were conducted to investigate the feasibility of this approach to the N-oxide (191). Treatment of the acid (190) with N,N'-carbonyldiimidazole in THF gave the imidazolide derivative (215) which, on addition to hydrogen peroxide (30% or 60%), afforded the N-oxide (191) in 70% and 85% yields respectively. On the other hand, the starting material was recovered unchanged when the acid (190) was treated with 60% hydrogen peroxide alone, indicating that the formation of the imidazolide (215) was required to generate the peroxycarboxylic acid (216) for the N-oxidation (Scheme 37). A mechanism for the formation of imidazolides has been postulated by Staab et al.119

Following this favourable result, the N-oxidation of monoacetate (206) was attempted. Streptonigrin o-acetate (206) in THF was first treated with N,N'-carbonyldiimidazole forming the imidazolide (217) (this compound has been isolated and characterised) which was then added to a solution of 30% hydrogen
peroxide. After one hour, mainly hydrolysis of the imidazolide (217) to the acid (206), together with traces of some polar compounds were evident from a thin-layer chromatogram of the reaction mixture. Acid work-up unexpectedly afforded two compounds which were separated after esterification with diazomethane. The minor components of the reaction mixture were not isolated.

The less polar major component, obtained in 40% yield, was identified as streptonigrin \(\alpha\)-acetate methyl ester (208). The second major component (30% yield) was assigned the isomeric \(N\)-acetyl structure (218) on detailed examination of its spectroscopic data. The EIMS showed a molecular ion at \(m/z\) 562 (\(C_{28}H_{26}N_4O_9\); CIMS showed \(m/z\) 563). Upfield shifts (0.41 and 0.15 ppm, respectively) were observed for the 11' (\(\delta\) 6.58 ppm) and 12' (\(\delta\) 6.76 ppm)III proton signals in the \(^1\)H NMR spectrum compared to that of the \(\alpha\)-acetate (208). The UV absorption maximum at 378 nm of the \(\alpha\)-acetate (208) is attributed to the chromophore of the A-, B- and C-rings.\(^{iv}\) In the UV spectrum of the \(N\)-acetyl derivative (218), a hypsochromic shift to 332 nm was observed, indicating that the C-ring was no longer coplanar with the A- and B-rings once the C-5' amino group was acetylated. This blue shift is also observed in the UV spectrum of the diacetyl derivative (209).

The formation of this \(N\)-acetyl derivative (210) could be attributed to the presence of the acid in the work-up procedure. This conclusion was substantiated by heating acetate (206) in acidic THF. The \(N\)- and \(\alpha\)-acetyl methyl esters (218) and (208) were obtained in 51% and 40% yields after esterification with diazomethane and purification. Similar acid-catalysed acyl migrations have been documented in the literature.\(^{120}\)

In subsequent reactions, higher concentrations of hydrogen peroxide were used to attempt to minimise hydrolysis of the imidazolide (217). No useful results, as observed by tlc, were obtained, however. Treatment of the imidazolide (217) with 60% hydrogen peroxide also resulted in hydrolysis of the imidazolide (217) together

\(^{iii}\) The resonances occur at \(\delta\) 6.99 and 6.91 ppm respectively in the \(\alpha\)-acetate (208) and at \(\delta\) 6.71 and 6.76 ppm in the methyl ester (144).

\(^{iv}\) The C-ring is nearly coplanar to the A- and B-rings due to intramolecular hydrogen bonding.
with traces of polar components. On the other hand, treatment of the imidazolide (217) with anhydrous hydrogen peroxide gave a complex mixture.

**Scheme 38**

![Scheme 38](image)

It was initially anticipated that the N-oxidation of 2-pyridinecarboxylic acid (190) *via* the imidazolide route would involve both intermolecular and intramolecular processes, possibly with preference for the intramolecular process. In view of the difficulties encountered (*vide supra*), however, it is plausible that formation of the N-oxide (191) proceeded predominantly *via* an intermolecular process. Hence, N-oxidation of the model system (190) was reinvestigated. A study using deuterium-labelled 2-pyridinecarboxylic acid was conducted to clarify the mechanism of the N-oxidation. In the case where an intermolecular mechanism predominates, a mixture of unlabelled and deuterated N-oxides, (191) and (191), would be obtained. Alternatively, formation of mainly unlabelled N-oxide (191), would indicate that an intramolecular N-oxidation is preferred.
Thus, the deuterio-2-pyridinecarboxylic acids (190a), were prepared by heating
the acid (190) in a sealed tube with deuterium oxide and a pre-reduced platinum
dioxide catalyst for several days.\textsuperscript{121} Hydrogen peroxide (30%) was added to a

\[
\begin{array}{c}
\text{N} \text{C}_2\text{H} \\
(190) \\
\end{array}
\xrightarrow{1. \text{D}_2\text{O} / \text{Pt cat.}} 
\begin{array}{c}
\text{N} \text{C}_2\text{H} \\
(190a) \\
\end{array}
\xrightarrow{2. \text{MeOH}}
\]

solution of unlabelled imidazolide (215) generated in the usual manner, followed by
an equivalent amount of a mixture of the deuterio-2-pyridinecarboxylic acids (190a).
After an hour, the reaction mixture was worked up using standard procedure. An
EIMS of the residue showed a mixture of non-deuterated and deuterated N-oxides,
(191) and (191a), in an approximately 1:1 ratio, indicating that the N-oxidation
reaction proceeded largely via an intermolecular pathway.

It is known\textsuperscript{122} that peroxyacetic acid, in the presence of a large excess of
sulphuric acid, undergoes a reversible acid catalysed transformation to acetyl
peroxide (219). We do not believe, however, that formation of deuterio-2-
pyridinecarboxylic acid N-oxide (191a) was, to any substantial extent, due to a
reversible equilibrium existing between a peroxyacid (216) and the acid (190a) to
give a peroxide (220) since the presence of a substantial amount of sulphuric acid
was required for this transformation in the reported example\textsuperscript{122} (see Scheme 39).

Therefore, the results from the experiment with deuterio-2-pyridinecarboxylic
acid (190a) indicated that the predominant mechanism involved in the N-oxidation
reaction was very likely an intermolecular process. Furthermore, depending on the
timing of bond forming and bond breaking processes, the intramolecular process
could well have the character of a 5-endo-tet process which would be disfavoured according to Balwin's rules. These observations presumably explain the difficulties encountered in attempts to synthesise the N-oxide derivative of streptonigrin by intramolecular oxidation.

\[
\begin{align*}
CH_3CO_2H + CH_3CO_3H & \xrightleftharpoons[k]{k-1} O \quad H_3C-C-O-O-C-CH_3 + H_2O \\
(219)
\end{align*}
\]

Scheme 39

6.3.2 The Quaternary Salt Route

In the previous section, the conversion of streptonigrin (80) to streptonigrone (82) via the N-oxide route was examined but difficulties were encountered in the synthesis of the crucial N-oxide intermediate (205). An alternative approach to the biomimetic conversion of streptonigrin (80) to streptonigrone (82) involving a quaternary pyridinium intermediate (221) was therefore investigated.

N-alkyl pyridone formation by alkaline oxidation of quaternary pyridinium salts is a known reaction, e.g. treatment of 1-methylpyridinium iodide (222) with alkaline potassium ferricyanide gave 1-methyl-2(1H)-pyridone (224). The mechanism is presumed to proceed by way of an intermediate "pseudo-base" (223). It has also been reported that 2-pyridinecarboxylic acid methyl ester quaternary salts, also undergo this alkaline oxidation to give 2-pyridone.
Hence, in our model studies, 2-pyridinecarboxylic acid methyl ester methosulfate salt (226) [prepared by heating the ester (225) and dimethyl sulphate\textsuperscript{128} in acetonitrile under reflux] and potassium ferricyanide were warmed in aqueous sodium hydroxide to afford 2-pyridone (224) in an unoptimised 40\% yield. Similarly, methyl 4-phenylpyridine-2-carboxylate (227) was synthesised according to literature procedure\textsuperscript{127} and its methosulfate salt (228) was prepared by heating the ester (227) with dimethyl sulphate\textsuperscript{128} in acetonitrile under reflux conditions. The methosulphate salt (228) was converted to the 2-pyridone (229) in 50\% yield (unoptimised) using the same methodology. Presumably, the mechanism parallels that involving the "pseudo-base" (223). Nucleophilic attack at C-2 by the hydroxide ion, followed by hydrolysis of the carbomethoxy group would give the \(\alpha\)-hydroxy acid (230). This intermediate (230) on decarboxylation and oxidation would furnish the desired 2-pyridone (224) (Scheme 40).

This alkaline oxidation reaction was also studied with the \(\beta\)-carboline model system, but was unsuccessful. The \(\beta\)-carbolinium salt (231) [prepared by heating methyl \(\beta\)-carboline-2-carboxylate (159) with dimethyl sulphate\textsuperscript{57} in acetonitrile under reflux] yielded only the carboxylic acid salt (232) even after prolonged
heating in aqueous sodium hydroxide and potassium ferricyanide (Scheme 41).

Presumably, the energy barrier for the oxidation of this system (231) to the indolo-α-pyridone (233) is too high to circumvent even with forcing reaction conditions.

**Scheme 40**

\[
\begin{align*}
R & \quad \text{CO}_2\text{Me} \\
\text{MeSO}_4 / \text{CH}_3\text{CN} & \quad \rightarrow \\
\text{R} & \quad \text{CO}_2\text{Me} \\
\text{NaOH} / \text{H}_2\text{O} / \text{K}_3\text{Fe(CN)}_6 & \quad \rightarrow \\
\text{R} & \quad \text{N} \quad \text{O} \\
\end{align*}
\]

(225) \( R=\text{H} \)
(226) \( R=\text{H} \)
(227) \( R=4\text{-Ph} \)
(228) \( R=4\text{-Ph} \)
(224) \( R=\text{H} \)
(229) \( R=4\text{-Ph} \)

**Mechanism**

Nevertheless, since the methosulphate salts, (225) and (227), were successfully converted to the corresponding 2-pyridones, (224) and (229), the extension of this second approach to the conversion of streptonigrin (80) to streptonigrone (82) was
investigated. As with the N-oxide route, it was anticipated that the nitrogen (N-1') associated with the 4-phenylpyridine moiety would be methylated in preference to the nitrogen (N-1) of the quinolinequinone moiety because of the deactivation of N-1 by the quinone system and by intramolecular hydrogen bonding. The syntheses of pyridinium salts have been well documented. Attempts to prepare the quaternary salt (221) of streptonigrin methyl ester and other derivatives using a variety of methylating agents were, however, unsuccessful.

Prolonged heating of streptonigrin methyl ester (144) and dimethyl sulphate in acetonitrile under reflux gave only 46% of the C-5' methylamine derivative (234), together with 30% of starting material. When the methyl ester (144) was heated at 100°C in a sealed tube with an excess of methyl iodide for 48 hours, a mixture of dimethylated (235) and trimethylated (236) compounds were obtained in 52 and 46% yields respectively. With an increase in reaction time (72 hours), 65% of the trimethyl (236) and 33% of the dimethyl (235) derivatives were obtained; formation of any quatemised material was not observed.

Quaternisation with alkylating agents such as methyl triflate (MeSO_3CF_3) and trimethyloxonium tetrafluoroborate (Me_3OBF_4) which have been used to quaternise hindered or deactivated sp^3 nitrogen, e.g., N,2,2,6,6-pentamethylpiperidine, and sp^2 nitrogen, e.g., 2,6-lutidine and 2,6-dimethoxycarbonylpyridine were also attempted. The 5'-amino and 8'-hydroxyl groups were first protected as acetyl derivatives as previously described and the

v. We have been unable to protect the 7-NH_2 group (vide supra).
resulting diacetate (209) was treated with methyl triflate\textsuperscript{131} and a hindered pyridine base (2,6-di-\textit{tert}-butylpyridine). No reaction was observed after 10 hours at room temperature whereas prolonged heating in THF gave a complex mixture from which was recovered 20\% of the starting material (209). Similar results were obtained on treatment of the diacetate (209) with trimethylxonium tetrafluoroborate\textsuperscript{130}. Further investigations were not pursued at this stage.

6.3.3 Oxidative Decarboxylation Route

Oxidative decarboxylation of \(\alpha\)-hydroxy acids\textsuperscript{132} and related compounds have been achieved selectively and under mild conditions using a number of reagents such as lead(IV) acetate\textsuperscript{133}, sodium bismuthate\textsuperscript{134} in aqueous acetic acid and sodium or calcium hypochlorite\textsuperscript{135,136}. In principle, a "one-pot" oxidative decarboxylation of streptonigrin (80) [as the "covalent hydrate" or \(\alpha\)-hydroxy acid (237)] to streptonigrone (82) (Scheme 42) could be achieved using one of the aforementioned reagents.

The feasibility of this approach was studied with a model, 2-pyridinecarboxylic acid (190). Attempts to effect oxidative decarboxylation (Scheme 42) of 2-pyridinecarboxylic acid (190) were, however, unsuccessful. Lead(IV) acetate was
unstable in the aqueous acidic medium required for the "covalent hydrate" (238) to exist, while no reaction was observed with sodium bismuthate or sodium or calcium hypochlorite. At this stage, this route for the conversion of the 2-pyridinecarboxylic acid to the 2-pyridone system was not pursued further. This approach was not investigated using the β-carboline system (159).

Failure to apply any of the methodology discussed in the previous sections for the biomimetic conversion of streptonigrin (80) to streptonigrone (82) prompted investigation into other areas. Radical reactions offered an alternative to the ionic reactions for the desired conversion of streptonigrin (80) to streptonigrone (82). These radical processes are advantageous in that they can generally be conducted under neutral conditions and are fairly selective in their mode of action.

6.3.4 Radical Decarboxylation Route

The radical decarboxylation of organic acids with or without the concomitant replacement by some other functional group has been studied in detail by Barton and coworkers. In their work, the thiohydroxamic esters, (241) or (242), vi derived from aliphatic or alicyclic carboxylic acids (RCO₂H) by reaction with suitable thiohydroxamic acids, (239) or (240), underwent efficient radical chain decarboxylation to the corresponding alkanes on heating with either tri-n-butylstannane or -butylmercaptan. Alternatively, the carbon radicals formed after decarboxylation could be intercepted to give a variety of products, often in

vi. These compounds are actually mixed anhydrides, but are called esters for convenience.
high yields, e.g., in the presence of diaryl disulphides,\textsuperscript{138} diselenides,\textsuperscript{138} ditellurides,\textsuperscript{138} halides\textsuperscript{139} or oxygen,\textsuperscript{140} the respective aryl sulphides, selenides, tellurides, alkyl halides and alcohols were obtained. With these thiohydroxamic esters, the idea of aromatization as an additional aid for smooth fragmentation was incorporated,\textsuperscript{141} as well as retaining the concept of thiocarboxyl reduction as a driving force for the expulsion of the carboxylate radical.\textsuperscript{141}

Conceptually, the same approach could be adopted for the biomimetic conversion of streptonigrin (80) to streptonigrone (82). It would involve synthesis of streptonigrin thiohydroxamic ester (243) which, on radical decarboxylation, could generate the pyridinium radical (244). The pyridinium radical (244) could then be intercepted by a radical trap with a suitable oxygen function, e.g., 2,2,6,6-tetramethylpiperidine-N-oxide (TEMPO) (246), giving compound (245). Removal of the TEMPO group with zinc in acetic acid,\textsuperscript{142} followed by hydrolysis could furnish the desired compound, streptonigrone (82) (Scheme 43).

Model studies with 2-pyridinecarboxylic acid (190) were conducted to examine the feasibility of this approach for the conversion of streptonigrin (80) to streptonigrone (82). Thus, the pyridinium salt (247), prepared by treatment of the thiohydroxamic acid (239) with phosgene, was added to a solution of 2-pyridinecarboxylic acid (190) in deoxygenated acetonitrile at room temperature. However, ester (248) was not isolated as decarboxylative rearrangement occurred to give a high yield of the dipyridyl sulphide (251). This result was not unexpected as Barton and coworkers\textsuperscript{137-142} had also observed similar rearrangements in various substrates. Attempts to trap the radical (249), formed by N-O fission and decarboxylation of the thiohydroxamic ester by \textit{in situ} addition of TEMPO (246) (5 mole equivalents) afforded 59\% of the ester (252), together with 22\% of the sulphide (251); none of the desired product (250) was observed.

Two possible mechanisms could be formulated for this unusual result. If a radical reaction had been involved, presumably homolysis of the C-O bond occurred to generate corresponding acyloxy (253) and nitroxide (254) radicals,\textsuperscript{vii} both of

\textsuperscript{vii.} The exceptional stability of nitrooxide radicals is known.\textsuperscript{144,145} According to thermochemical data, the electron delocalization energy for the N-O group in heterocyclic N-oxides is approximately 30 kcal/mol.
which are highly stabilized by delocalization of the unpaired electron. The acyloxy radical (253) could have been trapped by TEMPO (246) before it was able to decarbonylate (Scheme 44).

Alternatively, the reaction might have involved a transesterification process. Under the reaction conditions, TEMPO (246) could be reduced to the hydroxylamine (255) which could then react with the thiohydroxamic ester (248) via an addition-elimination reaction to furnish the ester (252). Neither mechanism proposed was substantiate experimentally.

**Scheme 44**

(A) C-O Bond Homolysis

(B) Transesterification
An alternative method for inducing radical decarboxylation would be to effect a "one-pot" decarboxylative halogenation of the type also reported by Barton and coworkers. They demonstrated that carboxylate esters derived from N-hydroxypyridine-2-thione (241) reacted with carbon tetrachloride, bromotrichloromethane or iodoform in a radical chain reaction [in which the chain carrier is the trichlorornethyl radical (\(\text{CCl}_3\))] giving the corresponding noralkyl chlorides, bromides or iodides in high yields. In principle, streptonigrin o-acetate (206) could be converted to the halide derivative (256) via a decarboxylative halogenation of the intermediate thiohydroxamic ester (243) in the presence of the appropriate solvent. The halide (256) could then be hydrolysed to give streptonigrone (82) (Scheme 45). The hydrolysis of pyridine halides to the corresponding pyridones is a well known reaction.

The feasibility of this approach for the conversion of streptonigrin (80) to streptonigrone (82) was investigated using a model, 2-pyridinecarboxylic acid (190).
Hence, the pyridinium salt (247) was added in the dark to a stirred solution of 2-pyridinecarboxylic acid (190) and pyridine in a mixture of deoxygenated acetonitrile and bromotrichloromethane (4:1). However, a complex mixture was obtained on work-up.

In view of this result, such radical processes for the conversion of streptonigrin (80) to streptonigrone (82) were not pursued further. The application of these radical processes was not investigated with the β-carboline (159) system.

6.3.5 Concluding Remarks

In this section 6.3, the biomimetic conversion of the antibiotic streptonigrin (80) or a β-carboline precursor (133) to streptonigrone (82) was investigated using several approaches. In the first approach, discussed in section 6.3.1, the model systems, 2-pyridine carboxylic acid (190) and β-carboline-3-carboxylate (159) were successfully converted to the corresponding 2-pyridone, (194) and indolo-α-pyridone (201) via the N-oxides, (191) and (198). In the second approach, discussed in section 6.3.2, the quaternary pyridinium salts, (226) and (228) were successfully converted to the 2-pyridones, (224) and (229) although the model study with the β-carbolinium salt (231) was unsuccessful. When either approach was extended to the conversion of streptonigrin (80) to streptonigrone (82), we were unable to synthesise the key intermediates, (205) or (221), required for the application of these methods. The model study with 2-pyridinecarboxylic acid (190) via the "one-pot" decarboxylative oxidation route (section 6.3.3) was unsuccessful.

The conversion of streptonigrin (80) to streptonigrone (82) via radical processes (section 6.3.4) was also investigated. Treatment of the model system, 2-pyridinecarboxylic acid (190), with the pyridinium salt (247), followed by TEMPO (247), gave the ester (252) and the sulphide (251), rather than the expected ester (250). A complex mixture was obtained when 2-pyridinecarboxylic acid (190) was treated with the pyridinium salt (247) and bromotrichloromethane.
CHAPTER 7

7.1 INTRODUCTION

The X-ray structure for the natural product, streptonigrin (80), was solved by Chiu and Lipscomb\(^\text{[47]}\) (see also Chapter 3). It was shown that each crystal contained only one enantiomer of the streptonigrin (80) molecule, and hence that the antibiotic was chiral. The chiroptic properties of streptonigrin (80) and its monoamide derivative (111) have been reported by Dekker and Gaal\(^\text{[71]}\). Although their results suggested that streptonigrin (80) was an L configuration, the absolute configuration remains to be determined.

The 3-pyridone analogue, streptonigrone (82), is also a natural product and co-occurs with streptonigrin (80) in an unidentified toxicogenic species. Alkaline metal ions, as described by Richards and co-workers\(^\text{[44]}\) to measure the chiroptic properties of streptonigrone (82), were unsuccessful due to the strong chelation of the antibiotic (82). However, streptonigrone (82) would be expected to be chiral and of the same absolute configuration as streptonigrin (80). An investigation of the enantiomeric purity of streptonigrone (82) using a combination of chemical and spectroscopic techniques is discussed in the following section. The partial isolation of streptonigrin (80) and of streptonigrone (82) was also investigated and is described in this chapter.

7.2 DETERMINATION OF THE ENANTIONERIC PURITY OF STREPTONIGRIN AND STREPTONIGRONE

7.2.1 The Use of Chiral Lanthanide 3d-4p- or 4f-Complexes

Paramagnetic lanthanide complexes have been used to facilitate spectral analysis of organic compounds by inducing chemical shifts stereospecifically in \(^{1}H\) NMR.
CHAPTER 7 : AN APPROACH TO THE DETERMINATION OF THE CHIRALITIES OF STREPTONIGRIN AND STREPTONIGRONE

7.1 INTRODUCTION

The X-ray structure for the natural product, streptonigrin (80), was solved by Chiu and Lipscomb\textsuperscript{47} (\textit{vide supra} Chapter 5). It was shown that each crystal contained only one enantiomer of the streptonigrin (80) molecule, and hence that the antibiotic was chiral. The chiroptical properties of streptonigrin (80) and its monoxime derivative (111) have been reported by Dohlakia and Gillard.\textsuperscript{53} Although their results suggested that streptonigrin (80) has an $S$ configuration, the absolute configuration remains to be determined.

The 2-pyridone analogue, streptonigrone (82) is also a natural product and co-occurs with streptonigrin (80) in an unidentified \textit{Streptomycetes} species. Attempts were made by Rickards and coworkers\textsuperscript{44} to measure the chiroptical properties of streptonigrone (82), but were unsuccessful due to the strong light absorption of the antibiotic (82). However, streptonigrone (82) would be expected to be chiral and of the same absolute configuration as streptonigrin (80). An investigation of the enantiomeric purity of streptonigrone (82) using a combination of chemical and spectroscopic techniques is discussed in the following sections. The axial chirality of streptonigrin (80) and of streptonigrone (82) was also investigated and is described in this chapter.

7.2 DETERMINATION OF THE ENANTIOMERIC PURITY OF STREPTONIGRIN AND STREPTONIGRONE

7.2.1 The Use of Chiral Lanthanide Shift Reagent

Paramagnetic lanthanide reagents have been used to facilitate spectral analysis of organic compounds by inducing chemical shifts stereospecifically in $^1$H NMR
spectroscopy. The protons of a substrate which has sufficiently polar groups to form a complex with the lanthanide reagent will experience induced shifts. The sizes of these lanthanide induced shifts (LIS) are partly dependent on the distance between the complexing site and the proton in question. Consequently, valuable information concerning the environment of particular hydrogen atoms in the substrate can be obtained.

The enantiomeric resolution of $^1$H NMR spectra of chiral substances in solution can be achieved in two ways, i.e. by use of an achiral lanthanide shift reagent (LSR) together with an optically pure additive, or by the use of a chiral LSR. Better enantiomeric resolution, however, has usually been observed with the latter reagent. Hence, it seemed feasible to determine the enantiomeric purity of streptonigrone (82) using a chiral LSR such as tris[3-(trifluoromethylhydroxymethylene)-$d$-camphorato europium III [Eu(tfc)$_3$].

The applicability of this method was studied with a model, 2,2'-dihydroxy-1,1'-binaphthyl (or binaphthol) (257) and with streptonigrin (80). With racemic binaphthol (257), one would expect to observe two sets of proton signals (one set for each enantiomer) on addition of a suitable amount of shift reagent, whereas, in the case of streptonigrin (80), only one set of resonances would be expected in the $^1$H NMR spectrum.

Upon addition of increasing amounts of Eu(tfc)$_3$ (up to 0.3 mole equivalents) to a solution of racemic binaphthol (257) in deuteriochloroform, shifts of the proton resonances to lower field were observed in the $^1$H NMR spectrum. Further addition of the shift reagent (0.4 mole equivalents) produced two sets of signals (one for each enantiomer), particularly for that of the hydroxyl groups, the separation between which increased with increasing concentrations of Eu(tfc)$_3$.

In the case of streptonigrin (80), progressive shifts of the proton resonances were also observed on addition of increasing amounts of the europium reagent. However, only one set of signals was observed with up to 1.6 mole equivalents of Eu(tfc)$_3$, consistent with the enantiomeric purity of the antibiotic (80).
Similarly, two sets of resonances are expected in the $^1$H NMR spectrum of streptonigrone (82) on addition of a suitable amount of the europium reagent if this antibiotic is a mixture of enantiomers, whereas, the presence of only one set of signals would be indicative of its enantiomeric purity. On addition of Eu(tfc)$_3$ (up to 1.6 mole equivalents) to streptonigrone (82), progressive downfield shifts of the proton resonances were observed without any separation of signals, indicating that the antibiotic consisted of only one isomer.

It is possible, however, that streptonigrone (82) is a mixture of enantiomers and that the induced shifts experienced by two the enantiomers are not sufficiently different to give rise to two separate sets of signals. There are many coordination sites available on streptonigrone (82) [and on streptonigrin (80)]. Hence, the coordination experienced by each individual site could be less, rendering the induced shifts less detectable. It was not possible to use a larger amount of the shift reagent due to considerable line broadening of the $^1$H NMR spectra. Thus, another method for checking the optical purity of the antibiotic (82) was investigated.

7.2.2 The Use of $\alpha$-Methylmandelate Esters

The phenomenon of chemical shift non-equivalence in the $^1$H NMR spectra of diastereomeric esters and amide derivatives has been widely utilized for the analysis of enantiomeric composition of secondary alcohols and amines. These derivatives of $\alpha$-methylmandelic acid and $\alpha$-methoxy-$\alpha$-trifluoromethylphenylacetic acid (MTPA or Mosher’s reagent) have been especially useful in this regard.$^{148a}$ Of particular interest is the use of these esters for the determination of the enantiomeric purity of biaryl systems.

Yamaguchi and coworkers$^{148b}$ reported the determination of enantiomeric purities of axially chiral biaryls with amino, carboxyl and hydroxyl groups by analysis of the $^1$H NMR spectra of the MTPA derivatives. The $^1$H NMR spectra of some of these diastereomeric MTPA derivatives showed unresolved or partially resolved methoxyl signals which separated into two sets of signals on progressive addition of an achiral LSR. Fully resolved methoxyl resonances were observed in the undoped spectra of
other diastereomeric pairs. The relative peak areas of the well separated methoxyl signals thus allowed accurate determination of the enantiomeric purity of the original biaryls.

This methodology was extended to determine the enantiomeric purity of streptonigrone (82), its applicability being established with racemic [(R) and (S)] 2,2'-dihydroxy-1,1'-binaphthyl (257), and with the streptonigrin (80). The readily available (R)-O-methylmandelic acid was used for the preparation of ester derivatives. With a mixture of binaphthol mono-mandelate esters [(R)(R) and (R)(S)], two separate sets of signals (one set for each diastereomer) should be observed in the $^1$H NMR spectrum, or if the signals are unresolved, separation of the resonances should be achievable by progressive addition of a shift reagent. In the case of streptonigrin (R)- or (S)-mandelate ester, only one set of resonances would be observed [The (R) and (S) configurations denote the chirality of the mandelate portion only as the absolute configuration of streptonigrin (80) is unknown at this stage]. The respective mandelate esters were thus prepared and their $^1$H NMR spectral data analysed.

Preparation of Racemic Binaphthol O-Methylmandelate Esters and Analysis of Their $^1$H NMR Spectra

The racemic binaphthol mono-mandelate esters were prepared as follows. Treatment of racemic binaphthol (257), with an excess of diazomethane gave the monomethyl and dimethyl ethers, (258) and (259), in 50% and 5% yield respectively after purification, together with recovered starting material (40%). The racemic monomethyl ether (258) was then treated with (R)-O-methylmandelic acid in the presence of DCC and DMAP to afford a mixture of (R)(R)- and (R)(S)-esters, (260) and (261), in 84% yield after purification (Scheme 46).

The $^1$H NMR spectrum of the diastereomeric mixture of esters, (260) and (261), recorded in deuteriochloroform, showed two well separated sets of signals for the aromatic methoxyls and for the mandelate moieties (methine proton and O-methyl), i.e. one set for each diastereomer, with the resonances of the (R)(R) diastereomer (260) occurring at higher field. This assignment was made by synthesising the (R)(R) isomer (260) and comparing the $^1$H NMR spectrum with that of the racemic mixture.
Preparation of Streptonigrin O-Methylmandelate Esters and Analysis of Their $^1$H NMR Spectra

The streptonigrin (R)-mandelate ester (262) was prepared in 63% yield with 95% enantiomeric excess (by $^1$H NMR spectroscopy) on treatment of the methyl ester (144) [which was prepared as previously described (refer Chapter 6)] with (R)-O-methylmandelic acid. DCC and DMAP.\textsuperscript{29} That esterification of the C-8' phenolic hydroxyl had occurred, was evident from the downfield shifts of the 11' and 12' protons ($\Delta\delta$ 0.13 and 0.24 ppm respectively) compared to the starting material (144). The presence of a small amount (5%) of the (S)-mandelate ester could be attributed to racemisation of the (R)-O-mandelic acid by DMAP\textsuperscript{29} under the reaction conditions used. The $^1$H NMR spectrum of the (R)-mandelate ester (262), recorded in deuteriochloroform, showed only one set of resonances, even on progressive addition of the shift reagent, tris(1,1,1,2,2,3,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium III, \textit{i.e.} Eu(fod)$_3$ [up to 1.4 mole equivalents (\textit{vide infra})]. Furthermore, the $^1$H NMR spectrum of the (S)-mandelate ester (263) [prepared in 70% yield with 95%
enantiomeric excess by treating the methyl ester (144) with (S)-O-methylmandelic acid in the presence of DCC and DMAP (Scheme 47) differed from that of the (R) diastereomer (262) (see Table 14), even in the absence of the shift reagent, and was therefore consistent with the enantiomeric purity of the original antibiotic (80).

Scheme 47

```
CO2Me
H2N
H2N
MeO
MeO
N
N
CO2Me
H2N
MeO
MeO

(i) HO2C-~H
Ph

(ii) HO2C-~H
Ph

(144)  (262)  (263)
```

Table 14: $^1$H NMR Spectral Data (in CDCl$_3$) of (R) and (S) Mandelate Esters (262) and (263).

<table>
<thead>
<tr>
<th>Assignment</th>
<th>(262)</th>
<th>(263)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-Me (s, 3H)</td>
<td>2.10</td>
<td>2.19</td>
</tr>
<tr>
<td>-OMe (mandelate moiety, s, 3H)</td>
<td>3.29</td>
<td>3.25</td>
</tr>
<tr>
<td>-OMe (all s, 3H each)</td>
<td>3.76</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>3.95</td>
<td>3.94</td>
</tr>
<tr>
<td></td>
<td>3.98</td>
<td>3.99</td>
</tr>
<tr>
<td></td>
<td>4.10</td>
<td>4.09</td>
</tr>
<tr>
<td>C-H (mandelate moiety, s, 1H)</td>
<td>4.80</td>
<td>4.75</td>
</tr>
<tr>
<td>7-NH$_2$ (bs, 1H)</td>
<td>5.12</td>
<td>5.12</td>
</tr>
<tr>
<td>11'-H (d, 1H, J=8Hz)</td>
<td>6.84</td>
<td>6.86</td>
</tr>
<tr>
<td>12'-H (d, 1H, J=8Hz)</td>
<td>7.0</td>
<td>6.98</td>
</tr>
<tr>
<td>4-H (d, 1H, J=8Hz)</td>
<td>8.45</td>
<td>8.44</td>
</tr>
<tr>
<td>3-H (d, 1H, J=8Hz)</td>
<td>8.95</td>
<td>8.94</td>
</tr>
<tr>
<td>Ar-H (mandelate portion, m, 5H)</td>
<td>7.1</td>
<td>7.1</td>
</tr>
</tbody>
</table>
In the case of streptonigrone (82), two alternatives should be considered. If the antibiotic (82) is a mixture of enantiomers, the $^1$H NMR spectrum of the (R)- [or (S)-] mandelate esters would show two sets of signals as observed for the binaphthol mandelate derivatives. (260) and (261). or if the resonances were unresolved, separation of the signals may be observed on progressive addition of a lanthanide shift reagent. The (R) and (S) configurations denote the chirality of the mandelate portion only as the configuration of streptonigrone (82) is unknown at this stage. On the other hand, if streptonigrone (82) is enantiomerically pure, only one set of signals would be observed. In addition, the $^1$H NMR spectra of the (R)- and (S)-mandelate diastereomers would, as in the case of the streptonigrin mandelate derivatives. (262) and (263), be different.

Difficulties were, however, encountered when attempting to prepare the mandelate ester (264). No significant reaction was observed on treatment of streptonigrone (82) with (R)-O-methylmandelic acid in the presence of DCC and DMAP in dichloromethane at 40°C. When the reaction temperature was increased to 80°C, a complex mixture, containing some starting material, was obtained (Scheme 48). A similar result was observed on treatment of streptonigrone (82) with (R)-O-methylmandelic acid in the presence of DCC, pyridine and N-hydroxybenzotriazole.29 A complex mixture was also obtained on treatment of the antibiotic (82) with the acid chloride of O-methylmandelic acid (prepared by slow addition of the acid to a suspension of oxalyl chloride in DMF) in the presence of pyridine29 (Scheme 48).

It is unclear why the reactivity of the phenolic hydroxyl of streptonigrone (82) differed significantly from that of streptonigrin (80). Further investigations using one of the other literature methods for preparing esters could prove more successful, but were not attempted at this stage.

---

i. The reaction conditions used were the same as those for the preparation of the streptonigrin O-methylmandelate esters. (262) and (263).
7.3 AN APPROACH TO DETERMINE THE CHIRALITIES OF STREPTONIGRIN AND STREPTONIGRONE

While the efforts to determine the enantiomeric purity of streptonigrone were in progress, the axial chirality of streptonigrin (80) [and of streptonigrone (82)] was also investigated. Yamaguchi et al.\textsuperscript{148} initially reported the use of MTPA derivatives in the presence of lanthanide shift reagents to determine the enantiomeric purities of chiral biaryls. In a subsequent paper,\textsuperscript{149} the same authors reported the determination of the absolute configuration of biaryls with amino, carboxyl and hydroxyl groups using the same methodology. The relative magnitudes of the lanthanide induced shifts of the methoxyl signals (MTPA moiety), \textit{i.e.} LIS\textsubscript{OMe}, were correlated to the absolute configuration of the biaryls.

A model was proposed (as shown in Fig. 23a and b) in which both the ester (OCO) linkage and the naphthalene ring bearing the ester function lie almost in the same plane, and the LSR coordinates with the oxygen atoms of the carbonyl and methoxyl groups. In the case of (R)(S)-binaphthol [Fig. 23a, where (R) and (S) denote the absolute configurations of the mandelate moiety and of the binaphthol.
respectively], the substituent A on the second naphthalene ring system is in close proximity to the carbonyl and methoxyl groups. When A is a polar group (e.g. OH, NH$_2$), coordination of A with the shift reagent is possible and this additional coordination would result in larger LIS$_{OMe}$ values compared to those of the (R)(R)-isomer (as shown in Fig. 23b).

Fig. 23a

![Fig. 23a](image)

Fig. 23b

![Fig. 23b](image)

It seemed feasible to utilize a similar method for the determination of the axial chirality of streptonigrin (80) and streptonigrone (82). In the case of streptonigrin (80) where there is only one enantiomer, both the (R) and (S) mandelate esters must be prepared to utilise this approach. For example, if streptonigrin (80) has (R) configuration, then the esters would correspond to the (R)(R) and (S)(R) isomers. Since the $^1$H NMR spectrum of the (S)(R) isomer is identical to that of the (R)(S) isomer, the same correlations as outlined for the binaphthol system [(R)(R) and (R)(S) isomers] can be applied but modified to realise that the mandelate stereocentre is inverted. The above should also apply for the determination of the axial chirality of streptonigrone (82) provided this compound is enantiomerically pure. Examination of several literature X-ray structure analysis data, e.g. for diacetylferferol (265), riacardin A diacetate (266), pristimerol bis(p-bromobenzoate) (267) and leprolomin (268), however, indicated that the model proposed by Yamaguchi and coworkers, in which the ester carbonyl was planar to the naphthalene ring bearing the ester function, was incorrect. The X-ray data showed that the ester carbonyls were in fact perpendicular to the plane of the naphthalene ring. To verify the perpendicular arrangement of the ester carbonyls, in particular that of the binaphthol esters, the (R)(R)-mandelate derivative (260) was prepared as previously described with
greater than 99% enantiomeric excess (by \textsuperscript{1}H NMR spectroscopy), and the relative spatial arrangement of the molecule determined by an X-ray structure analysis.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig24.png}
\caption{ORTEP Drawing of (R)(R) Ester (260).
\textsuperscript{ii}}
\end{figure}

7.3.1 Model Study with (R)-2,2'-Dihydroxy-1,1'-Binaphthyl

\textit{X-Ray Structure Analysis and Low Temperature NMR Spectroscopy}

Suitable crystals were obtained from ethyl acetate and hexane by the liquid diffusion technique. An X-ray crystal structure analysis using the DIRECT method\textsuperscript{ii} showed the crystals were orthorhombic with the space group P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}. A perspective ORTEP drawing of the (R)(R) ester (260) is presented in Fig. 24. The X-ray structure analysis showed that the ester carbonyl was indeed perpendicular to the naphthalene ring bearing the ester functionality, and therefore that the model proposed by Yamaguchi and his collaborators\textsuperscript{149} was incorrect.

\textsuperscript{ii} The author is grateful to Drs. V. Parthasarathi, G. B. Robertson and A. C. Willis for performing the X-ray structure analysis.
Fig. 24: ORTEP Drawing of \((R)(R)\)-Mandelate Ester (260)

The X-ray analysis also showed that there were two conformations of the ester linkage (see Figs. 25a and b) of the \((R)(R)\)-mandelate ester (260). In one conformation, the carbonyl group is above the plane of the naphthalene ring bearing the ester function while in the other, the carbonyl group is below; in both conformations, the carbonyl is perpendicular to the plane of the naphthalene ring. In the solid state, lattice packing effects may explain the existence of the two conformations of the ester linkage. In solution, however, rapid exchange between the two orientations of the ester linkage would be expected, and thus only one set of signals was detected in the \(^1\)H NMR spectrum even upon reduction of temperature to \(-90^\circ C\).
Stereopairs of \((R)(R)\)-Mandelate Ester (260) Showing the Two Conformations of the Ester (OCO) Linkage

Lanthanide Shift Experiment

Although the model proposed by Yamaguchi and his collaborators\(^{149}\) was incorrect, the empirical correlation of chirality with induced shifts drawn from their \(^1\)H NMR experiments was probably valid. As a comparison, the \(^1\)H NMR spectrum of a mixture of the \((R)(R)\)- and \((R)(S)\)-mandelate derivatives, (260) and (261), was recorded in the presence of a shift reagent, Eu(fod)\(_3\). The aromatic methoxyl as well as the \(\alpha\)-methyl signal of the mandelate moiety were used as probes in order to
correlate the lanthanide induced shifts $[\text{LIS}_\text{OMe(naphthyl)}]$ and $[\text{LIS}_\text{OMe(mandelate)}]$ with the chiralities of the biaryl compounds.

On progressive addition of Eu(fod)$_3$, downfield shifts of different magnitude were observed for the methoxyl signals of the two diastereomers. The aromatic methoxyl signal of the (R)(R) diastereomer (260) [which appears at higher field than that of the (R)(S) isomer (261) in the absence of the shift reagent] was shifted further downfield, passing over the signal of the (R)(S) diastereomer (261), giving a $\Delta\text{LIS}_\text{OMe}$ value (see Fig.26) of 0.26 ppm (for a one mole equivalent of the europium reagent). The $\Delta\text{LIS}_\text{OMe}$ value being the difference between the $\text{LIS}_\text{OMe}$ of the (R)(R) derivative (260) and that of diastereomer (261). On the other hand, the $\alpha$-methyl resonance (mandelate portion) of the (R)(S) (261) derivative, which occurred at lower field than that of the (R)(R) isomer (260), shifted further downfield compared to diastereomer (260) on progressive addition of Eu(fod)$_3$. The $\Delta\text{LIS}_\text{OMe}$ value in this case was -0.15 ppm (Fig.27) for a one mole equivalent of the shift reagent.

The above $^1$H NMR configurational correlation, i.e. of chirality with induced shifts, could be explained by the following models (Figs.28a and b) proposed on the basis of the X-ray crystal structure of the (R)(R)-mandelate derivative (260). The magnitude of the induced shifts clearly reflect primary binding to the methoxyl (mandelate moiety) and carbonyl (or oxygen of the ester linkage since there are two possible conformations of the ester linkage) groups. In the case of the (R)(R) diastereomer (260), the aromatic methoxyl is in the vicinity of the primary binding site, thus allowing coordination of the europium reagent to the aromatic methoxyl as well as to the oxygens of the primary binding site (Fig.28a). This would therefore result in a larger $\text{LIS}_\text{OMe(naphthyl)}$ value than for the alternate diastereomer (261) in which the aromatic methoxyl is further removed from the primary binding site (see Fig.28b). However, coordination of the shift reagent to a third oxygen (i.e. the aromatic methoxyl) in the (R)(R) diastereomer (260) (Fig. 28a) would result in decreased coordination of the shift reagent to the primary binding site. in particular to the methoxyl of the mandelate moiety, and hence would afford a smaller $\text{LIS}_\text{OMe(mandelate)}$ value than for the alternate diastereomer (261) (Fig.28b).
Fig. 26  Binaphthol (R)(R)- and (R)(S)-O-Methylmandelate esters (260) and (261) : Graph of Induced Chemical Shifts (δ in ppm) of Aromatic Methoxyl Resonance vs Molar Ratio of Eu(fod)₃

Fig. 27  Binaphthol (R)(R)- and (R)(S)-O-Methylmandelate esters (260) and (261) : Graph of Induced Chemical Shifts (δ in ppm) of O-Methyl (Mandelate Moiety) Resonance vs Molar Ratio of Eu(fod)₃
7.3.2 An Attempt to Determine the Axial Chirality of Streptonigrin

The $^1$H NMR spectra of the (R)- and (S)-mandelate esters, (262) and (263), were examined in the presence of a shift reagent, Eu(fod)$_3$.

Lanthanide Shift Experiment:

Only the $\alpha$-methyl signals of the mandelate moiety were used as probes because the C-5' amine resonance is not observed in $^1$H NMR spectra of either streptonigrin (80) or derivatives recorded in deuteriochloroform. On successive addition of Eu(fod)$_3$, downfield shifts were observed for the $\alpha$-methyl signals of both the (R) and (S) esters, (262) and (263). The $\alpha$-methyl resonance of the (R)-ester (262) was, however, shifted slightly further downfield, giving a $\Delta$LIS$_{OMe}$ of 0.08 ppm for one mole equivalent of the shift reagent (see Fig. 31), indicating that the absolute configuration of streptonigrin (80) is as shown in Fig. 30, i.e. S. This result agrees with the absolute configuration reported by Dohlakia and Gillard.$^{53}$
The following models, shown in Figs. 29 and 30, were postulated to explain the NMR configurational correlation. As can be seen in Fig. 29, coordination of the shift reagent with two oxygens and a nitrogen seems possible in the (R)-mandelate ester (262). The additional coordination would decrease the proximity of the shift reagent with the methoxyl group (mandelate moiety), and would result in a smaller $\text{LIS}_{OMe(\text{mandelate})}$ value compared to that of (S) diastereomer (263). If the configuration of streptonigrin (80) is as shown in Fig. 30, a larger $\text{LIS}_{OMe(\text{mandelate})}$ value for the (R)-mandelate ester (262) would be expected instead.

It is difficult, however, to establish unambiguously the axial chirality of the antibiotic (80) based on this small $\Delta\text{LIS}_{OMe}$ value. Attempts to increase the separation between the $\alpha$-methyl resonances of the (R) and (S) esters (262) and (263) with increased concentrations of the shift reagent were unsuccessful due to rapid signal broadening. A further difficulty in establishing the axial chirality of streptonigrin (80) arises from its multiple binding sites.

Figure 29

![Figure 29](image)

Figure 30

![Figure 30](image)
As mentioned in Chapter 5, the conversion of streptonigrin (80) to streptonigrone (82) was investigated, not only as a biomimetic model, but also as a means of correlating the chiralities of the two antibiotics (80) and (82). The biomimetic conversion involved only the substituent at C-2' of streptonigrin (80), and therefore the configuration of the molecule is unlikely to be affected. Hence, if the conversion of streptonigrin (80) to streptonigrone (82) was achieved and the chirality of streptonigrin (80) determined, then the absolute configuration of streptonigrone (82) could be inferred. However, investigations to date of the conversion of streptonigrin (80) to streptonigrone (82) have been unsuccessful (refer Chapter 6).
7.4 CONCLUDING REMARKS

In this chapter, an investigation of the enantiomeric purity of streptonigrone (82) using a combination of spectroscopic and chemical techniques was discussed. Two approaches were investigated.

The first approach involved the use of a chiral lanthanide shift reagent such as Eu(tfc)$_3$ to facilitate the enantiomeric resolution of $^1$H NMR spectra of chiral substrates. The applicability of this method was studied with racemic binaphthol (257) and with the antibiotic streptonigrin (80) and the results obtained were consistent with the enantiomeric composition of the two compounds. (257) and (80). In the case of streptonigrone (82), only one set of signals was observed with up to 1.6 mole equivalents of Eu(tfc)$_3$, indicating that this compound consisted of only one isomer.

The second approach utilised the chemical shift non-equivalence in $^1$H NMR spectra of esters of $o$-methylmandelic acid. The applicability of this method was studied with racemic binaphthol (257) and with streptonigrin (80). A $^1$H NMR spectrum of a mixture of the binaphthol mandelate esters, (260) and (261), showed two well resolved sets of signals (one for each diastereomer), whereas in the case of streptonigrin ($R$)-mandelate ester (262), only one set of resonances was observed, consistent with the enantiomeric purity of the original antibiotic (80). In addition, the $^1$H NMR spectra of the ($R$)- and ($S$)-mandelate esters were different. To date, attempts to prepare streptonigrone ($R$)-mandelate ester (264) were, however, unsuccessful.

An approach to determine the absolute configuration of streptonigrin (80) and of streptonigrone (82) was also discussed in this chapter. This approach, involving the use of $o$-methyl mandelate derivatives in the presence of lanthanide shift reagents, was studied with ($R$)-binaphthol (257) and with streptonigrin (80). In the study with ($R$)-binaphthol (257), the aromatic methoxyl signal of the ($R$)($R$) diastereomer (260) was shifted further downfield than the ($R$)($S$) diastereomer (261), giving a $\Delta$LIS$_{OMe}$ value of 0.26 ppm, whereas the $o$-methyl resonance (mandelate portion) of the ($R$)($S$)-ester shifted further downfield compared to diastereomer (260) on addition of Eu(fod)$_3$, giving a $\Delta$LIS$_{OMe}$ value of -0.15 ppm.
The $^1$H NMR configurational correlation was explained by models (Figs. 28a and b) proposed on the basis of an X-ray crystal structure of the $(R)(R)$-mandelate ester (260). The results of the X-ray analysis also showed that another model proposed by Yamaguchi et al.$^{149}$ for the correlation of chirality with induced shifts in $^1$H NMR spectra of MTPA esters was incorrect. The X-ray structure analysis clearly showed that the carbonyl of the ester linkage was perpendicular to the naphthyl ring bearing the ester functionality rather than planar as proposed by the Japanese workers, and that there were two conformations of the ester linkage.

In the case of streptonigrin mandelate esters, downfield shifts were observed for the $\alpha$-methyl signals of both the $(R)$ and $(S)$ esters (262) and (263), giving a $\Delta\text{LIS}_{\text{OMe}}$ of 0.08 ppm on successive addition of Eu(fod)$_3$, indicating that the absolute configuration of streptonigrin (80) is as shown in Fig. 30, i.e. $S$. This result, although in agreement with the chirality reported by Dohlakia and Gillard$^{53}$ for streptonigrin (80), could not be established unambiguously due to the small $\Delta\text{LIS}_{\text{OMe}}$ value observed.
CHAPTER 8

EXPERIMENTAL

1.2.3.4-Tetrahydror suspension of 3H-captrofuran-3H-captrofuran-3H-captrofuran-3H-captrofuran (157)

A mixture of di-ethyl ether (100 ml) (10.2 g, 956 mmol), sodium hydroxide (1.0 g, 0.05 mol) and water (50 ml) was added and cooled and formalin (30%, 3.0 g, 0.05 mol) was then added. The mixture was stirred at room temperature for 2 h, filtered under reflux for 3 h, then cooled and neutralized with 5M aqueous hydrochloric acid (6.5 ml) to pH 7. The precipitate was filtered, washed with water, methanol and diethyl ether, and dried to give the tetrahydro-3H-captrofuran-3H-captrofuran-3H-captrofuran-3H-captrofuran (157) in a yield of 9.7 g.

MP: 300°C (dec.) (lit. 84-325°C, dec.).

1H NMR (CDCl3): DCH2: 6.66 (m, 0H, Ar-H), 4.38 (m, 1H, CH2), 4.3 (m, 1H, CH2), 3.93 (m, 2H, CH2) ppm.

EIMS: 208 (M+), 192 (M+H) 190, 185, 171, 167, 129, 125. (C6H10O2, 131)

1.2.3.4-Tetrahydror suspension of 3H-captrofuran-3H-captrofuran-3H-captrofuran-3H-captrofuran (157)

Concentrated sulphuric acid (1.84 ml) was slowly added to a suspension of the solid (157) (1.0 g, 4.65 mmol) in methanol (20 ml). The reaction mixture was then heated under reflux for 16 h. The reaction mixture was then filtered, poured into ice-water and neutralized with concentrated sulphuric acid. The resulting mixture was extracted with ethyl acetate. The crude was washed with water, dried over sodium sulphate, and concentrated. The crude 157 was recrystallized from methanol (12.7 ml) to give 157 (2.04 g) after recrystallization from methanol: diethyl ether:

MP: 128-130°C (lit. 126-128°C)

1H NMR: DCD: 7.3 (s, 0H, 9-N), 7.3 (d, 0H, 3-N), 4.1 (m, 1H, CH2), 4.1 (m, 1H, CH2), 3.91 (m, 1H, CH2-OCH3), 3.8 (m, 1H, CH2-COOCH3), 3.8 (m, 2H, CH2-Cl) ppm.

EIMS: 320 (M+), 285 (M+H) 271, 269, 255, 233, 191, 177 (157), 141 (C9H14O2, 100)

CHAPTER 8
GENERAL TOPICS

For general procedure, see Chapter 4.

EXPERIMENTAL

1,2,3,4-Tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid (157)

A mixture of dl-tryptophan (130) (10.2 g, 0.05 mol), sodium hydroxide (2.0 g, 0.05 mol) and water (20 mL) was stirred until clear and formalin (30%, 5.0 g, 0.05 mol) was then added. The mixture was stirred at room temperature for 2 h., heated under reflux for 3 h., then cooled and neutralized with 6M aqueous hydrochloric acid (8.3 mL) to pH 5. The precipitate was collected, washed with water, methanol and dichloromethane, and dried to give the tetrahydro-β-carboline acid (157) in 90% yield (9.7 g).

MP 300°C (dec.) (Lit.84 305°C, dec.).

1H NMR (CD3OD + DCl) 6.66 (m, 4H, Ar. H), 4.59 (m, 2H, CH2N), 4.5 (m, 1H, CHCO2H), 3.4 (m, 2H, CH2CH) ppm.

EIMS 216 (M+, C12H12N2O2, 40.4%), 171 (C11H12N2, 19.2), 168 (43.4), 143 (C10H9N, 100), 115 (C8H5N, 14.1).

1,2,3,4-Tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (158)

Concentrated sulphuric acid (1 mL) was slowly added to a suspension of the acid (157) (1.0 g, 4.63 mmol) in methanol (20 mL). The reaction mixture was then heated under reflux for 10 h., concentrated, poured into ice and neutralized with sodium carbonate. The resulting mixture was extracted with dichloromethane. The extracts were washed with water, dried over sodium sulphate and evaporated. The methyl ester (158) was obtained in 87.5% yield (0.93 g) after recrystallisation from methanol / chloroform.

MP 188 - 190°C (Lit.84 190 - 192°C).

1H NMR CDCl3 7.8 (s, 1H, 9-NH), 7.0 - 7.5 (m, 4H, Ar. H), 4.15 (s, 1H, CH2N), 3.8 (m, 1H, CHCO2Me), 3.8 (s, 3H, CO2CH3), 3.0 (m, 2H, CH2CH) ppm.

EIMS 230 (M+, C13H14N2O2, 55.8%), 171 (C11H11N2, 47.5), 143 (C10H9N, 100).
9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (159)

To a cooled, rapidly stirred solution of tetrahydro-β-carboline methyl ester (158) (0.23 g, 1 mmol) in glacial acetic acid (4 mL) was added lead tetraacetate (0.89 g, 2 mmol) and the mixture was stirred for 15 min. Oxalic acid (1 g) was added and stirring was continued for 1 h. The precipitate formed was collected, washed sparingly with dichloromethane (20 mL), and neutralized with sodium carbonate. The reaction mixture was filtered and the residue washed with dichloromethane. The aqueous layer was further extracted with dichloromethane (3 x 15 mL). The combined dichloromethane extracts were dried over sodium sulphate, evaporated to a solid which was recrystallized from methanol to give the β-carboline ester (159) in 30% yield (68 mg).

MP 250 - 253°C (dec.) (Lit. 84-86 255 - 257°C, dec.).

1H NMR CDCl3 9.80 (bs, 1H, 9-NH), 9.10 (s, 1H, 1-H), 8.90 (s, 1H, 4-H), 8.2 (d, 1H, Jп = 8 Hz, 5-H), 7.65 (m, 2H, 7-H and 8-H), 7.35 (m, 1H, 6-H), 4.10 (s, 3H, CO2CH3) ppm.

EIMS 226 (M+, C13H10N2O2, 31.8%), 195 (C12H7N2O, 3.8), 168 (C11H7N2, 100), 140 (20.1).

Microanalysis

C13H10N2O2 Calculated C (69.02) H (4.42) N (12.39)
Found C (69.35) H (4.48) N (12.41).

Alternative Methods for the Oxidation of the Tetrahydro-β-carboline ester (158) to the β-carboline ester (159)

(i) A suspension of tetrahydro-β-carboline ester (158) (200 mg, 0.87 mmol) and powdered sulphur (100 mg) in xylene (10 mL) was heated under reflux. The reaction was monitored by tlc (silica, 30% methanol in chloroform). After 48 h, the reaction mixture was cooled and evaporated to dryness. Flash chromatography (silica, 5% methanol in chloroform) of the crude material gave 40% (79 mg) of the β-carboline ester (159) and 45% of the unchanged starting material (158).

(ii) The tetrahydro-β-carboline ester (158) (200 mg, 0.87 mmol) and 10% palladium on charcoal (80 mg) in cumene (5 mL) was heated under reflux for 48 h. The palladium catalyst was then removed by filtration. After the solvent was removed under reduced pressure, the residue was flash chromatographed (silica, 5% methanol in chloroform) to afford the pure β-carboline ester (159) in 60% yield (118 mg).
(iii) 2,3-Dichloro-5,6-dicyanobenzoquinone (108 mg, 0.48 mmol) was added slowly to a cooled, stirred solution of the tetrahydro-β-carboline ester (158) (100 mg, 0.44 mmol) in THF (10 mL). After 20 min., the reaction mixture was evaporated to dryness. Purification was achieved by flash chromatography of the crude material (silica, 5% methanol in chloroform) to afford the β-carboline ester (159) in 75% yield (81 mg).

9H-Pyridol[3,4-b]indole-3-carboxylic acid (167)

A suspension of the β-carboline methyl ester (159) (100 mg, 0.44 mmol) in 5% aqueous sodium hydroxide solution (2 mL) was heated for 8 h. to give a clear solution. The mixture was then acidified with acetic acid to pH 3. The precipitate formed was collected, washed with methanol and dried under vacuum to give the β-carboline acid (167) in 90% yield (84 mg) after recrystallisation from methanol.

MP >300°C (dec.) (Lit.85 318 - 320°C, dec.).

1H NMR  

\[
d_d{DMSO} \quad 8.95 \text{(s, 1H, 1-H)}, \quad 8.90 \text{(s, 1H, 4-H)}, \quad 8.38 \text{(d, 1H, } J_o = 10 \text{ Hz, 5-H)}, \quad 7.65 \text{(m, 2H, 7-H and 8-H)}, \quad 7.30 \text{(m, 1H, 6-H) ppm.}
\]

EIMS  212 (M+, C_{12}H_8N_2O_2, 40.9%), 168 (C_{11}H_7N_2, 100), 140 (35.6).

Concentrated sulphuric acid (50 µL) was added to a stirred suspension of β-carboline methyl ester (159) (10 mg, 43.5 µmol) in ethanol (or n-butanol) and water (100 µL : 70 µL). The reaction mixture was heated under reflux for 8 h., cooled, diluted with water (500 µL), neutralized to pH ~6 with sodium carbonate and extracted with ethyl acetate (3 x 1 mL). The extracts were combined, dried over sodium sulphate and evaporated to give the ethyl ester (165) in 75% yield (8 mg) [72% yield (8.5 mg) for the n-butyl ester (166)].

9H-Pyridol[3,4-b]indole-3-carboxylic acid, ethyl ester (165)

MP  208 - 211°C (Lit.85 207 - 208°C).

1H NMR  

\[
CDCl_3 \quad 9.60 \text{(bs, 1H, 9-NH)}, \quad 9.05 \text{(s, 1H, 1-H)}, \quad 8.85 \text{(s, 1H, 4-H)}, \quad 8.15 \text{(d, 1H, } J_o = 8 \text{ Hz, 5-H)}, \quad 7.55 \text{(m, 2H, 7-H and 8-H)}, \quad 7.30 \text{(m, 1H, 6-H)}, \quad 4.48 \text{(q, 2H, } J = 8 \text{ Hz, CH}_2\text{CH}_3), \quad 1.4 \text{(t, 3H, } J = 8 \text{ Hz, CH}_2\text{CH}_3) \text{ ppm.}
\]

EIMS  240 (M+, C_{14}H_{12}N_2O_2, 17.2), 168 (C_{11}H_7N_2, 100), 140 (15.4).
9H-Pyrido[3,4-b]indole-3-carboxylic acid, n-butyl ester (166)

**MP**  229 - 232°C.

**1H NMR**  CDCl₃  9.8 (bs, 1H, 9-NH), 9.12 (s, 1H, 1-H), 8.90 (s, 1H, 4-H), 8.22 (d, 1H, Jₒ = 8 Hz, 5-H), 7.65 (m, 2H, 7-H and 8-H), 7.40 (m, 1H, 6-H), 4.50 (t, 2H, J = 8 Hz, CO₂CH₂CH₂), 1.75 (m, 4H, CH₂CH₂CH₃), 1.00 (t, 3H, J = 8 Hz, CH₂CH₃) ppm.

**EIMS**  268 (M⁺, C₁₆H₁₆N₂O₂, 9.1 %), 195 (5.8), 168 (C₁₀H₈N₂, 100), 140 (2.1).

Attempted Hydrolytic Cleavage of 9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (159)

The β-carboline methyl ester (159) (10 mg, 43.5 µmol) was suspended in 3M aqueous sulphuric acid (500 µL) and the reaction mixture was warmed gently with stirring until a homogeneous solution was obtained. The reaction mixture was then heated under reflux for 12 h., cooled, neutralized with sodium carbonate and extracted with ethyl acetate (4 x 2 mL). The extracts were combined, dried over sodium sulphate and evaporated to dryness to afford the β-carboline acid (167) in 45% yield.

Under more acidic conditions (e.g. 9M aqueous sulphuric acid, 12M aqueous hydrochloric acid) and longer reaction times (60 h.), decomposition of the substrate was observed.

Preparation of 6-Hydroxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (174)

A mixture of dl-5-hydroxytryptophan (177) (1.2 g, 5.5 mmol) and 37% aqueous formaldehyde (0.5 mL) in methanol (7 mL) and water (20 mL) containing acetic acid (0.1 mL) was stirred under nitrogen at room temperature overnight. The solids were collected, washed with icewater and dried to give 6-hydroxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid in 80% yield (1.1 g).

6-Hydroxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid

**MP**  300°C (dec.) (Lit.91 305°C, dec.).

**1H NMR**  d₆-DMSO  7.10 (d, 1H, Jₒ = 8.5 Hz, 8-H), 6.80 (d, 1H, Jₚ = 2 Hz, 5-H), 6.59 (dd, 1H, Jₒ = 8.5 Hz, Jₚ = 2 Hz, 7-H), 4.20 ( m, 2H, CH₂N), 3.63 (m, 1H, CHCO₂H), 2.95 (m, 2H, CH₂CH) ppm.
The tetrahydro-β-carboline acid (1 g, 4.3 mmol) was dissolved in saturated methanolic hydrogen chloride solution (15 mL) and the mixture was heated under reflux for 8 h. under nitrogen. The reaction mixture was then cooled, concentrated to 1/3 volume, neutralized with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate (4 x 10 mL). The extracts were combined, dried over sodium sulphate and evaporated to dryness. Purification was achieved by flash chromatography (neutral alumina, 5% methanol in chloroform) to give 84.9% (0.88 g) of 6-hydroxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid methyl ester.

6-Hydroxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid methyl ester (174)

MP 130 - 132°C (Lit.91 130 - 131°C).
1H NMR d6-DMSO 10.35 (bs, 1H, Ar-OH), 8.55 (bs, 1H, 9-NH), 7.04 (d, 1H, J = 8.8 Hz, 8-H), 6.65 (d, 1H, Jm = 2.2 Hz, 5-H), 6.50 (dd, 1H, J0 = 8.8 Hz, Jm = 2.2 Hz, 7-H), 3.90 (m, 2H, CH2N), 3.65 (m, 1H, CHCO2Me), 3.65 (s, 3H, CO2CH3), 2.78 (m, 2H, CH2CH) ppm.
EIMS 246 (M+, C13H14N2O3, 58.6%), 187 (43.3), 159 (100).

The tetrahydro-β-carboline methyl ester (0.5 g, 2.03 mmol) and 10% palladium on charcoal (0.4 g) in cumene (50 mL) and diglyme (50 mL) were heated under reflux for 3 days. The palladium catalyst was removed by filtration and the solvent was evaporated under reduced pressure. Purification by column chromatography (neutral alumina, methanol in chloroform, gradient elution) afforded 35% (0.17 g) of the β-carboline ester (174).

6-Hydroxy-9H-pyrido[3,4-b]indole-3-carboxylic acid methyl ester (174)

MP 254 - 258°C (Lit.91 249 - 253°C).
1H NMR d6-DMSO 8.88 (s, 1H, 1-H), 8.78 (s, 1H, 4-H), 7.62 (d, 1H, Jm = 2.2 Hz, 5-H), 7.49 (d, 1H, J0 = 8.5 Hz, 8-H), 7.10 (dd, 1H, J0 = 8.5 Hz, Jm = 2.2 Hz). 3.89 (s, 3H, CO2CH3) ppm.
EIMS 242 (M+, C13H10N2O3, 50.5%), 184 (C11H7N2O, 100).
Microanalysis C13H10N2O3 Calculated C (64.46) H (4.13) N (11.57)
Found C (64.80) H (4.22) N (11.68).
Attempted Oxidation of 6-hydroxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (174) to the quinoneimine (175)

(i) Manganese dioxide

To a stirred solution of 6-hydroxy-β-carboline (174) (5 mg, 20.7 µmol) in THF (1 mL) was added commercial manganese dioxide (130 mg, 2.07 mmol). After 10 h. stirring, the excess manganese dioxide was filtered off and unchanged starting material (174) was recovered quantitatively.

The above experiment was repeated with 6-hydroxy-β-carboline (174) (5 mg, 20.7 µmol) and freshly prepared manganese dioxide (13 mg, 0.21 mmol) in THF (0.5 mL). After 20 min., the manganese salt was filtered off and the filtrate was concentrated to give a complex mixture.

(ii) Fremy's salt

Fremy's salt (6.1 mg, 22.7 µmol) in pH 6.8 phosphate buffer (0.5 mL) was added to a stirred solution of 6-hydroxy-β-carboline (174) (5 mg, 20.7 µmol) in THF (0.5 mL). After 10 h., the reaction mixture was diluted with saturated aqueous sodium chloride solution (1 mL) and extracted with ethyl acetate (3 x 1 mL). The extracts were dried over sodium sulphate and evaporated to recover quantitatively the unchanged starting material (174).

The above experiment was repeated in acetonitrile (0.5 mL) and pH 6.8 phosphate buffer (0.5 mL) and worked up as previously described. However, on concentration of the extracts, a black-brown tar was obtained.

(iii) Silver (I) oxide

To a stirred solution of 6-hydroxy-β-carboline (174) (5 mg, 20.7 µmol) in THF (0.5 mL) was added commercial silver (I) oxide (23.9 mg, 0.1 mmol). After 15 h., the silver salt was filtered off and the filtrate was concentrated to give only unchanged starting material (174) in quantitative yield.

(iv) Palladium on charcoal

10% palladium on charcoal (5 mg) was added to a stirred solution of 6-hydroxy-β-carboline (174) (5 mg, 20.7 µmol) in acetone (400 µL) and water (200 µL). No reaction was observed after 3 days and the unchanged starting material (174) was recovered quantitatively.
(v) **Nickel peroxide**

Nickel peroxide (5 mg) was added to a stirred solution of 6-hydroxy-β-carboline \((174)\) (5 mg, 20.7 µmol) in THF (0.5 mL) at room temperature. No reaction was observed after 24 h. and the unchanged starting material \((174)\) was recovered in quantitative yield.

(vi) **20% Cerium (IV) on silica**

The 20% cerium (IV) on silica reagent (10 mg) which was prepared according to literature procedure,\(^9\) was added to a stirred solution of 6-hydroxy-β-carboline \((174)\) (5 mg, 20.7 µmol) in acetonitrile (0.5 mL). No reaction was observed after 10 h. and the unchanged starting material \((174)\) was recovered in quantitative yield.

(vii) **Benzeneseleninic anhydride**

A solution of 6-hydroxy-β-carboline \((174)\) (10 mg, 41 µmol) in dry THF (2 mL) was added dropwise to a suspension of benzeneseleninic anhydride (16 mg, 41 µmol) in THF (3 mL) at room temperature. After 5 min., tlc analysis (silica, 50% ethyl acetate in acetone) of the reaction mixture showed it to be a complex mixture.

(viii) **Potassium ferricyanide**

A solution of 6-hydroxy-β-carboline \((174)\) (10 mg, 41.3 µmol) in acetonitrile (3 mL) and 30% aqueous hydrogen peroxide (70 µL, 0.62 mmol) were added to a solution of potassium hexacyanoferrate (III) (1 mg) in water (1 mL). After stirring at room temperature overnight, the reaction mixture was diluted with ethyl acetate (6 mL) and washed with water and then saturated aqueous sodium chloride solution. The reddish-brown coloured organic fraction was dried over sodium sulphate and evaporated to afford a brown tar.

The above experiment was repeated and UV and mass spectra of the reddish-brown organic fraction (in ethyl acetate) were obtained.

**UV**

| MeOH | 500 (br), 280 nm. |

**EIMS**

| 256 (M⁺, C₁₃H₈N₂O₄, 12.8%), 241 (19.6), 228 (12.3), 197 (14.0), 121 (9.1), 135 (7.7), 57 (100), 43 (65.4), 28 (58.6). |
(ix) **Swern Oxidation**

Dimethylsulphoxide (7.1 µL, 91 µmol) was added to a stirred solution of oxalyl chloride (5.8 µL, 45 µmol) in THF (1.0 mL) at -30°C. After 5 min., the 6-hydroxy-β-carboline (174) (10 mg, 44.3 µmol) in dry THF (2 mL) was added and stirring was continued for 2 h. at -10°C. A tlc analysis of the reaction mixture showed the presence of unchanged starting material (174) only.

(x) **Lead dioxide**

Lead tetraacetate (2.5 g) was distributed in centrifuge tubes and rubbed with water (23 mL) until it was turned into a brown oxide. After centrifugation, the residue was washed with water (until the water liquor was neutral to litmus), followed by acetone (4 x 10 mL) and diethyl ether (4 x 10 mL). The residue was then collected, dried in the oven overnight at 100°C and cooled in a dessicator before use.

The freshly prepared lead dioxide (30 mg, 0.21 mmol) was added to a stirred solution of the 6-hydroxy-β-carboline (174) (10 mg, 41.3 µmol) in THF (2 mL). After 20 min., the lead dioxide was filtered off and washed several times with methanol. The organic layers were combined and evaporated to dryness. Flash chromatography (neutral alumina, 5% methanol in chloroform) of the residue afforded the unstable methoxy compound (181) in 15% yield (1.7 mg) as an amorphous solid.

**6-Hydroxy-5-methoxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (181)**

\[ \text{1H NMR } d_6-\text{acetone } 8.90 (s, 1H, 1-H), 8.45 (s, 1H, 4-H), 7.68 (d, 1H, J = 10 Hz, 7-H\*), 6.15 (d, 1H, J= 10 Hz, 8-H\*), 3.92 (s, 3H, CO\text{2CH}_3), 3.35 (s, 3H, O\text{CH}_3) \text{ ppm.} \]

* Interchangeable

EIMS 272 (M+, C\text{14H12N2O4}, 100%), 257 (16.8), 214 (C\text{12H10N2O2}, 84.4).

HRMS C\text{14H12N2O4} requires 272.0797 found 272.0798.

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**Attempted Oxidation of 9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (159) with Fremy's salt**

A solution of Fremy's salt (14.8 mg, 55 µmol) in pH 6.8 phosphate buffer (1.6 mL) was added to a stirred solution of β-carboline methyl ester (159) (5 mg, 22 µmol) in THF (400 µL). After 15 h., no reaction was observed and the unchanged starting material (159) was recovered in quantitative yield.
Oxidation of 9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (159) with Fenton's reagent

Ferrous sulphate (10 mg, 0.44 mmol) in 0.2M aqueous sulphuric acid (0.5 mL), followed by 30% hydrogen peroxide (250 µL, 2.2 mmol) were added to a stirred solution of β-carboline ester (159) (50 mg, 0.22 mmol) in 0.2M aqueous sulphuric acid (1 mL) at room temperature. The reaction mixture was then warmed gently (40°C) using a water-bath. After 7 h., the mixture was cooled, neutralized with cold, saturated aqueous sodium bicarbonate solution and extracted with cold ethyl acetate (3 x 5 mL). The combined extracts were dried over sodium sulphate and evaporated to dryness. Flash chromatography (silica, 5% methanol in chloroform) of the complex mixture afforded 10% of unchanged starting material (159) and 5% (2.7 mg) of a mixture of the 6- and 8-hydroxy-β-carboline, (174) and (182) as an amorphous solid. Attempts to separate the two hydroxylated compounds, (174) and (182), using HPLC were unsuccessful.

8-Hydroxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (182)

1H NMR d6-acetone 8.98 (d, 1H, Jp = 0.7 Hz, 1-H), 8.85 (d, 1H, Jp = 0.7 Hz, 4-H), 7.80 (dd, 1H, Jo = 8 Hz, Jm = 2 Hz, 5-H), 7.14 (t, 1H, J = 8 Hz, 6-H), 7.05 (dd, 1H, Jo = 8 Hz, Jm = 2 Hz, 7-H) ppm.

EIMS 242 (M+, C13H10N2O3, 50.5%), 184 (C11H7N2O, 100).

6-Hydroxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (174)

1H NMR d6-acetone 8.82 (d, 1H, Jp = 0.7 Hz, 1-H), 8.78 (d, 1H, Jp = 0.7 Hz, 4-H), 7.73 (d, 1H, Jm = 2.2 Hz, 5-H), 7.54 (d, 1H, Jo = 8.8 Hz, 6-H), 7.19 (dd, 1H, Jo = 8.8 Hz, Jm = 2.2 Hz, 7-H) ppm.

EIMS 242 (M+, C13H10N2O3, 50.5%), 184 (C11H7N2O, 100).

Reaction of 9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (159) with Udenfriend’s Reagent

Ferrous sulphate (10 mg, 29 µmol), ethylenediaminetetraacetic acid (EDTA) (56 mg, 0.14 mmol) and ascorbic acid (56 mg, 0.31 mmol) in pH 6.8 phosphate buffer (6 mL) were added to a stirred solution of the β-carboline methyl ester (159) (30 mg, 0.13 mmol) in THF (4 mL) at ambient temperature. The reaction mixture was then stirred overnight. However, unchanged starting material was recovered quantitatively.

The experiment was repeated using pH 5.5 phosphate buffer. The reaction
mixture was stirred overnight at 50°C, then cooled and extracted with ethyl acetate (3 x 10 mL). The extracts were combined, dried over sodium sulphate and evaporated to dryness. Flash chromatography (silica, 5% methanol in chloroform) of the crude material provided 46% (17.7 mg) of the tetrahydrofuryl compound (187) and 40% of unchanged starting material (159).

1-Tetrahydrofuryl-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (187)

MP 257 - 259°C.

$^1$H NMR $d_6$-acetone 10.8 (bs, 1H, 9-NH), 8.80 (s, 1H, 4-H), 8.36 (dd, 1H, $J_D = 8$ Hz, $J_M = 1$ Hz, 5-H), 7.74 (dd, 1H, $J_O = 8$ Hz, $J_M = 1$ Hz, 8-H) ppm.

EIMS 296 (M+, C$_{17}$H$_{16}$N$_2$O$_3$, 19.8%), 253 (82.8), 237 (61.6), 225 (44.8), 205 (25.2), 193 (16.5), 180 (30.4), 167 (100), 166 (97.5).

HRMS Microanalysis 
C$_{17}$H$_{16}$N$_2$O$_3$ requires 296.1161 found 296.1159

Attempted preparation of 6-hydroxy-8-methoxy-9H-pyrido[3,4-b]indole-3-carboxylic acid, methyl ester (182)

Freshly prepared lead dioxide (90 mg, 62 mmol) was added with stirring, to a mixture of the 6- and 8-hydroxy-β-carboline, (174) and (182), (30 mg, 12.4 mmol) in THF (6 mL) at room temperature. After 20 min., the excess lead dioxide was filtered off and washed several times with methanol. The filtrate and washings were combined and evaporated to dryness. Flash chromatography (neutral alumina, 5% methanol in chloroform) of the complex mixture gave the 5-methoxy compound (181) in 5% yield (0.5 mg).
Pyridine-2-carboxylic acid N-oxide (191)

Potassium hydroxide (0.56 g, 10 mmol) was added with shaking to pyridine-2-carboxylic acid (190) (1.23 g, 10 mmol) in methanol (7 mL). The reaction mixture was then evaporated to dryness and the residue dissolved in acetic acid (6 mL). 30% aqueous hydrogen peroxide (1.2 mL, 13 mmol) was added and the reaction mixture was warmed at 70-80°C for 3 h. More 30% aqueous hydrogen peroxide (1 mL) was added and the mixture was heated at 70-80°C overnight. The solvent was removed in vacuo and the residue dissolved in 2M aqueous hydrochloric acid (10 mL). The mixture was then evaporated to dryness and the residue was reextracted with methanol. The extracts were dried and evaporated to give the N-oxide (191) in 78% yield (1.1 g) after recrystallisation from methanol.

MP 161 - 163°C. (Lit.156 162°C)

^1H NMR d6-DMSO 8.75 (m, 1H), 8.30 (m, 1H), 7.90 (m, 2H) ppm.

EIMS 139 (M+, C₆H₅N0₃, 24.1%), 123.1 (C₆H₅NO₂, 1.0), 95 (C₅H₅NO, 100), 78 (C₅H₄N, 88.3).

2-Acetoxypyridine (193)

The N-oxide (191) (100 mg, 0.72 mmol) was added portionwise to a stirred solution of acetic anhydride (680 µL, 7.2 mmol) and triethylamine (200 µL, 1.4 mmol) kept at 40°C. After 1 h., the reaction mixture was cooled and filtered through a column of neutral alumina (60% ethyl acetate in hexane). The eluent was then analyzed by GC/MS.

GC/MS 137 (M+, C₇H₇N0₂, 2%), 95 (C₅H₅NO, 100), 67 (C₄H₅N, 45).

For a larger scale preparation, e.g. N-oxide (191) (1 g, 7.2 mmol), the reaction mixture was first diluted with ethyl acetate and washed with 5% aqueous sodium bicarbonate solution then 1M aqueous hydrochloric acid, followed by saturated aqueous sodium chloride solution. Purification was achieved by distillation (Kugelrohr) to provide the 2-acetoxypyridine (193) in 60% yield (0.59 g).

BP 110°C at 10mm Hg.

^1H NMR CDCl₃ 8.40 (dd, 1H, J₀ = 10 Hz, J₉ = 2 Hz, 6-H), 7.30 (m, 1H, 5-H), 7.22 (m, 1H, 4-H), 7.08 (dd, 1H, J₀ = 10 Hz, J₉ = 2 Hz, 3-H) ppm.

EIMS 137 (M+, C₇H₇N0₂, 5%), 95 (C₅H₅NO, 100), 67 (C₄H₅N, 49.5).
2-(1H)-Pyridone (194)

A solution of 2-acetoxypyridine (193) (50 mg, 0.36 mmol) in 5% aqueous potassium carbonate solution (0.5 mL) and methanol (0.5 mL) was stirred at room temperature for 2 h. The reaction mixture was then neutralized with 6M aqueous hydrochloric acid and the aqueous fraction was continuously extracted with ethyl acetate. The extracts were combined, dried over sodium sulphate and concentrated to afford the 2-pyridone (194) in 70% yield (24 mg) after recrystallisation from methanol and ethyl acetate.

MP 102 - 105°C. (Lit. 105 - 107°C)

1H NMR CDCl3 7.50 (m, 1H, 4-H), 7.40 (dd, 1H, J_o = 9 Hz, J_m = 2 Hz, 6-H), 6.60 (dd, 1H, J_o = 9 Hz, J_m = 2 Hz, 3-H), 6.35 (m, 1H, 5-H) ppm.

EIMS 95 (M+, C5H5NO, 100%), 67 (C4H5N, 43.5%).

9H-Pyrido[3,4-b]indole-3-carboxylic acid, methyl ester N-oxides (197)

(i) Potassium hydroxide (0.13 g, 2.4 mmol) was added, with stirring to the β-carboline acid (167) (0.5 g, 2.4 mmol) in methanol (10 mL). The methanol was then evaporated and the residue was dissolved in acetic acid (3 mL). 30% aqueous hydrogen peroxide (2.5 mL) was added and the mixture was warmed at 70° - 80°C. After 3 h., more 30% aqueous hydrogen peroxide (2.5 mL) was added and the mixture was heated at 80°C for 9 h. The mixture was cooled, evaporated to dryness in vacuo, and the residue was dissolved in 6M aqueous hydrochloric acid. The mixture was then evaporated to dryness, redissolved in methanol, filtered and the filtrate was evaporated to give quantitatively a mixture of unchanged starting material (167) and some of the desired N-oxide (10% by 1H NMR).

(ii) p-Nitroperoxybenzoic acid (405 mg, 2.2 mmol) was added slowly to a stirred solution of the β-carboline methyl ester (159) (200 mg, 0.9 mmol) in dichloromethane (10 mL) and saturated aqueous sodium bicarbonate solution (10 mL). After 2 h., the precipitate formed was filtered and collected, washed with water then dried in vacuo. Recrystallization from methanol gave 71% (155 mg) of the N-oxide (197).

MP >300°C (dec.).

1H NMR d6 DMSO 8.60 (s, 1H, 1-H), 8.50 (s, 1H, 4-H), 8.14 (d, 1H, J_o = 8 Hz, 5-H), 7.60 (d, 1H, J_o = 8 Hz, 8-H), 7.42 (m, 1H, 7-H), 7.18 (m, 1H, 6-H) ppm.

EIMS 242 (M+, C13H10N2O3, 1%), 226 (C13H10N2O2, 5.8), 168 (C11H8N2, 100).
Microanalysis

\[ \text{C}_{13}\text{H}_{10}\text{N}_{2}\text{O}_{3} \]

Calculated: C (64.46)  H (4.13)  N (15.70)

Found: C (64.75)  H (4.20)  N (15.81).

9-H-Pyrido[3,4-b]indole-3-carboxylic acid N-oxide (198)

Potassium superoxide (26.4 mg, 0.37 mmol) was added to a stirred mixture of the β-carboline methyl ester (197) (30 mg, 0.12 mmol) and 18-crown-6 (10.9 mg, 41 µmol) in dry benzene (3 mL) under argon. The resulting mixture was stirred overnight, then cautiously poured into water (2 mL) and acidified with 6M aqueous hydrochloric acid to pH 4. The precipitate was collected by filtration and dried to give 85.5% (23.4 mg) of the β-carboline acid (198) after recrystallisation from methanol.

MP >300°C (dec.).

\[ ^1\text{H NMR} \quad \delta_{\text{d}-\text{DMSO}}\]

12.00 (bs, 1H, \( \text{CO}_2\text{H} \)), 9.20 (s, 1H, 1-H), 9.09 (s, 1H, 4-H), 8.48 (d, 1H, \( J_o = 9 \text{ Hz} \), 5-H), 7.15 (m, 2H, 7-H and 8-H), 7.28 (m, 1H, 6-H) ppm.

EIMS 228 (M+, \( \text{C}_{12}\text{H}_{3}\text{N}_{2}\text{O}_{3} \), <1%), 212 (\( \text{C}_{12}\text{H}_{3}\text{N}_{2}\text{O}_{2} \), 5.0), 184 (54.2), 168 (100).

2,9-H Pyrido[3,4-b]indol-3-one (201)

The N-oxide acid (198) (20 mg, 88 µmol) was added slowly to a stirred solution of acetic anhydride (0.2 mL, 1.8 mmol) and triethylamine (30 µL, 0.35 mmol) at room temperature. The reaction mixture was then warmed gently at 40°C. After 3 h., the mixture was cooled, diluted with ethyl acetate (5 mL) and washed with 5% aqueous sodium bicarbonate solution, 1M aqueous hydrochloric acid and saturated aqueous sodium chloride solution. The organic fraction was dried over sodium sulphate and evaporated to dryness to provide a mixture of the acetate (199) and the diacetyl derivative (200).

Without separation, the mixture of (199) and (200) was treated with 5% aqueous potassium carbonate solution (100 µL) in methanol (0.5 mL). After 20 min. stirring at room temperature, the mixture was neutralized with 2M aqueous hydrochloric acid, diluted with saturated aqueous sodium chloride solution (1 mL) and extracted with ethyl acetate (4 x 10 mL). The combined extract was dried over sodium sulphate and evaporated to afford the indolo-α-pyridone (201) in 52.2% (8.5 mg) overall yield after recrystallisation from methanol.
MP >300°C (dec.)

\( ^1\)H NMR CD\textsubscript{3}OD 8.28 (d, 1H, J\textsubscript{O} = 8 Hz, 5-H), 8.25 (s, 1H, 1-H), 7.60 (m, 3H, 4-H, 7-H and 8-H), 7.27 (m, 1H, 6-H) ppm.

EIMS 184 (M\textsuperscript{+}, C\textsubscript{11}H\textsubscript{8}N\textsubscript{2}O, 100%), 168 (11.8), 156 (C\textsubscript{10}H\textsubscript{8}N\textsubscript{2}, 29.1), 129 (31.0).

HRMS C\textsubscript{11}H\textsubscript{8}N\textsubscript{2}O requires 184.0637 found 184.0636

4-(2-Acetyl-3,4-dimethoxyphenyl)-5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-3-methyl-2-pyridinecarboxylic acid (206)

Acetic anhydride (75 µL, 0.79 mmol) was added to a stirred solution of streptonigrin (80) (20 mg, 39.5 µmol) and N,N-dimethylaminopyridine (DMAP) (38.6 mg, 0.32 mmol) in dichloromethane (1 mL) at 0°C. After 0.5 h., the mixture was diluted with ethyl acetate (5 mL) and stirred with saturated aqueous sodium bicarbonate solution (5 mL). The bicarbonate fraction was acidified with 2M aqueous hydrochloric acid to pH 4 and extracted with ethyl acetate (4 x 2 mL). The ethyl acetate extracts were combined then washed with saturated aqueous sodium chloride solution, dried over sodium sulphate and evaporated. Purification using flash chromatography (pH 6.8 buffer-washed silica, 2% methanol in chloroform) gave the acetate (206) in 72% yield (15.6 mg) as an amorphous solid.

IR CHCl\textsubscript{3} 3680, 3600, 3510, 3460, 3400, 3250 (br), 1750 (s), 1685, 1640, 1615, 1590 cm\textsuperscript{-1}.

UV CHCl\textsubscript{3} 490, 378, 297 nm.

\( ^1\)H NMR CDCl\textsubscript{3} 8.68 (d, 1H, J = 8 Hz, 3-H), 8.48 (d, 1H, J = 8 Hz, 4-H), 7.01 (d, 1H, J = 8 Hz, 11'-H), 6.89 (d, 1H, J = 8 Hz, 12'-H), 5.13 (bs, 2H, 7-NH\textsubscript{2}), 4.10, 3.97 and 3.91 (alls, each 3H, 3 x OCH\textsubscript{3}), 2.46 (s, 3H, 3'-CH\textsubscript{3}), 2.05 (s, 3H, OCOCH\textsubscript{3}) ppm.

EIMS 518 (C\textsubscript{26}H\textsubscript{22}N\textsubscript{4}O\textsubscript{8}, 1.4%), 504 (C\textsubscript{26}H\textsubscript{24}N\textsubscript{4}O\textsubscript{7}, 94.7), 489 (16.7), 474 (18.0), 461 (C\textsubscript{24}H\textsubscript{21}N\textsubscript{4}O\textsubscript{6}, 40.6), 447 (91.8), 445 (100), 431 (34.7), 415 (35.5).

CIMS 549 [M\textsuperscript{+}+1, (C\textsubscript{27}H\textsubscript{24}N\textsubscript{4}O\textsubscript{9})+1].

Methyl 5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-2-pyridine carboxylate (144)

Ethereal diazomethane was added dropwise to a cooled (-10° - 0°C), stirred
solution of streptonigrin (80) (20 mg, 39.5 µmol) in dry dichloromethane (2 mL). After 0.5 h., the mixture was allowed to warm up to room temperature and then evaporated to dryness. Flash chromatography of the residue (pH 6.8 buffer-washed silica, 1% methanol in chloroform) afforded the methyl ester (144) in 99% yield (20 mg) as an amorphous solid.

IR CHCl₃  3860, 3510, 3470, 3395, 1710 (s), 1680, 1640, 1620, 1590 cm⁻¹.
UV CHCl₃  490, 376, 294 nm.
MeOH  375 (ε 15000), 246 (ε 40000) nm.
¹H NMR CDCl₃  8.98 (d, 1H, J = 8.5 Hz, 3-H), 8.41 (d, 1H, J = 8.5 Hz, 4-H), 6.76 (d, 1H, J = 8.5 Hz, 12'-H), 6.71 (d, 1H, J = 8.5 Hz, 11'-H), 5.08 (bs, 2H, 7-NH₂), 4.08, 3.98, 3.97 and 3.94 (all s, each 3H, CO₂CH₃ and 3 x OCH₃), 2.32 (s, 3H, 3'-CH₃) ppm.
EIMS  520 (M⁺, C₂₆H₂₄N₄O₃, 100%), 503 (C₂₆H₂₃N₄O₇, 21.0), 487 (28.6), 473 (C₂₅H₂₁N₄O₆, 35.1), 471 (24.9), 459 (C₂₄H₁₉N₄O₆, 38.1), 445 (61.0), 443 (66.3), 431 (C₂₃H₁₉N₄O₅, 61.4), 415 (18.1), 402 (24.0).
HRMS C₂₆H₂₄N₄O₈ requires 520.1594 found 520.1594

Methyl 4-(2-acetyl-3,4-dimethoxyphenyl)-5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-3-methyl-2-pyridine carboxylate (208)

A cooled (-10° - 0°C), stirred solution of the acetate (206) (20 mg, 36 µmol) in dry dichloromethane (2 mL) was treated dropwise with ethereal diazomethane. After stirring for 0.5 h., the excess diazomethane and solvent were removed under reduced pressure. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue gave the ester (208) in 82% yield (16.6 mg) as an amorphous solid.

IR CHCl₃  3680, 3510, 3460, 3400, 1765 (s), 1710 (s), 1680, 1640, 1620, 1590 cm⁻¹.
UV CHCl₃  500 (ε 2700), 378 (ε 32700), 298 (sh, ε 36500) nm.
¹H NMR CDCl₃  8.79 (d, 1H, J = 8.5 Hz, 3-H), 8.42 (d, 1H, J = 8.5 Hz, 4-H), 6.99 (d, 1H, J = 8.5 Hz, 12'-H), 6.91 (d, 1H, J = 8.5 Hz, 11'-H), 5.10 (bs, 2H, 7-NH₂), 4.08, 3.98, 3.96 and 3.90 (all s, each 3H, CO₂CH₃ and 3 x OCH₃), 2.27 (s, 3H, 3'-CH₃), 2.02 (s, 3H, OCOCH₃) ppm.
EIMS  562 (M⁺, C₂₇H₂₆N₄O₉, 100%), 547 (C₂₇H₂₃N₄O₉, 5.5), 532 (C₂₇H₂₄N₄O₈, 32.0), 520 (C₂₆H₂₃N₄O₈, 21.3), 504 (C₂₆H₂₄N₄O₆, 31.5), 487 (30.2), 473 (C₂₆H₂₀N₄O₈, 40.0), 471 (28.5), 459 (C₂₆H₂₁N₄O₇, 30.0), 445 (43.8), 443 (54.5), 431 (38.1), 415 (21.2), 402 (20.0).
HRMS  C₂₈H₂₆N₄O₉  requires 562.1700  found 562.1700

Attempted Preparation of Streptonigrin N-Oxide (205)

\( p \)-Nitroperoxybenzoic acid (1.1 mg, 5.4 \( \mu \)mol) was added to a cooled (0°C), stirred solution of acetate (208) (3 mg, 5.4 \( \mu \)mol) in THF (250 \( \mu \)L) and saturated aqueous sodium bicarbonate solution (250 \( \mu \)L). The starting material was recovered unchanged after 3 h..

The above experiment was repeated with excess \( p \)-nitroperoxybenzoic acid (3.3 mg, 16.2 \( \mu \)mol). A complex mixture was observed from tlc (non-fluorescent silica, 5% methanol in chloroform) and the reaction mixture was not worked up for further investigation.

Acetic anhydride (0.13 mL, 1.42 mmol) was added to a stirred solution of the acetate (208) (8 mg, 14.2 \( \mu \)mol) in ethyl acetate (2 mL). The mixture was then heated under reflux. After 48 h., the reaction mixture was cooled, diluted with ethyl acetate (5 mL), washed with saturated aqueous sodium bicarbonate solution (3 \( \times \) 1 mL) then saturated aqueous sodium chloride solution (1 mL). The organic fraction was dried over sodium sulphate and evaporated to dryness. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) afforded the diacetyl derivative (209) in 83.1% yield (7 mg) and a trace amount of the triacetyl derivative (210) as amorphous solids.

Methyl-5-acetamido-4-(2-acetyl-3,4-dimethoxyphenyl)-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-3-methyl-2-pyridine carboxylate (209)

IR  CHCl₃  3680, 3615, 3395, 1765 (s), 1730 (s), 1685, 1640, 1615, 1590 cm⁻¹.
UV  CHCl₃  500, 328, 295 (sh)
\(^1\)H NMR  CDCl₃  8.75 (d, 1H, \( J = 8.5 \) Hz, 3-H), 8.49 (d, 1H, \( J = 8.5 \) Hz, 4-H), 7.10 (d, 1H, \( J = 8.5 \) Hz, 11'-H), 6.96 (d, 1H, \( J = 8.5 \) Hz, 12'-H), 5.17 (bs, 2H, 7-NH₂), 4.10, 4.00, 3.94 and 3.88 (all s, each 3H, CO₂CH₃ and 3 x OCH₃), 2.30 (s, 3H, 3'-CH₃), 1.98 and 1.89 (both s, each 3H, OCCOCH₃ and NCOCH₃) ppm.
EIMS  604 (M⁺, C₃₀H₂₈N₄O₁₀, 8.2%), 562 (C₂₈H₂₆N₄O₉, 100), 547 (C₂₇H₂₃N₄O₉, 12.5), 532 (C₂₇H₂₄N₄O₈, 30.0), 520 (10.3), 504 (C₂₆H₂₄N₄O₆, 50.1), 487 (35.0), 473 (42.5), 459 (20.0), 445 (45.5), 415 (18.4), 402 (12.3).
Methyl 5-diacetamido-4-(2-acetyl-3,4-dimethoxyphenyl)-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-3-methyl-2-pyridine carboxylate (212)

The diacetyl derivative (209) (3.5 mg, 5.8 µmol) in dry DMF (200 µL) was added to potassium hydride (0.7 mg, 14.5 µmol) in dry DMF (200 µL) with stirring at room temperature. After 10 min., freshly distilled acetyl chloride (1 µL, 14.5 µmol) was then added to the dark green solution at -20°C. The mixture was stirred for 20 min., diluted with dichloromethane (1 mL) and allowed to warm up to room temperature. The reaction mixture was then quenched with pH 6.8 phosphate buffer solution and washed with copious amount of water. The organic fraction was then dried over sodium sulphate and concentrated. Purification was achieved by flash chromatography (pH 6.8 buffer-washed silica, chloroform) to give 53.4% (2.0 mg) of the triacetyl derivative (212) and 14% of the starting material (209).

IR CHCl₃ 3685, 3615, 3515, 3400, 1765 (s), 1735, 1685, 1640, 1615, 1590 cm⁻¹.
UV CHCl₃ 490 (br), 315, 295 (sh) nm.
¹H NMR CDCl₃ 8.60 (d, 1H, J = 8.5 Hz, 3'-H), 8.48 (d, 1H, J = 8.5 Hz, 4'-H), 6.90 (d, 1H, J = 8.5 Hz, 11'-H), 6.78 (d, 1H, J = 8.5 Hz, 12'-H), 5.18 (bs, 2H, 7-NH₂), 4.09, 4.06, 3.95 and 3.88 (alls, each 3H, CO₂CH₃ and 3 x OCH₃), 2.30, 2.27, 2.24 and 2.07 (alls, each 3H, 3'-CH₃, OCOCH₃ and 2 x NCOCH₃) ppm.
EIMS 646 (M⁺, C₃₂H₃₀N₄O₁₁, 8.3%), 630 (10.1), 604 (C₃₀H₂₃N₄O₁₀, 18.4), 562 (C₂₈H₂₆N₄O₉, 37.5), 547 (9.8), 532 (10.0), 504 (26.3), 503 (26.5), 487 (21.0), 473 (25.7), 457 (12.0), 445 (25.5), 415 (10.1), 402 (5.5).
HRMS C₃₂H₃₀N₄O₁₁ requires 646.1911 found 646.1911

Attempted Preparations of Streptonigrin Triacetyl Derivative (213)

(i) Freshly distilled acetyl chloride (5 µL, 73 µmol) was added to a cooled (0°C), stirred solution of streptonigrin (80) (3.7 mg, 7.3 µmol) and pyridine (5.5 µL, 73 µmol) in dry dichloromethane (0.2 mL). After 5 min., a complex mixture was observed on tlc (non-fluorescent silica, 5% methanol in chloroform).

(ii) A mixed anhydride, prepared in situ by treating potassium acetate (0.7 mg,
7.5 µmol) in acetone with methyl chloroformate (0.7 µL, 7.5 µmol) and a catalytic amount of N-methyl morpholine at -10°C, was added to a solution of the diacetyl derivative (209) (3 mg, 3 µmol) in acetone at 5°C. The reaction was kept at 5°C for 20 min. and then allowed to warm up to room temperature. After 3 h., tlc analysis (non-fluorescent silica, 5% methanol in chloroform) of the reaction mixture showed only the presence of starting material.

Preparation of Anhydrous Hydrogen Peroxide

30% aqueous hydrogen peroxide (100 mL) was mixed with p-cymene (200 mL) in a round bottom flask and the mixture was distilled under vacuum (10 mm Hg) at 70°C. The concentrated hydrogen peroxide (approximately 80% as determined by standard potassium iodide titration147) was dissolved in ether and dried once over sodium sulphate and twice over anhydrous calcium sulphate to give anhydrous hydrogen peroxide, the concentration of which was also determined using standard potassium iodide titration.147

2-Pyridinylcarbonyl-1H-imidazole (215)

N,N'-Carbonyldiimidazole (725 mg, 4.5 mmol) in dry THF (15 mL) was added to a cooled, stirred solution of pyridine-2-carboxylic acid (500 mg, 4.1 mmol) in dry THF (10 mL). After 1 h., the reaction mixture was evaporated to dryness. Purification was achieved by reverse-phase MPLC (acetonitrile) to give the unstable imidazolide (215) in 90% yield (640 mg) as an oil.

$^1$H NMR  CDCl$_3$  8.95 (bs, 1H, NCHN), 8.78 (d, 1H, $J_o = 8$ Hz, 6-H), 8.25 (d, 1H, $J_o = 8$ Hz, 3-H), 7.99 (m, 1H, 4-H), 7.98 (bs, 1H, NCH), 7.60 (m, 1H, 5-H), 7.14 (bs, 1H, NCH) ppm.

EIMS  173 (M$^+$, C$_9$H$_7$N$_3$O, 15.9%), 106 (C$_6$H$_4$NO, 46.6), 78 (C$_5$H$_4$N, 100), 68 (C$_2$H$_3$N$_2$, 46.0), 51 (44.4).

HRMS  C$_9$H$_7$N$_3$O requires 173.0589 found 173.0610

Microanalysis  
C$_9$H$_7$N$_3$O  Calculated C (64.42) H (4.07)  
Found C (64.85) H (4.40).
Pyridine-2-carboxylic acid, N-oxide (191)

The imidazolide (215) (150 mg, 0.87 mmol) in THF (3 mL) was added to a cooled (0°C), stirred solution of 60% aqueous hydrogen peroxide (1 mL, 16.2 mmol). After 2 h., the mixture was concentrated to 1/3 volume and partitioned between dichloromethane (5 mL) and 5% aqueous sodium bicarbonate solution (5 mL). The bicarbonate soluble fraction was acidified with 2M aqueous hydrochloric acid to pH 3 and extracted several times with ethyl acetate. The extracts were combined, dried over sodium sulphate and evaporated to afford the N-oxide (191) in 84% yield (101.6 mg) after recrystallisation from methanol and ethyl acetate. With 30% aqueous hydrogen peroxide, the yield of the N-oxide (191) was 70.8% (85.6 mg). The typical data of the N-oxide (191) has previously been reported (vide supra).

2-(4-(2-Acetyl-3,4-dimethoxyphenyl)-5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)--3-methyl-2-pyridinylcarbonyl]-1H-imidazole (217)

To a cooled, stirred solution of the streptonigrin-0-acetate (206) (8 mg, 15 µmol) in dry dichloromethane (0.5 mL) was added N,N'-carbonyldiimidazole (9.2 mg, 73 µmol) in dry dichloromethane (1 mL). After 1.5 h., the mixture was evaporated to dryness. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue gave the streptonigrin imidazolide (217) in 79.3% yield (7.1 mg) as an amorphous solid.

UV 490, 375, 332, 308 nm.

\[ ^1 \text{H NMR} \text{ CDCl}_3 8.68 (d, 1H, J = 8.5 \text{ Hz}, 3-H), 8.39 (d, 1H, J = 8.5 \text{ Hz}, 4-H), 8.37 (bs, 1H, NCHN of imidazole), 7.75 (bs, 1H, NCH of imidazole), 7.16 (bs, 1H, NCH of imidazole), 7.02 (d, 1H, J = 8.5 \text{ Hz}, 11'-H), 6.94 (d, 1H, J = 8.5 \text{ Hz}, 12'-H), 5.10 (bs, 2H, 7-NH), 4.09, 3.97 and 3.92 (alls, each 3H, 3 x OCH3), 2.26 (s, 3H, 3'-CH3), 2.08 (s, 3H, 8'-OCOCH3) ppm.

EIMS 598 (M+, C_{30}H_{26}N_6O_8, 1.5%), 531 (C_{27}H_{23}N_4O_8, 71.3), 503 (C_{26}H_{23}N_4O_7, 24.8), 488 (9.0), 473 (C_{25}H_{21}N_4O_6, 7.8), 461 (C_{24}H_{21}N_4O_6, 31.8), 431 (17.2), 68 (C_{3}H_{4}N_2, 100).

HRMS C_{30}H_{26}N_6O_8 requires 598.1812 found 598.1811

The imidazolide (217), generated in situ as previously described, from N,N'-carbonyldiimidazole (4 mg, 21 µmol) and acetate (206) (2.3 mg, 4.2 µmol) in dichloromethane, was added to a cooled (0°C) solution of 60% aqueous hydrogen peroxide. The reaction mixture was stirred vigorously for 1 h. and then partitioned between
dichloromethane and 5% aqueous sodium bicarbonate solution. The bicarbonate soluble fraction was acidified to pH 3 with 2M aqueous hydrochloric acid and extracted with ethyl acetate. The extracts were dried over sodium sulphate and evaporated to dryness. The residue was redissolved in dichloromethane and excess ethereal diazomethane was added to the stirred solution at 0°C. After 20 min., the reaction mixture was evaporated to dryness. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue provided N-monoacetylated streptonigrin methyl ester (218) and streptonigrin acetate methyl ester (208) in 41% (1.0 mg) and 30% (0.7 mg) yields respectively over the two steps.

Methyl 5-acetamido-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-(2-hydroxy-3,4-dimethoxy)-3-methyl-2-pyridine carboxylate (218)

IR CHCl₃ 3680, 3520, 3400, 3022, 1730, 1685, 1645, 1615, 1590 cm⁻¹.
UV CHCl₃ 500 (ε 1500), 332 (ε 18500), 276 (sh, ε 25900) nm.
¹H NMR CDCl₃ 8.84 (d, 1H, J = 8.5 Hz, 3-H), 8.49 (d, 1H, J = 8.5 Hz, 4-H), 6.76 (d, 1H, J = 8.5 Hz, 12'-H), 6.57 (d, 1H, J = 8.5 Hz, 11'-H), 6.18 (s, 1H, 5'-NH), 5.16 (bs, 2H, 7-NH₂), 4.12, 4.01, 3.96 and 3.92 (all s, each 3H, 3 x OCH₃ and CO₂CH₃), 2.32 (s, 3H, 3'-CH₃), 1.95 (s, 3H, 5'-HNCOCH₃) ppm.
EIMS 562 (M⁺, C₂₈H₂₆N₄O₉, 64.0%), 547 (3.5), 532 (8.4), 520 (3.3), 503 (46.4), 487 (12.5), 473 (21.6), 459 (6.9), 445 (26.7), 431 (5.8), 28 (100).
HRMS C₂₈H₂₆N₄O₉ requires 562.1700 found 562.1700

The spectroscopic data for methyl 5-diacetamido-4-(2-acetyl-3,4-dimethoxyphenyl)-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-3-methyl-2-pyridine carboxylate (208) has been discussed previously.

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Reaction of Streptonigrin O-acetate (206) with Hydrochloric Acid

6M aqueous hydrochloric acid (50 µL) was added to a solution of the streptonigrin acetate (206) (3 mg, 5.5 µmol) in dichloromethane (0.5 mL) at room temperature. The mixture was stirred vigorously overnight, then diluted with dichloromethane (2 mL), washed with water (1 mL), dried over sodium sulphate and concentrated. The residue was redissolved in dichloromethane (0.5 mL) and treated with diazomethane. After 20 min., the mixture was evaporated to dryness. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue afforded 58.4% (1.8 mg) of the N-acetyl derivative methyl ester (218) and 19.7% (0.6 mg) of the O-acetate methyl ester (208) over two steps. Both
compounds display identical spectroscopic features and chromatographic behaviour to their corresponding samples prepared by an alternative route as described previously.

Preparation of Pre-Reduced Platinum Catalyst

Platinum dioxide hydrate (30 mg) was stirred in water (1 mL) under an atmosphere of hydrogen until completely reduced (i.e. until uptake of hydrogen had ceased). The water was then evaporated in vacuo and the catalyst was stored in a dessicator until required for use.

Deuterio-pyridine-2-carboxylic acid

Pyridine-2-carboxylic acid (30 mg, 0.22 mmol) and the pre-reduced platinum catalyst (5 mg) in D₂O (1 mL) were heated in a sealed tube at 100°C for 3 - 6 days. The catalyst was filtered off and the filtrate was concentrated. The residue was then dissolved in methanol and evaporated to dryness (repeated several times) to give, in quantitative yield, a mixture of deuterio-pyridine-2-carboxylic acids.

Reaction time : 3 days

% D₀ = 31.7%  % D₁ = 39.1%  % D₂ = 16.5%
% D₃ = 9.5%  % D₄ = 3.2%

Reaction time : 6 days

% D₀ = 1.2%  % D₁ = 6.9%  % D₂ = 29.6%
% D₃ = 56.6%  % D₄ = 5.7%

The above values were calculated from the intensity of peaks in the EIMS as a percentage of the total yield obtained.

Reaction of 2-pyridine-imidazolide (215) with Hydrogen Peroxide in the Presence of Deuterio-2-pyridine-2-Carboxylic Acid

N,N'-carbonyldiimidazole (26 mg, 0.16 mmol) in dry THF (1 mL) was added to a cooled, stirred solution of pyridine-2-carboxylic acid (20 mg, 0.16 mmol) in dry THF (1 mL). After 1 h., half of the reaction mixture (1 mL) was transferred to an excess of 30% aqueous hydrogen peroxide (0.5 mL) at 0°C. This mixture was stirred for 1 h., then diluted with dichloromethane and extracted with 5% aqueous sodium bicarbonate solution.
The bicarbonate soluble fraction was acidified to pH 3 with 6M aqueous hydrochloric acid and continuously extracted with ethyl acetate. The combined extract was dried over sodium sulphate and evaporated to give 36% yield (8 mg) of the N-oxide (191).

The other half of the above reaction mixture containing the imidazolide (215) was also added to an excess of aqueous 30% hydrogen peroxide (0.5 mL) at 0°C. After 5 min., deuterio-pyridine-2-carboxylic acid (10 mg) was added and the resulting mixture was stirred for another h.. The reaction mixture was then worked up as previously described to give a mixture of the deuterio- and unlabelled pyridine-2-carboxylic acids, (190a) and (191), and the deuterio- and unlabelled pyridine-2-carboxylic acid N-oxides, (191a) and (191), in 35.5% total yield.

**N-oxides**

<table>
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<th>% D0</th>
<th>% D1</th>
<th>% D2</th>
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<td>52.5%</td>
<td>8.8%</td>
<td>12.3%</td>
</tr>
<tr>
<td>21.1%</td>
<td>5.3%</td>
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</table>

The above values were calculated from the intensity of peaks in the EIMS. The ratio between the deuterio- and unlabelled N-oxide is approximately 1:1.

**Pyridine-2-carboxylic acid, methyl ester (225)**

A solution of pyridine-2-carboxylic acid (190) (0.5 g, 4.1 mmol) in methanol (3.2 mL) and concentrated sulphuric acid (0.5 mL) was heated under reflux for 6 h.. The reaction mixture was then cooled, neutralized with 25% aqueous sodium carbonate solution and extracted with dichloromethane (4 x 10 mL). The extracts were combined, dried over sodium sulphate and concentrated to an oil. Purification was achieved by flash chromatography (silica, 10% methanol in chloroform) to afford the methyl ester (225) as an oil in 75% yield (0.42 g).

**1H NMR**  CDCl3  8.77 (dd, 1H, Jθ = 8 Hz, Jm = 2 Hz, 6-H), 8.15 (dd, 1H, Jθ = 8 Hz, Jm = 2 Hz, 3-H), 7.79 (m, 1H, 4-H), 7.50 (m, 1H, 5-H), 4.00 (s, 3H, CO2CH3) ppm.

**EIMS**  137 (M+, C7H7NO2, 48.9%), 78 (C5H7N, 100).

**2-Methoxycarbonyl-1-methyl-pyridinium methosulphate (226)**

Dimethyl sulphate (42 µL, 0.44 mmol) was added to a stirred solution of the
methyl ester (225) (50 mg, 0.37 mmol) in THF (1 mL) and the mixture was heated under reflux for 6 h. The reaction mixture was then cooled and the solvent was evaporated in vacuo to give a quantitative yield of the methosulphate salt (226) as an oil.

\[
\begin{align*}
\text{1H NMR} & \quad \text{CDCl}_3 \quad 9.31 \text{ (dd, } J_\theta = 8 \text{ Hz, } J_m = 2 \text{ Hz, 6-H)}, \ 8.78 \text{ (m, 1H, 4-H)}, \ 8.60 \text{ (dd, } 1H, J_\theta = 8 \text{ Hz, } J_m = 2 \text{ Hz, 5-H)}, \ 8.28 \text{ (m, 1H, 3-H)}, \ 4.67 \text{ (s, 3H, NCH}_3\text{)}, \ 4.09 \text{ (s, 3H, CO}_2\text{CH}_3\text{)}, \ 3.57 \text{ (s, 3H, CH}_3\text{SO}_4\text{)} \text{ ppm.}
\end{align*}
\]

N-Methyl-2-(1H)-pyridone (224)

The methosulphate salt (226) (50 mg, 0.19 mmol) was added slowly to a cooled, stirred solution of potassium ferricyanide (125 mg, 0.38 mmol) and sodium hydroxide (22.8 mg, 0.57 mmol) in water (1 mL). The mixture was then warmed gently at 40°C using a water bath. After 3 h, the mixture was evaporated to dryness and extracted into methanol. The extract was then evaporated to afford the 2-pyridone (224) in 65% yield (12 mg) after recrystallisation from methanol and ethyl acetate.

\[
\begin{align*}
\text{MP} & \quad 103 - 1070\text{C} \quad (\text{Lit.}156 105 - 1070\text{C}).
\end{align*}
\]

\[
\begin{align*}
\text{1H NMR} & \quad \text{CDCl}_3 \quad 7.50 \text{ (m, 1H, 4-H)}, \ 7.40 \text{ (dd, } 1H, J_\theta = 9 \text{ Hz, } J_m = 2 \text{ Hz, 6-H)}, \ 6.60 \text{ (dd, 1H, } J_\theta = 9 \text{ Hz, } J_m = 2 \text{ Hz, 3-H)}, \ 6.35 \text{ (m, 1H, 5-H)} \text{ ppm.}
\end{align*}
\]

\[
\begin{align*}
\text{EIMS} & \quad 95 \text{ (M}^+, \text{ C}_5\text{H}_5\text{NO, 100%),} \ 67 \text{ (C}_4\text{H}_5\text{N, 45).}
\end{align*}
\]

Preparation of 4-phenylpyridine-2-carboxylic acid methyl ester (227)

\[
p\text{-Nitroperoxybenzoic acid (3.5 g, 19 mmol) was added slowly to a stirred solution of 4-phenylpyridine (2 g, 12.9 mmol) in dichloromethane (10 mL) and saturated aqueous sodium bicarbonate solution (10 mL). After 5 h. stirring, the organic fraction was separated from the aqueous fraction and washed with saturated aqueous sodium chloride solution, dried over sodium sulphate and evaporated to give 4-phenylpyridine N-oxide in 90% yield (1.98 mg) after recrystallisation from ethyl acetate and methanol.}
\]

4-Phenylpyridine N-oxide

\[
\begin{align*}
\text{MP} & \quad 150 - 1530\text{C} \quad (\text{Lit.}127 153-1550\text{C}).
\end{align*}
\]

\[
\begin{align*}
\text{1H NMR} & \quad \text{CDCl}_3 \quad 8.25 \text{ (m, 2H),} \ 7.50 \text{ (m, 7H)} \text{ ppm.}
\end{align*}
\]

A mixture of 4-phenylpyridine N-oxide (1g, 5.8 mmol) and dimethyl sulphate
(0.5 mL, 5.8 mmol) was heated at 70°C for 6 h. The mixture was cooled and crushed ice was added. A solution of potassium cyanide (456 mg, 7.0 mmol) in water (2.5 mL) was slowly added into the iced mixture at 0°C - 10°C. After standing overnight at 0°C, the resulting precipitate was filtered and collected to provide 4-phenylpyridine-2-carbonitrile in 37.8% yield (0.43 g).

4-Phenylpyridine-2-carbonitrile

MP 97 - 100°C (Lit.127 100-102°C).

$^1$H NMR CDCl$_3$ 8.75 (dd, 1H, $J_o$ = 8 Hz, 6-H), 7.93 (d, 1H, $J_m$ = 2 Hz, 3-H), 7.72 (dd, 1H, $J_o$ = 8 Hz, $J_m$ = 2 Hz, 5-H), 7.50 (m, 5H, Ar.-H) ppm.

4-Phenyl-2-pyridinecarbonitrile (240 mg, 1.37 mmol) was added to a solution of 3M aqueous sodium hydroxide (4 mL) and the mixture was heated under reflux for 15 h. The reaction mixture was then cooled, acidified to pH 3 with 2M aqueous hydrochloric acid and evaporated to dryness. The residue was extracted with methanol and the extract was evaporated to provide 80% yield (236 mg) of 4-phenylpyridine-2-carboxylic acid after recrystallisation from ethyl acetate and methanol.

4-Phenylpyridine-2-carboxylic acid

MP 152 - 154°C (Lit.127 155-157°C)

$^1$H NMR CD$_3$OD 8.70 (d, 1H, $J_o$ = 6 Hz, 6-H), 8.38 (d, 1H, $J_m$ = 1.8 Hz, 3H), 7.90 (dd, 1H, $J_o$ = 6 Hz, $J_m$ = 1.8 Hz, 5-H), 7.82 (m, 2H, Ar.-H), 7.55 (m, 3H, Ar.-H) ppm.

EI/MS 199 (M+, C$_{12}$H$_9$NO$_2$, 47%), 155 (C$_{11}$H$_9$N, 100).

Microanalysis

C$_{12}$H$_9$NO$_2$ Calculated C (72.36) H (4.52) N (7.04)

Found C (72.40) H (4.58) N (7.10).

4-Phenylpyridine-2-carboxylic acid, methyl ester (227)

A solution of 4-phenylpyridine-2-carboxylic acid (200 mg, 1.0 mmol) in methanol (6 mL) and concentrated sulphuric acid (0.5 mL) was heated under reflux for 6 h. The mixture was cooled, neutralized with 25% aqueous sodium carbonate solution and extracted with ethyl acetate (4 x 10 mL). The extracts were combined, dried over sodium sulphate and evaporated to afford the methyl ester (227) in 80.4% (184 mg) yield as an oil after purification by flash chromatography (silica, 5% methanol in chloroform).
\( ^1H \text{NMR} \quad \text{CDCl}_3 \quad 8.78 \text{ (d, } 1H, J_o = 6 \text{ Hz, } 6-H), 8.38 \text{ (d, } 1H, J = 1.5 \text{ Hz, } 3-H), 7.70 \text{ (m, } 3H, 5-H \text{ and Ar.-H), } 7.50 \text{ (m, } 3H, \text{ Ar.-H), } 4.00 \text{ (s, } 3H, \text{ CO}_2\text{CH}_3) \text{ ppm.} \\
\text{CD}_3\text{OD} \quad 8.70 \text{ (d, } 1H, J_o = 6 \text{ Hz, } 6-H), 8.39 \text{ (d, } 1H, J_m = 1.5 \text{ Hz, } 3-H), 7.90 \text{ (dd, } 1H, J_o = 6 \text{ Hz, } J_m = 1.5 \text{ Hz, } 5-H), 7.78 \text{ (m, } 2H, \text{ Ar.-H), } 7.53 \text{ (m, } 3H, \text{ Ar.-H), } 4.00 \text{ (s, } 3H, \text{ CO}_2\text{CH}_3) \text{ ppm.} \\
\text{CD}_3\text{OD} \quad 8.70 \text{ (d, } 1H, J_o = 6 \text{ Hz, } 6-H), 8.39 \text{ (d, } 1H, J_m = 1.5 \text{ Hz, } 3-H), 7.90 \text{ (dd, } 1H, J_o = 6 \text{ Hz, } J_m = 1.5 \text{ Hz, } 5-H), 7.78 \text{ (m, } 2H, \text{ Ar.-H), } 7.53 \text{ (m, } 3H, \text{ Ar.-H), } 4.00 \text{ (s, } 3H, \text{ CO}_2\text{CH}_3) \text{ ppm.} \\
\text{2-Methoxycarbonyl-1-methyl-4-phenylpyridinium methosulphate (228)} \\
4-Phenylpyridine-2-carboxylic acid methyl ester (227) (200 mg, 0.9 mmol) and dimethyl sulphate (0.1 mL, 1.1 mmol) in THF (1 mL) were heated under reflux for 6 h.

The resulting precipitate was collected by filtration and recrystallized from methanol and ethyl acetate to provide the methosulphate salt (227) in 75% yield (240 mg).

\( ^1H \text{NMR} \quad \text{CD}_3\text{OD} \quad 9.09 \text{ (d, } 1H, J_o = 6.6 \text{ Hz, } 6-H), 8.85 \text{ (d, } 1H, J_m = 1.5 \text{ Hz, } 3-H), 8.55 \text{ (dd, } 1H, J_o = 6.6 \text{ Hz, } J_m = 1.5 \text{ Hz, } 5-H), 8.08 \text{ (m, } 2H, \text{ Ar.-H), } 7.70 \text{ (m, } 3H, \text{ Ar.-H), } 4.95 \text{ (s, } 3H, \text{ NCH}_3), 4.58 \text{ (s, } 3H, \text{ CH}_3\text{SO}_4), 4.15 \text{ (s, } 3H, \text{ CO}_2\text{CH}_3) \text{ ppm.} \\
\text{Microanalysis} \\
\text{C}_{15}\text{H}_{17}\text{N}_6\text{O}_6\text{S} \quad \text{Calculated C (53.09) H (5.01) N (4.13)} \\
\text{Found C (53.33) H (5.29) N (4.22).} \\
\text{N-Methyl-4-phenyl-2-(1H)-pyridone (229)} \\
The methosulphate salt (227) (150 mg, 0.44 mmol) was added to a cooled solution of potassium ferricyanide (290 mg, 0.88 mmol) and sodium hydroxide (53 mg, 1.32 mmol) in water (10 mL). The mixture was warmed at 40°C in a water bath. After 6 h., the reaction mixture was cooled and evaporated to dryness. The residue was extracted with methanol and the extract was concentrated. The crude solid was recrystallized from methanol and ethyl acetate to give the N-methyl-pyridone (229) in 71% yield (57.8 mg).

\( ^1H \text{NMR} \quad \text{CDCl}_3 \quad 7.58 \text{ (m, } 2H, \text{ Ar.-H), } 7.45 \text{ (m, } 3H, \text{ Ar.-H), } 7.37 \text{ (d, } 1H, J_o = 8 \text{ Hz, } 6-H), 6.80 \text{ (d, } 1H, J_m = 2 \text{ Hz, } 3-H), 6.45 \text{ (dd, } J_o = 8 \text{ Hz, } J_m = 2 \text{ Hz, } 5-H), 3.55 \text{ (s, } 3H, \text{ NCH}_3) \text{ ppm.} \\
\text{EIMS} \quad 185 \text{ (M+, } \text{C}_{12}\text{H}_{11}\text{NO, } 87.5\%), 155 \text{ (C}_{11}\text{H}_9\text{N, } 100).
9H-Pyrido [3,4-b]indole-3-carboxylic acid, methyl ester, methosulphate (231)

The β-carboline ester (159) (25 mg, 0.11 mmol) and dimethyl sulphate (12.6 µL, 0.13 mmol) in THF (1 mL) were heated under reflux for 8 h. The precipitate formed was collected by filtration, washed with a small amount of cold THF and dried in vacuo to give the methosulphate salt (231) in 77% yield (30 mg) after recrystallisation from methanol and ethyl acetate.

**MP** 221 - 224°C (dec.).

**1H NMR**

CD3OD 9.41 (s, 1H, 1-H), 9.38 (s, 1H, 4-H), 8.54 (d, 1H, J = 10 Hz, 5-H), 7.90 (m, 2H, 7-H and 8-H), 7.58 (m, 1H, 6-H), 4.75 (s, 3H, NCH3), 4.16 (s, 3H, CO2CH3), 3.70 (s, 3H, CH3SO4) ppm.

**Microanalysis**


9H-Pyrido [3,4-b]indole-3-carboxylic acid, methosulphate salt (232)

The methosulphate salt (231) (10 mg, 28 µmol) was added to a stirred solution of potassium ferricyanide (18.7 mg, 56 µmol) and sodium hydroxide (3 mg, 84 µmol) in water (0.5 mL). The mixture was warmed at 40°C using a water bath. After 8 h, the reaction mixture was cooled, acidified to pH 5 with 2M aqueous hydrochloric acid and concentrated. The residue was extracted with methanol and the extract was evaporated to dryness to afford the β-carboline methosulphate salt (232) in 78% yield (7.4 mg).

**MP** >300°C (dec.).

**1H NMR**

CD3OD 8.75 (s, 1H, 1-H), 8.65 (s, 1H, 4-H), 8.20 (d, 1H, J = 8 Hz, 5-H), 7.65 (m, 2H, 7-H and 8-H), 7.37 (m, 1H, 6-H), 4.60 (s, 3H, NCH3), 3.74 (s, 3H, CH3SO4) ppm.

**Methyl 6-(7-amino-5,6-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-5-N-methylamino-2-pyridine carboxylate (234)**

Dimethyl sulphate (1.8 µL, 19 µmol) was added to a stirred solution of streptonigrin methyl ester (144) (2 mg, 3.8 µmol) in acetonitrile (1 mL) and the mixture was heated under reflux overnight. The reaction mixture was then cooled and evaporated to dryness. The resulting complex mixture was subjected to flash chromatography (pH 6.8
buffer-washed silica, chloroform) to isolate the monomethylated derivative (234) in 46.3% yield (1 mg) as an amorphous solid.

IR  CHCl₃  3680, 3600, 3510, 3400, 3250, 1710, 1680, 1640, 1620, 1590 cm⁻¹.
UV  CHCl₃  450 (br), 306 nm.
¹H NMR  CDCl₃  9.02 (d, 1H, J = 8.5 Hz, 3-H), 8.41 (d, 1H, J = 8.5 Hz, 4-H), 6.78 (d, 1H, J = 8.5 Hz, 12'-H), 6.56 (d, 1H, J = 8.5 Hz, 11'-H), 5.10 (bs, 2H, 7-NH₂), 4.09, 3.97, 3.96 and 3.94 (all s, each 3H, CO₂CH₃ and 3 x OCH₃), 2.45 (bs, 3H, 5'-NCH₃), 2.28 (s, 3H, 3'-CH₃) ppm.
EIMS  534 (M⁺, C₂₇H₂₆N₄O₃, 100%), 519 (21.0), 504 (26.5), 487 (22.2), 485 (24.1), 474 (30.5), 459 (33.5), 444 (25.0), 430 (18.5).
HRMS  C₂₇H₂₆N₄O₃ requires 534.1751 found 534.1751

When the reaction time was increased to 72 h., 65.5% (1.6 mg) of the N,N,N'-trimethyl (236) and 33.2% (0.8 mg) of the N,N-dimethyl derivatives (235) were obtained.

6-(7-Amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-5,N,N-dimethylamino-2-pyridine carboxylate (235)
Methyl-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-5,N,N-dimethylamino-6-(7-N-methyl-amino-5,8-dioxo-2-quinolinyl)-2-pyridine carboxylate (236)

IR CHCl₃ 3680, 3630, 3510, 3390, 1720, 1680, 1630, 1610, 1580 cm⁻¹.

UV CHCl₃ 480 (br), 323 nm.

¹H NMR CDCl₃ 8.44 (d, 1H, J = 8.5 Hz, 3-H), 8.04 (d, 1H, J = 8.5 Hz, 4-H), 6.64 (d, 1H, J = 8.5 Hz, 12'-H), 6.59 (d, 1H, J = 8.5 Hz, 11'-H), 5.70 (bs, 1H, 7-NHMe), 3.95 (s, 3H, OCH₃), 3.94 (s, 6H, 2 x OCH₃), 3.93 (s, 3H, OCH₃), 3.30 (d, 3H, J = 6 Hz, 7-NHCH₃), 2.30 [s, 6H, 5'-N(CH₃)₂], 2.27 (s, 3H, 3'-CH₃) ppm.

EI/MS 562 (M⁺, C₂₉H₃₀N₄O₈, 5.0%), 548 (14.1), 533 (7.1), 517 (100), 503 (18.9), 502 (45.9), 487 (22.3), 472 (9.6), 457 (20.9), 442 (59.1), 428 (14.9), 414 (26.1), 399 (8.9).

HRMS C₂₉H₃₀N₄O₈ requires 562.2064 found 562.2068

Attempted Preparation of Streptonigrin Quaternary Salt (221)

(i) Methyl triflate (1.9 µL, 16.5 µmol) was added to a solution of the diacetyl derivative (209) (2 mg, 3.3 µmol) and 2,6-di-tert-butylpyridine (3.4 mg, 16.5 µmol) in dry dichloromethane (1 mL). The mixture was stirred at room temperature and the reaction was monitored by tlc (non-fluorescent silica, 5% methanol in chloroform). No reaction was observed after 6 h. The mixture was then heated under reflux. After 6 h, the reaction mixture was cooled and evaporated to dryness to give a complex mixture. 20% of the unchanged starting material (209) was recovered by flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue.

(ii) The diacetyl derivative (209) (2 mg, 3.3 µmol) in dry dichloromethane (1 mL) was treated with trimethylxonium tetrafluoroborate (1.0M solution in dichloromethane, 38 µL, 33 µmol) and the mixture was stirred at room temperature. The reaction was monitored by tlc (non-fluorescent silica, 5% methanol in chloroform). No reaction was observed after 5 h. The reaction mixture was then heated under reflux for an additional 5 h, cooled and evaporated to dryness to give a complex mixture. Flash chromatography (pH 6.8 buffer-washed silica, chloroform) of the residue recovered 27% of the unchanged starting material (209).
Attempted Cleavage of α-Hydroxy acids

(i)  Pyridine-2-carboxylic acid (190) (0.5 g, 4.1 mmol) was dissolved in a mixture of acetonitrile, acetic acid and water (3 : 1 : 1 mL) and stirred at room temperature for 20 min. 5% Aqueous sodium hypochlorite solution (commercial bleach) (12 mL, 8.12 mmol) was added slowly at 0°C. The mixture was then allowed to warm up to room temperature, stirred for an additional 5 h., acidified with 6M aqueous hydrochloric acid and continuously extracted with ethyl acetate. The extracts were combined, dried over sodium sulphate and evaporated to recover the unchanged starting material (190) in quantitative yield.

Similar results were obtained with freshly prepared 12% aqueous sodium and calcium hypochlorite solutions and extended reaction times.

(ii) Pyridine-2-carboxylic acid (190) (0.5 g, 4.1 mmol) was stirred in a mixture of water and acetic acid (8 : 2 mL) for 0.5 h. Sodium bismuthate (0.5 g, 4.1 mmol) was added portionwise to the vigorously stirred mixture at 0°C. After 8 h., phosphoric acid (0.5 mL) was added to the reaction mixture and the precipitate formed was filtered off. The filtrate was acidified to pH 5 with 2M aqueous hydrochloric acid and then evaporated to dryness. The residue was extracted with methanol and the extract was concentrated to give a quantitative yield of the unchanged starting material (190).

Reaction of N-Hydroxypyridinethione (239) with Phosgene

20% Phosgene in toluene (10 mL) was added slowly to a stirred solution of N-hydroxypyridinethione (239) (2 g, 15.7 mmol) in dry benzene (10 mL) at 50°C. The resulting precipitate was filtered and collected, washed with small amount of cold benzene and then dried in vacuo to provide the pyridinium chloride salt (247) as a white solid.

MP 100-102°C

Di-2-pyridyl sulphide (251)

The pyridinium chloride salt (247) (162 mg, 0.85 mmol) was added to a stirred solution of pyridine-2-carboxylic acid (190) (100 mg, 0.81 mmol) and pyridine (0.7 mL, 0.85 mmol) in deoxygenated acetonitrile (20 mL) at room temperature under argon. After 3 h., the precipitate was removed and the filtrate was concentrated. Purification by flash chromatography (silica, 5% methanol in chloroform) afforded the dipyridyl sulphide (251) in 82% yield (125 mg).
MP 215 - 219°C  (Lit. 219 - 221°C).

$^1$H NMR CDCl$_3$ 8.30 (dd, 2H, $J_o = 7$ Hz, $J_m = 1.5$ Hz), 7.62 (dd, 2H, $J_o = 7$ Hz, $J_m = 1.5$ Hz), 7.25 (m, 4H) ppm.

$^{13}$C NMR CDCl$_3$ 149.1, 138.6, 126.5, 122.5, 121.8 ppm.

Microanalysis

C$_{10}$H$_8$N$_2$S  Calculated C (63.81) H (4.28) N (14.88)  

The above reaction was repeated with 2,2,6,6-tetramethylpiperidine-N-oxide (TEMPO) (133 mg, 0.85 mmol). Purification of the reaction mixture by flash chromatography (Chromatatron, 2 mm plate, gradient elution with chloroform and methanol) afforded 19% (42.3 mg) of the piperidyl ester (252) and 60.5% (97 mg) of the sulphide (251) after recrystallisation from ethyl acetate and hexane.

With an increased amount of TEMPO (665 mg, 4.25 mmol), 59% (131 mg) of the ester (252) and 23% (36.8 mg) of the sulphide (251) were obtained.

2,2,6,6-Tetramethylpiperidyl-2-pyridine carboxylate (252)

MP 94 - 98°C.

IR  CCl$_4$ 2980, 2940, 1780, 1750 (s, C=O), 1590, 1570 cm$^{-1}$.

$^1$H NMR CDCl$_3$ 8.80 (dd, 1H, $J_o = 6.5$ Hz, $J_m = 1$ Hz, 6-H), 8.10 (dd, 1H, $J_o = 6.5$ Hz, $J_m = 1$ Hz, 3-H), 7.85 (m, 1H, 4-H), 7.47 (m, 1H, 5-H), 1.70 [m, 6H, (CH$_2$)$_3$], 1.28 (s, 6H, 2 x CH$_3$), 1.14 (s, 6H, 2 x CH$_3$) ppm.

$^{13}$C NMR CDCl$_3$ 165.0, 150.2, 148.1, 136.7, 126.6, 124.8, 60.5, 39.0, 31.9, 28.9, 17.0 ppm.

EI MS 262 (M$^+$, C$_{15}$H$_{22}$N$_2$O$_2$, 3%), 140 (C$_9$H$_{18}$N, 78.9), 78 (C$_5$H$_4$N, 100), 44 (CO$_2$, 30.5).

CIMS 263 (M$^+$+1, 100%).

Microanalysis

C$_{15}$H$_{22}$N$_2$O$_2$  Calculated C (68.70) H (8.39) N (10.69)  
Found C (68.74) H (8.60) N (10.55).

An excess of freshly prepared diazomethane in ether was added in portions to a stirred solution of racemic binaphthol (257) (1 g, 3.5 mmol) in dry THF (10 mL) at 0°C. The reaction mixture was then allowed to warm up to room temperature. After 3 h., the reaction mixture was evaporated to dryness. Flash chromatography of the residue (silica; ethyl acetate / hexane / dichloromethane, 1 : 7 : 2) afforded the O-methyl ether (258) (56%, 0.59 g), the dimethyl ether (259) (1%, 11 mg) and starting material (257) (37%).
(R)/(S)-2-Hydroxy-2'-methoxy-1,1'-binaphthyl (258)

MP 155-157°C (Lit. 152-153°C).

1H NMR CDCl₃ 8.0 (M, 4H, Ar-H), 7.25 (M, 8H, Ar-H), 4.92 (bs, 1H, OH), 3.80 (s, 3H, -OCH₃).

EIMS 300 (M⁺, C₂₁H₁₆O₂, 100%), 285 (8), 268 (14), 239 (14).

(R)/(S)-2,2'-Dimethoxy-1,1'-binaphthyl (259)

MP 191-194°C.

1H NMR CDCl₃ 7.90 (m, 4H, Ar-H), 7.25 (m, 8H, Ar-H), 3.76 (s, 6H, -OCH₃).

EIMS 314 (M⁺, C₂₂H₁₈O₂, 100%), 299 (4), 284 (4), 268 (55).

The above experiment was repeated with (R)-binaphthol (257) (100 mg, 0.35 mmol). Flash chromatography of the residue, as described previously, gave 38% (40 mg) of the monomethyl ether (258) and 62% of the starting material.

MP 90-93°C.

1H NMR CDCl₃ 8.0 (m, 4H, Ar-H), 7.25 (m, 8H, Ar-H), 4.90 (bs, 1H, Ar-OH), 3.86 (s, 3H, OCH₃).

Preparation of Racemic 2-Methoxy-2-(2-methoxy-2-phenylacetyl)-1,1'-binaphthyl (260) and (261)

N, N'-Dimethylaminopyridine (162 mg, 1.4 mmol) was added to a stirred solution of racemic binaphthol-O-methyl ether (258) (100 mg, 0.33 mmol), dicyclohexylcarbodiimide (274 mg, 1.4 mmol) and (R)-O-methylmandelic acid (122 mg, 0.74 mmol) in dichloromethane (2 mL) at 0°C. The reaction mixture was then allowed to warm up to 40°C. After 4 h., the dicyclohexylurea formed was filtered off and the filtrate evaporated to dryness. Purification was achieved by successive preparative tlc (silica, 20x20 cm plates, 30% ethyl acetate in hexane) to afford 84% (124 mg) of the O-methyl- mandelate ester (260).

MP 133-136°C.

1H NMR CDCl₃ 7.89 (m, 8H, Ar-H), 7.20 (m, 16H, Ar-H), 4.52 (s, 1H, C-H of mandelate ester *), 4.46 (s, 1H, C-H of mandelate ester **), 3.62 (s, 3H, Ar-OCH₃ *), 3.56 (s, 3H, Ar-OCH₃ **), 3.08 (s, 3H, OCH₃ of mandelate ester *), 2.97 (s, 3H, -OCH₃ of mandelate ester **).
The above two experiments were repeated with (R)-binaphthol (100 mg, 0.33 mmoL) and the mandelate ester (261) formed was purified as described previously and recrystallised from ethyl acetate and hexane.

** (R)-2-Methoxy-2-[2-(R)-2-methoxy-2-phenylacetyl]-1,1'-binaphthyl (260)

** Microanalysis

Calculated C (80.34), H (5.39).

Found C (80.22), H (5.62).

Methyl 5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl)-4-{3,4-dimethoxy-2-[2-(R)-2-methoxy-2-phenylacetyl]}-3-methyl-2-pyridine carboxylate (262)

N, N'-Dimethylaminopyridine (4.6 mg, 38.4 µmoL) was added to a stirred solution of streptonigrin methyl ester (144) (5 mg, 9.6 µmoL), dicyclohexylcarbodiimide (8 mg, 38.4 µmoL) and (R)-O-methylmandelic (3.6 mg, 21.2µmoL) in dichloromethane (1 mL). The reaction mixture was then warmed at 40°C overnight. The dicyclohexylurea was filtered off and the filtrate evaporated to dryness. Successive preparative chromatography (silica, 20x20 cm plates, 2% methanol in chloroform) gave the mandelate ester (262) in 63% yield (4 mg) (95% e.e. by 1H NMR spectroscopy) as an amorphous solid. Further purification was achieved by HPLC (reverse-phase C8 steel column, 60% methanol in water).

UV 500 (ε 1550), 376 (ε 19000, 298 (sh, ε 20100) nm.

1H NMR C6D6 8.65 (d, 1H, J = 8 Hz, 3-H), 8.45 (d, 1H, J = 8 Hz, 4-H), 7.1 (m, 5H, Ar-H of mandelate portion), 7.4 (d, 1H, J = 8 Hz, 11’-H), 6.54 (d, 1H, J = 8 Hz, 12’-H), 5.12 (bs, 2H, 7-NH2), 4.80 (s, 1H, C-H of mandelate portion), 4.10, 3.98, 3.95 and 3.76 (all s, all 3H, all OCH3), 3.29 (s, 3H, OCH3 of mandelate portion), 2.10 (s, 3H, 10-CH3).
The above reaction was repeated with streptonigrin methyl ester (144) (5 mg, 9.6 µmol) and with (S)-O-methylmandelic acid (3.6 mg, 21.2 µmol). The mandelate ester (263) was obtained in 70% (4.5 mg) yield with 95% e.e. after purification.

^1H NMR CDCl₃ 8.94 (d, 1H, J = 8 Hz, -H), 8.44 (d, 1H, J = 8 Hz, -H), 7.1 (m, 5H, Ar-H of mandelate portion), 6.98 (d, 1H, J = 8 Hz, -H), 6.86 (d, 1H, J = 8 Hz, -H), 5.12 (bs, 2H, 7-NH₂), 4.75 (s, 1H, C-H of mandelate portion), 4.09, 3.99, 3.94 and 3.63 (all s, all 3H, all OCH₃), 3.25 (s, 3H, OCH₃ of mandelate portion), 2.19 (s, 3H, 10-CH₃).

EIMS 668 (C₃₅H₃₃N₄O₁₀, 3%), 638 (1), 607 (1.5), 591 (3), 561 (1), 547 (2.5), 121 (C₈H₉O, 100).

HRMS Found 668.

Lanthanide Shift Experiment

Successive 300 MHz ^1H NMR spectra of a mixture of (R) (R) - and (R)/(S)-binaphthol-O-methylmandelate esters, (260) and (261), in anhydrous deuteriochloroform, were recorded with increasing amounts of Eu (fbc)₃. The changes in chemical shifts, i.e. LISOMe(naphthyl) and LISOMe(mandelate) for each diastereomer, (260) and (261), were expressed graphically as a function of molar ratio of LSR to the substrate (see Figs. 26 and 27). The above experiment was repeated with streptonigrin (R)-O-methylmandelate ester (262) and streptonigrin (S)-O-methylmandelate ester (263). The induced shifts are shown in Fig. 31.

Attempted Preparation of Streptonigrone-(R)-O-Methylmandelate Ester (264)

(i) N, N'-Dimethylaminopyridine was added to a stirred solution of streptonigrone (1.6 mg, 3.35 µmol), (R)-O-methylmandelic acid (0.6 mg, 3.68 µmol) and DCC (1.4 mg, 6.70 µmol) in dichloromethane (1 mL) at room temperature. The reaction mixture was warmed at 40°C. The reaction temperature was then slowly increased to 80°C. After another 10 h., a complex mixture together with some starting material (82) were observed (by tlc, non-fluorescent SiO₂, 10% methanol in chloroform).
(ii) N-Hydroxybenzotriazole (1.8 mg, 11.5 µmol), pyridine (0.9 µL, 11.5 µmol) and DCC (2.4 mg, 11.5 µmol) were added sequentially to a solution of streptonigrone (82) (5 mg, 10.5 µmol) and (R)-O-methylmandelic acid (2 mg, 11.5 µmol) in dichloromethane. The reaction mixture was then warmed at 40°C and monitored by TLC (non-fluorescent SiO₂, 10% methanol in chloroform). No significant reaction was observed after 10 h. However, a complex mixture together with some starting material was observed by TLC after 30 h. The starting material (82) was recovered in 40% yield.

(iii) (R)-O-Methylmandelic acid (1.2 mg, 7.4 µmol) was added to a white suspension, which was prepared by slow addition of oxalyl chloride (0.85 µL, 7.4 µmol) to DMF (0.57 µL, 7.4 µmol) in 20 µL of dry THF at 0°C. After 5 min., a solution of streptonigrone (3.2 mg, 6.7 µmol) and pyridine (0.6 µL, 7.4 µmol) in dry THF (200 µL) was added over a 5 min. period. The resulting solution was then stirred at 0°C and monitored by TLC (non-fluorescent SiO₂, 10% methanol in chloroform). A complex mixture, together with some starting material, was observed (by TLC) after 10 min. The reaction was not pursued further at this stage.
APPENDIX (ii)

Table 1: Selected Bond Lengths (Å) (with esd's)

<table>
<thead>
<tr>
<th>Bond Length</th>
<th>Value</th>
<th>ESD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(20)-O(1)</td>
<td>1.398</td>
<td>(5)</td>
</tr>
<tr>
<td>C(21)-O(1)</td>
<td>1.329</td>
<td>(1)</td>
</tr>
<tr>
<td>C(21)-O(2)</td>
<td>1.229</td>
<td>(1)</td>
</tr>
<tr>
<td>C(21) -C(22)</td>
<td>1.556</td>
<td>(5)</td>
</tr>
</tbody>
</table>

Disordered atoms

<table>
<thead>
<tr>
<th>Bond Length</th>
<th>Value</th>
<th>ESD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(20)-O'(1)</td>
<td>1.493</td>
<td>(6)</td>
</tr>
<tr>
<td>C'(21)-O'(1)</td>
<td>1.329</td>
<td>(1)</td>
</tr>
<tr>
<td>C'(21)-O'(2)</td>
<td>1.229</td>
<td>(1)</td>
</tr>
<tr>
<td>C'(21)-C(22)</td>
<td>1.550</td>
<td>(6)</td>
</tr>
</tbody>
</table>

Table 2: Selected Bond Angles (deg.) (with esd's)

<table>
<thead>
<tr>
<th>Bond Angle</th>
<th>Value</th>
<th>ESD</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(1)-C(21)-C(22)</td>
<td>110.17</td>
<td>(22)</td>
</tr>
<tr>
<td>C(20)-O(1)-C(21)</td>
<td>113.05</td>
<td>(25)</td>
</tr>
<tr>
<td>O(1)-C(21)-O(2)</td>
<td>123.03</td>
<td>(01)</td>
</tr>
<tr>
<td>C(22)-C(21)-O(2)</td>
<td>126.78</td>
<td>(22)</td>
</tr>
</tbody>
</table>

Table 3: Selected Torsional Angles (deg.) (with esd's)

<table>
<thead>
<tr>
<th>Torsional Angle</th>
<th>Value</th>
<th>ESD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(12)-C(11)-C(2)-C(1)</td>
<td>-79.7</td>
<td>(6)</td>
</tr>
<tr>
<td>C(12)-C(11)-C(2)-C(3)</td>
<td>96.8</td>
<td>(6)</td>
</tr>
<tr>
<td>C(11)-C(2)-C(3)-O(4)</td>
<td>1.0</td>
<td>(7)</td>
</tr>
<tr>
<td>C(20)-C(11)-C(2)-C(1)</td>
<td>102.0</td>
<td>(6)</td>
</tr>
<tr>
<td>C(20)-C(11)-C(2)-C(3)</td>
<td>-81.5</td>
<td>(6)</td>
</tr>
<tr>
<td>C(11)-C(20)-O(1)-C(21)</td>
<td>111.2</td>
<td>(4)</td>
</tr>
<tr>
<td>C(19)-C(20)-O(1)-C(21)</td>
<td>-83.5</td>
<td>(5)</td>
</tr>
<tr>
<td>C(20)-O(1)-C(21)-C(22)</td>
<td>179.0</td>
<td>(3)</td>
</tr>
<tr>
<td>C(20)-O(1)-C(21)-O(2)</td>
<td>-2.3</td>
<td>(2)</td>
</tr>
</tbody>
</table>
REFERENCES

52. R. W. Rickards, private communication.
ERRATA

p.-xi-, line 5 should be '2,3-dichloro-5,6-dicyano-1,4-benzoquinone'.
line 6 should be '4-(N,N-dimethylamino)pyridine'.
line 7 should be 'N,N-dimethylformamide'.

p.18, structure 38 (Scheme 2) should be 'Enz-S-C(O)-' and not 'Enz-S-O-C(O)-'.

p.20, line 20 should be '9th National ...'.

p.23, and p.61, line 1 should be 'Gram-positive bacterium (Bacillus subtilis)'.

p.31, Table 4 and p.43, Table 5 - The assignment of resonances for C-7 and C-9 should be interchanged.

p.31, penultimate line should read 12a-OCH₃.

p.43, Table 5 should read 'C-1, 190.7, 2.8-fold; C-3, 167.8, 3.3-fold; C-4a, 83.8, 2.6-fold; C-5a, 140.5, 3.0-fold; C-6a, 129.3, 3.1-fold; C-8, 158.1, 3.1-fold; C-10, 138.9, 3.8-fold; C-11, 167.0, 3.2-fold; C-12, 195.6, 2.9-fold; Ester C=O, 171.0, 2.6-fold.'

p.49, Figure 17 should be 12a-OR and 4a-0Me.
Table 7, line 1 should be 'C-4 (69.7)'.
lines 1-3 should be 'Thus, a sample of tetracenomycin X was exchanged several times with d₄-methanol and combined with an unexchanged sample of the antibiotic. A 5% w/v solution of tetracenomycin X in d₆-DMSO was then prepared, and the solution was treated with anhydrous calcium sulphate to remove residual water.'

p.50, The scale is missing. The peaks on the left-hand side of both the fully coupled and ¹H-decoupled ¹³C NMR spectra should be δ 87.0 ppm, whereas the peaks on the right-hand side should be δ 83.8 ppm.

p.66, structure 40 (Scheme 9) should be 'Enz-S-C(O)-' and not 'Enz-S-O-C(O)-'.

p.68, Scheme 11, carboxyl terminus of polyketide chain should have '-CO₂H'.

p.73, line 6 should be '¹⁶O¹⁸O₂'.
line 19 should be 'Table 9i'.
line 28 should be 'Table 9ii'.

p.75, lines 2 and 3 should be 'Table 10i and ii'.

p.82, line 18 should be 'N-methyl-N-nitroso- ...'.

p.84 and 85,
¹³C NMR data for CDCl₃ should be '158.14 (.., 3J = 4.4 Hz, ..), 138.87 (.., 2J = 5.9 Hz, ..), 129.32 (.., 2J = 5.0 Hz, ..), 121.15 (.., 3J = 5.9 Hz, ..), 107.37 (.., 3J = 4.4 Hz, ..), 69.67 (.., 2J = 4.0 Hz, ..)' and for d₆-DMSO should be '157.52 (.., 3J = 4.4 Hz, ..), 137.17 (.., 2J = 5.9 Hz, ..), 128.68 (.., 2J = 5.0 Hz, ..), 120.15 (.., 3J = 5.9 Hz, ..) 108.61 (.., 3J = 4.4 Hz, ..), 69.56 (.., 2J = 4.0 Hz, ..).'

p.85, lines 2, 3, 11, 12 and 30 - The assignment of resonances for C-7 and C-9 should be interchanged.

p.85, line 16 and p.87, lines 1 and 21 should be 'C₁₉H₁₇O₈'.

p.85, from line 27, the ¹³C enrichments should read '195.6 (C-12, 2.9-fold ), 190.7 (C-1, 2.8-fold), 171.0 (9-ÇO₂Me, 2.6-fold), 167.8 (C-3, 3.3-fold), 167.0 (C-11, 3.2-fold), 158.1 (C-8, 3.1-fold), 140.5 (C-5a, 3.0-fold), 138.9 (C-10, 3.8-fold), 129.3 (C-6a, 3.1-fold), 83.8 (C-4a, 2.6-fold).'

p.86, line 26 should be 'MeOH / HCl 403 (ε 10000), 388 (ε 9200)'.
p.87, penultimate line should be '40.7% (8.8 mg)'.
final line should be '44.9% (10.5 mg)'.
p.88, line 11 and p.90, line 10 - The assignment of resonances for C-7 and C-9 should be interchanged.
line 19 should be '[...,2-methoxy-2-phenylacetoxy]...'.
line 20 should be 'IR CHCl3 ...'.
line 26 should be '85% (5.7 mg) of the ester (49)'.
p.91, line 11 and last line - The assignment of resonances for C-7 and C-9 should be interchanged.
line 18 should be '652 (M+ i.e. C33H30O13 + 18, 1%)'.
line 23 should be '-2-methoxy-2-phenylacetoxy[1]'.
line 25 should be '80.5% yield (5.4 mg)'.
p.92, line 11 should be '652 (M+ i.e. C33H30O13 + 18, 4%)'.
p.95, footnote should be '1,4,11-Trihydroxy-3,8-dimethoxy-9-methoxycarbonyl-10-methylnaphthacene-5,12-quinone'.
p.97, ref. 10a and throughout this thesis, Tett. Lett. should be Tetrahedron Letters.
ref. 12 should be '9th National RACI Conference'.
ref. 15 should be 'R. W. Rickards'.
ref. 16a should be 'S. Sternhell'.
p.98, ref. 17b should be 'Bu' Lock'.
ref. 23b should be 'W. Steglich and G. Hofle, Angew. Chem. Int. Ed., 8, 981 (1969)'.
ref. 27a should be 'Vol. 2, 3rd Ed., Chapman and Hall, London, p. 141 (1975)'.
ref. 31 should be 'Holden-Day, San Francisco, p.1 (1965)'.
ref. 34b, should be 'Biological Oxidations', Colloquium der Gesellschaft fur Biologische Chemie, Springer-Verlag, Berlin.'.
ref. 39b should be 'Wiley-Interscience, New York'.
p.100, line 7 should be 'biosynthesis57-65'.
p.109, penultimate line should be 'Dholakia and Gillard'.
p.111, line 2 should be 'Weinreb and coworkers54'.
p.114, penultimate line should be 'Karpov and Romanova'.
p.118, line 1 should be '[U-13C6]glucose-6-phosphate (135)62'.
p.119, line 7 should be 'β-carboline (140)'.
p.123, line 13 should be '(144-147)46'.
p.126, line 1 should be 'widely studied57-65'.
p.132, line 2 should be 'oxidation of'
lines 7 and 9 should be '8-hydroxy derivative (182)'. 
p.133, line 19 should be 'quinoneimine (175)'.

p.136, penultimate line should be 'Gould and coworkers60'.

p.141, line 15 - the solvent should be 'dichloromethane'.
line 17 - the yield should be '72%'.

p.142, footnote should be 'δ 6.17 ppm'.

p.143, line 2 should be 'δ 1.98 ppm'.
line 16 should be '53.4%'.

p.144, line 3 should be 'tetraacetyl derivative (212)'.

p.145, lines 5 and 6 should be '71% and 84% yields respectively'.

p.146, line 19 should be 'N-acetyl derivative (218)'.

p.147, line 11 should be 'deuteriated N-oxides (191) and (191a)'.

p.152, structure (144), R=H.
line 16 should read '(MeSO_3CF_3)_130'.
line 17 should read '((MeO_2BF_4)_131'.

p.154, line 15 and p.155, line 22 should be 'Barton and coworkers137-141'.

p.156, Scheme 43, structure (82), for R_1 should have extra bond next to hydroxy group to connect benzene ring with pyridine nucleus.

p.157, line 8 should be 'substantiated in structure (206)'.

p.158, Scheme 45, structure (206), the '8-OH' should be '8-OAc'.

p.160, line 5 and p.174, final line should be 'Dholakia and Gillard'.

p.164, line 2, p.165, line 1 and p.169, line 1 should be 'diastereomeric excess'.

p.167, final line should be 'MTPA moiety'.

p.175, Figure 30, for structures (262) and (263), 'R' should be 'Q'.

p.178, penultimate line should be 'Dholakia'.

p.179, line 10 should be '171 (C_{11}H_{11}N_2, 19.2)'.

p.180, line 14 should be '168 (C_{11}H_8N_2, 100)'.
line 16 should be 'C (69.01), H (4.46 ...'.

p.181, final line and p.182, line 6 should be '168 (C_{11}H_8N_2, 100)'.

p.183, line 12, delete '(174)'.
line 30 should be '184 (C_{11}H_8N_2O, 100)'.

p.186, line 4 should be '(10 mg, 41.3 µmol)'.

p.187, lines 18 and 23 should be '184 (C_{11}H_8N_2O, 100)'.

p.188, lines 8 and 9, the ^1H NMR data should be '7.65 (m, 1H, 7-H), 7.35 (m, 1H, 6-H), 3.75 (m, 3H, -CH_2-O- and -CH-) and 1.80 (m, 4H, -CH_2-)'.

p.192, line 7 should be '4-(2-acetoxy- ...'.

p.193, line 7 should be 'CHCl_3 376, 249 nm'.
line 17 should read '-(2-acetoxy-3,4- ...'.
line 32 should read '520 (C_{26}H_{24}N_4O_8, 21.3), 506 (C_{25}H_{22}N_4O_8, 31.5)'.

penultimate line should read '473 (C_{25}H_{21}N_4O_6, 40.0) and '459 (C_{24}H_{19}N_4O_6, 30.0)'.

p.192, line 7 should be '4-(2-acetoxy- ...'.
line 20 should be 'Methyl 5-acetamido-4-(2-acetoxy- ...
line 21 should be '3-methyl-2-pyridinecarboxylate'.
line 23 should be '295 (sh) nm'.
penultimate line should be '506 (C_{15}H_{22}N_{4}O_{8} , 50.1)'.

line 2 should be '-4-(2-acetoxy-3,4- ...
lines 3 and 12, '(212)' should be '(210)'.
line 24 should be '68 (C_{4}H_{8}N, 46.0)'.
line 8 should be '85% yield (101.6 mg)'.
line 10 should be '70% yield (85.6 mg)'.
line 12 should be '2-[4-(2-Acetoxy-3,4- ...'

line 9, '(191)' should be '(190)'.
line 24 should be '90% yield (1.98 g)'.
line 10, should be 'methanosulphate salt (228)'.
lines 25 and 26 should be '(7-amino-5,8-dihydro- ...
line 21 should be 'Methyl 6-(7-amino ...'
lines 1 and 2 should be 'Methyl 4-(2-hydroxy-...
line 23 should be '9 5 % d.e.'.

lines 9 and 10 should be 'Purification of the reaction mixture by chromatography'.
line 3, 'M' should be 'm'.
line 16 should be 'Preparation of [1,1'-(R),2''-(R)]-2-Methoxy-2'-(2''-methoxy-2''-phenylacetox)-
1,1'-binaphthyl and [1,1'-(S),2''-(R)]-2-Methoxy-2'-(2''-methoxy-2''-phenylacetox)-1,1'-binaphthyl'.
line 5, '(261)' should be '(260)'.
lines 7 and 16 should be '-phenylacetox)'.
line 19 should be '-methylmandelic acid'.
line 23 should be '95% d.e.'. lines 1 and 12 should be '668 (C_{35}H_{33}N_{4}O_{10} , 3%)'.
lines 3 and 14 should be 'Requires 668.2118  Found 668.2119'.
line 6 should be '95% d.e.'.

ref. 50 should be 'Wiley, New York, p.48 (1973)'.
ref. 101 should be 'Vol. 1', Wiley, New York, p. 533 (1967)'.
ref. 114a should be 'Sternhell'.
ref. 120a should be 'Wiley-Interscience, New York, p.992 (1963)'.

ref. 129 should be 'M. Fieser, 'Reagents for Organic Synthesis', Vol. 8, Wiley-Interscience, New York'.
ref. 135a should be 'Acta Chemica Scandinavica, (B), 38, 343 (1984)'.
ref. 135c should be '... Synthesis', Vol. 1, Wiley, New York, p.1085 (1967)'.


ref. 152 should be 'Whiting'.


ref. 157 should be "*References*, 4th Ed., Eyre and Spottiswoode, London (1965)."