SYNTHESIS AND STRUCTURAL STUDIES OF BIOACTIVE NATURAL PRODUCTS

A thesis submitted for the degree of
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Research School of Chemistry

by
Annemarie Ward
(April 1995)
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.

Annemarie Ward

(April 1995)
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ABSTRACT

The work described in this thesis is divided into two parts. The first part details the approaches to and synthesis of (±)-iso-trans-trikentrin B. Initially model studies of the synthetic pathway were carried out using the more readily accessible cis-1,3-dimethylindan as described in Chapter 2. Once a stereoselective procedure to trans-dimethylindan was achieved, the synthesis of (±)-iso-trans-trikentrin B was undertaken following the route established for the model cis-isomer.

The correct regiochemistry of the four carbon sidechain, the cyclopentane and pyrrole around the aryl ring was accomplished using extensive modifications of the procedures previously used to synthesise cis- and trans-trikentrin A. Friedel Crafts acylation of trans-dimethylindan using butyryl chloride regioselectively placed the C4 sidechain in the desired 5-position on the aryl ring. Lithiation of the arene was directed ortho- to the hydroxylated butyl group, as expected. A formyl group could then be introduced into the correct position for annulation of the pyrrole ring and subsequent decarboethoxylation.

Removal of the MOM protecting group from the benzylic alcohol with dimethylboron bromide and quenching with base introduced the E-double bond in the sidechain to give the target (±)-iso-trans-trikentrin B (Chapter 3).

The second part of this thesis describes the isolation and structural identification of a number of biologically active components from cowvine, Ipomoea lonchophylla, and the seeds and leaves of the avocado plant, Persea americana var. Guatemalan.
A fraction from *Ipomoea lonchophylla* which was acutely toxic to mice contained a mixture of resin glycosides. The structure of the major component from the mixture was elucidated using high field NMR, mass spectrometry, chemical studies and comparisons with known resin glycosides. It was found to be a 3,11-dihydroxytetradecanoic acid glycosidically bonded through the C-3 hydroxyl group to a branched hexasaccharide containing two quinovosyl, two glucosyl, one fucosyl and one rhamnosyl unit. The sugars were esterified with C5 acid moieties (Chapter 4).

High field NMR and mass spectrometry showed that a component in *Persea americana* seeds which was cardiotoxic to mice had the same or a similar structure to a trimeric proanthocyanidin (polymeric hydroxyflavanol) that had been found in the seeds of the fruit of *Persea gratissima* (Chapter 5).

Two biologically active fractions were isolated from the chloroform extract of the leaves of *P. americana*. The fraction which caused milk reduction in lactating mice was confirmed as a mixture composed mainly of C50, C55, C60 and C65 polyisoprenols by comparing its spectroscopic data with that reported in the literature. The other fraction caused necrosis of the mammary tissue in lactating mice. The active compound was identified as the known (12Z, 15Z)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate by comparison of its optical rotation value, ultraviolet, infrared, NMR and mass spectral data with that previously reported (Chapter 5).
# TABLE OF CONTENTS

## PART ONE: SYNTHESIS OF (±)-ISO-TRANS-TRIKENTRIN B

### CHAPTER 1: INTRODUCTION  
1.1 Indole Compounds of Marine Origin  
   1.1.1 Background  
1.1.2 Synthetic Pathways to the Trikentrins  
   1.1.2.1 Non-Asymmetric Syntheses of the Trikentrins  
   1.1.2.2 Asymmetric Syntheses of the Trikentrins  

### CHAPTER 2: SYNTHETIC APPROACHES TO  
2.1 Previous Approaches  
2.2 Current Approaches  
2.3 *cis*-1,3-Dimethylindan (14) as a Starting, Model Compound  
   2.3.1 Synthesis of *cis*-1,3-Dimethylindan (14)  
2.4 Construction of the Four Carbon Unit Sidechain  
2.5 Attempted Formylations of the Butyl-1,3-dimethyl-indan Compounds  
   2.5.1 A Classical Method  
   2.5.2 Lithiation Attempts  
      2.5.2.1 Lithiation of the MOM ether (63)  
      2.5.2.2 Lithiation of the Hydroxybutylindan (56)  
   2.5.3 Formylation and Elaboration of (56)  
      2.5.3.1 Formylation  
      2.5.3.2 Attempted Formation of the Pyrrole Ring  
2.5.4 Alternative Approach to the Formylindan Intermediate (73)  
2.6 Annulation and Elaboration of the Pyrrole Ring  
   2.6.1 Formation of the Azidocinnamate (77)  
   2.6.2 Characterisation of (79) and (80)  
   2.6.3 Optimisation of Cyclisation Conditions  
2.7 Removal of the Ethyl Carboxylate Functionality from (79)  
   2.7.1 General Approaches
2.7.2 Hydrolysis of (79) and Subsequent Decarboxylation
2.7.3 Decarboethoxylation Method

2.8 Attempts to Introduce the Double Bond in the C4 Sidechain of (85)
2.8.1 Attempted Deprotection and Dehydration
2.8.2 Protection of the Indole Nitrogen of (79) and (85)
2.8.3 Cleavage of the MOM Group from (97)

CHAPTER 3: SYNTHESIS OF
(±)-ISO-TRANS-TRIKENTRIN B (5)
3.1 Synthesis of (±)-trans-1,3-Dimethylindan (17)
3.1.1 Background
3.1.2 Approaches
3.2 Construction of the Four Carbon Unit Sidechain
3.3 Formation of the Indole
3.3.1 Approach to the Indole (103)
3.3.2 Synthesis of the Indole (103)
3.4 Removal of the Carboxylate Group from (103)
3.5 Deprotection of the MOM Group and the Introduction of the
Double Bond in the C4 Sidechain of (112)
3.5.1 Proposed Approach
3.5.2 Synthesis of (±)-iso-trans-Trikentrin B (5)

EXPERIMENTAL 96

APPENDIX 145

REFERENCES 151

PART TWO: STRUCTURAL STUDIES OF BIOACTIVE NATURAL PRODUCTS

CHAPTER 4: A CHEMICAL INVESTIGATION OF THE CAUSE
OF THE 'DUMB LAMB SYNDROME'
4.1 Introduction
4.2 Isolation, Characterisation and Structural Elucidation of the
Resin Glycoside
4.2.1 Extraction Procedures and Isolation of the Toxic Extract
4.2.2 Acid Hydrolysis of the Resin Glycosides from the Toxic Extract

4.2.3 Alkaline Hydrolysis of the Resin Glycosides from the Toxic Extract

4.2.3.1 Derivatisation of the Ether Extract

4.2.3.2 Isolation and Analysis of the Alkaline Hydrolysed Resin Glycoside

4.2.4 Determination of the Position of Linkage of the Oligosaccharide to the Dihydroxytetradecanoic acid

4.2.5 Determination of the Positions of Linkage of the Hexose and Deoxyhexose Units

4.2.6 NMR Assignments leading to the Structure of (6)

4.2.7 Determination of the location of the C5 acids

EXPERIMENTAL

REFERENCES

CHAPTER 5: BIOLOGICALLY ACTIVE COMPONENTS OF PERSEA AMERICANA

5.1 Introduction

5.2 Toxic Extract of P. americana seeds

5.3 Biologically Active Fractions of P. americana Leaves

5.3.1 Fraction 1

5.3.2 Fraction 2

EXPERIMENTAL

REFERENCES
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
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<td>azobisisobutyronitrile</td>
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PART ONE

SYNTHESIS OF (±)-ISO-TRANS-TRIKENTRIN B
CHAPTER 1.
INTRODUCTION

1.1 INDOLE COMPOUNDS OF MARINE ORIGIN

1.1.1 Background

Tyrian purple was one of the most expensive commodities of the ancient world. The high cost was due to the fact that one mollusc yields only a couple of drops of dye-producing secretion. The dye production from molluscs is believed to date back to around 1600 BC, when the process was discovered by the Cretans. It was found that when the yellow-white fluid was placed on wool or linen and exposed to sunlight, it would undergo several colour changes and finally become a purple-red colour. To make the dye colour-fast, the fabric was washed in soap and water.¹,²

Tyrian purple was also the earliest product from a marine metabolite whose chemical constitution was correctly deduced and proven.³ In 1909, Friedländer⁴ extracted 1200 snails (Murex brandaris) and after carrying out several chemical tests and analyses, he deduced the chemical constitution of the dye was a symmetrical dibromoindigotin. Two of the symmetrical compounds had been synthesised by 1909, the 5,5'-dibromoisomer⁵ and the 6,6'-dibromoisomer (1)⁶,⁷ and based on the published properties Friedländer concluded that Sachs' 6,6'-isomer (1) was the better choice. He synthesised this compound from a new route via 2-amino-4-bromobenzoic acid and proved the synthetic and natural dyes to be identical by solubility and colour tests as well as their visible absorption spectra.
It was not until 1968 that Baker and Sutherland showed by chemical means and spectral data that indole (2) was the true metabolic precursor of Tyrian purple.

Except for the extraordinary case of Friedländer's work, the isolation and structural determination of marine indoles and their derivatives is a field developed relatively recently. It was not until the 1970s that work in this area expanded rapidly. A wide variety of natural compounds has been found, including structurally novel heterocycles with significant biological activity. A number of reviews have been written over the last twenty years covering the literature on the biological activity, structural elucidation and synthesis of marine indoles.

Recently, a class of novel indole alkaloids have been isolated from *Trikentrion flabelliforme* and *Axinella* sponges. The structures of some members of this class of compounds, the trikentrins (3a – 7a) and the herbindoles (8a – 10a), are given in Figure 1.
Figure 1. Absolute configurations of naturally occurring trikentrins and herbindoles.*

The five new trikentrin indoles were isolated from an aqueous acetone extract of the sponge *Trikentrion flabelliforme*, collected from the coastal waters off Darwin.

* The naturally occurring enantiomer will be denoted by the letter 'a', while the optical isomer will be given the letter 'b'.
Nonhem Territory, Australia. The structures of the trikentrins were elucidated by spectroscopic analysis but the absolute stereochemistry was not ascertained. Natsume and co-workers\textsuperscript{16,17,18} carried out asymmetric syntheses and determined the absolute configurations of the natural trikentrins to be those shown in Figure 1.

A Western Australian sponge, *Axinella* sp., yielded three polyalkylated indoles called herbindoles ((8a) - (10a), Figure 1). Natsume and co-workers\textsuperscript{18,19} were also able to determine the absolute configurations of these by synthesising herbindoles with known absolute configurations.

This class of marine alkaloids have attracted interest mainly due to their biological activities and interesting structures. The trikentrins were found to exhibit growth inhibitory activity against the gram positive bacteria *Bacillus subtilis*,\textsuperscript{14} while the herbindoles are fish antifeedants and showed cytotoxicity against KB cells.\textsuperscript{15} A unique aspect of their structure is the cyclopentane moiety fused to the aryl ring of the indole nucleus. Another unusual feature is the lack of substitution at C-3. Secondary metabolites incorporating indole moieties are not uncommon among natural products isolated from sponges but these all contain C-3 substituents and are considered to be derived from tryptophan.\textsuperscript{14} This suggests a biogenetic pathway for the trikentrins and herbindoles that may not involve tryptophan. Kornprobst and co-workers\textsuperscript{20} have speculated that metabolite (11), isolated from an axinellid sponge *Trikentrion loeve*, could provide a clue to the origin of these novel indole alkaloids. Apart from this metabolite, there are no other reported compounds that could be possible intermediates in the biogenesis of the trikentrins and herbindoles that have been isolated from either *Trikentrion* or *Axinella* sponges.
A recent review by Garson\textsuperscript{12} points out why there are relatively few publications in the domain of the biosynthesis of marine metabolites. The field is a new and emerging one in that the development of high field nuclear magnetic resonance (NMR) and mass spectral techniques has greatly aided biosynthetic experiments. Garson\textsuperscript{12} also explains some of the problems associated with the biosynthetic studies on marine life relate to the difficulties in collecting sufficient quantities of pure metabolites and the low rates of biosynthesis \textit{in vivo}.

It is not uncommon in the literature to find hypotheses about the formation of marine metabolites that are based on mechanisms that have been well established for secondary metabolites in terrestrial animals and plants. However, as Garson\textsuperscript{12} points out:

'(the) lack of experimental evidence prevents the confirmation of this assumption.

There are some striking differences between terrestrial and marine metabolism. Halogens and isocyanide groups appear frequently as substituents in the metabolites of algae or sponges yet these functionalities are rarely observed as products of terrestrial metabolism. Marine metabolites often differ in absolute stereochemistry from their terrestrial counterparts.'
1.1.2 Synthetic Pathways to the Trikentrins

1.1.2.1 Non-Asymmetric Syntheses of the Trikentrins

The first total synthesis of a trikentrin was carried out by Monahan and MacLeod\textsuperscript{21,22} who successfully constructed (±)-cis- and trans-trikentrins A (3) and (4). The dimethylindan was formed early in the scheme by the novel approach of radical cyclisation of 2-(2-bromophenyl)pent-4-en-2-ol (12) (Scheme 1). To obtain the cis-dimethyl geometry on the indan, water was eliminated from the indanol mixture using acidic conditions to give 1,3-dimethyl indene (13). The indene was then hydrogenated using 10% palladium on carbon as the catalyst, giving predominantly the (±)-cis-dimethyl isomer (14) (9 : 1, cis : trans).

Friedel-Crafts acylation of the dimethylindan yielded the desired 5-substituted indan with no trace of the 4-substituted product. To construct the indole moiety, the reduced ethyl compound (15) was regiospecifically formylated using dichloromethyl methyl ether and titanium tetrachloride to give (16).

Elaboration of the indole ring was achieved by treating the formylinadan (16) with ethyl azidoacetate, followed by thermolysis of the azide (Scheme 2). Hydrolysis of the ester to the acid and flash vacuum pyrolytic (FVP) decarboxylation completed the synthesis in an overall yield of 18% over 12 steps.
Scheme 1
This synthetic strategy was extended to furnish the \textit{trans}-isomer. To obtain the required intermediate \((\pm)\)-\textit{trans}-dimethylindan (17), the two dimethylindanol diastereomers were separated by medium pressure liquid chromatography (MPLC) and the \((\pm)\)-\textit{trans}-dimethylindanol was hydrogenolysed with retention of configuration using the slightly alkaline W7 Raney nickel.
Subsequently, the same manipulations and reaction conditions as those used to convert (±)-cis-dimethylindan (14) into (±)-cis-trikentrin A (3) were used to convert (±)-trans-dimethylindan (17) into (±)-trans-trikentrin A (4). The overall yield was 2%.

Yasukouchi and Kanematsu\textsuperscript{23} published the first total synthesis of (±)-cis-trikentrin B (6) based on the intramolecular Diels-Alder reaction of a 2,3-disubstituted allenic dienamide (18). This impressive reaction sets up the regiochemistry by correctly placing the ethyl group, the pyrrole moiety and a masked cyclopentane ring around the potential aryl ring (Scheme 3, (19) $\rightarrow$ (20)).

The synthesis proceeded from propargyl alcohol which was converted to the bicyclic ester (21) in four steps using the method of Hooz and Layton.\textsuperscript{24} The ester (21) was reduced to the aldehyde which was condensed with propargylamine, followed by protection of the nitrogen to give the dienamide (19). A mixture of dienamide (19), formaldehyde, dioxane, diisopropylamine and cuprous bromide was heated to reflux. The resulting allene (18) was found to undergo an intramolecular Diels-Alder reaction to give the adduct (20). Aromatisation, oxidation and alkaline hydrolysis followed by a side-chain extending Wittig reaction afforded the diol (22) (Scheme 3).
Sodium periodate oxidation of (22) gave the dialdehyde. It was reduced to the diol which was transformed to the dimesylate (23) and subsequent treatment with activated zinc dust and sodium iodide gave a mixture of (±)-(E)-cis-trikentrin B (6)
and (±)-(Z)-cis-trikentrin B (2:1) (Scheme 4). The (E)-isomer was separated from the (Z)-isomer by silica gel chromatography. The overall yield of this 18 step synthesis of (±)-cis-trikentrin B was 0.5%.

Scheme 4

An alternative approach to the synthesis of (±)-cis- and (±)-trans-trikentrins A (3) and (4), respectively was published by Boger and Zhang. It was based on two sequential heteroaromatic azadiene Diels-Alder reactions, which also provides a general approach to the preparation of other substituted indoles.
Initially, the aryl ring and fused cyclopentane were constructed. This was achieved by treatment of the tetrazine (24) with pyrrolidine enamine (25) to form the adduct (26) as a single diastereomer (Scheme 5).

Acid catalysis gave the 1,2-diazine (27) possessing (±)-cis-dimethyl stereochemistry. The synthesis continued with the oxidation of (27) to the corresponding sulfone (28) and subsequent reaction with (29) under pressure gave the allene (30).
Acylation of the secondary amine of (30) followed by mild thermolysis provided N-acetyl-cis-trikentrin A, presumably via an initial intramolecular allene 1,2-diazine Diels-Alder reaction with loss of nitrogen and thermal isomerisation (Scheme 6).
Methanesulfinic acid was subsequently eliminated and deprotection of the indole gave (±)-cis-trikentrin A (3) in an overall yield of 17% in 7 steps.

Deliberate epimerisation of the bis-sulfone (28) with triethylamine at 65°C gave the (±)-trans-dimethyl isomer (31) (40%) together with recovered (±)-cis-isomer (28) (55%) (Scheme 7). Similar manipulations to those carried out on the (±)-cis-dimethyl compounds were applied to (31) to give (±)-trans-trikentrin A (4) in an overall yield of 12% in 8 steps.

Scheme 7
Asymmetric Syntheses of the Trikentrins

As previously mentioned, it was not until Natsume and co-workers\textsuperscript{16,17,18} synthesised the trikentrins that the absolute stereochemistry of this class of compounds was determined. (6S, 8R)-cis-Trikentin A (3b) and (6R, 8R)-trans-trikentrin A (4b) were synthesised from (R)-3-methyladipic acid, thereby establishing that the natural cis- and trans-trikentin A (3a) and (4a), respectively, have the opposite configurations.\textsuperscript{16,18}

The dimethyl ester of (R)-3-methyladipic acid was cyclised to form methyl carboxylates (32) and (33) (Scheme 8). Methylation, successive removal of the methyl carboxylate group and subsequent silylation gave a mixture of silyl enol ethers (34) and (35). These compounds were coupled with N-phenylsulfonylpyrrole to give a mixture of 2-pyrrolecyclopentanones (36), (37) and (38), chromatographic purification of which gave a mixture of the desired isomers (36) and (37) (1 : 4) in a 37\% yield.

The carbon side chain was added by condensation of the lithium enolate of 2-butanoine N,N-dimethylhydrazone with (36) and (37) (Scheme 9). Subsequent sulfuric acid catalysed cyclisation reaction of (39) gave the desired indole (40) (73\%, \textit{cis} : \textit{trans} = 4 : 3) and the dehydration product (41) (12\%). Alkaline hydrolysis of (40) followed by chromatographic separation afforded (6S, 8R)-\textit{cis}-trikentin A (3b) and (6R, 8R)-\textit{trans}-trikentin A (4b). The overall yields for the 10 step syntheses starting from the methyl carboxylates (32) and (33) were 5.5\% and 4\% for \textit{cis}- and \textit{trans}-trikentrins respectively.

The optical rotation values for the synthetic trikentrins (3b) and (4b) were opposite to those obtained for the natural compounds establishing the absolute configurations
of natural cis-trikentrin A (3a) and trans-trikentrin A (4a) to be (6R, 8S) and (6S, 8S) respectively.

Scheme 8
(6S, 8R)-cis-trikentrin A (3b)  (6R, 8R)-trans-trikentrin A (4b)
Recently, Natsume and co-workers\textsuperscript{17,18} also established the absolute structures of \textit{iso-trans}\textsuperscript{-}trikentrin B (5a) as (6$S$, 8$S$), \textit{cis}\textsuperscript{-}trikentrin B (6a) as (6$R$, 8$S$) and \textit{trans}\textsuperscript{-}trikentrin B (7a) as (6$S$, 8$S$) by synthesising the enantiomer of \textit{iso-trans}\textsuperscript{-}trikentrin B (5b), \textit{cis}\textsuperscript{-}trikentrin B (6a) and the enantiomer of \textit{trans}\textsuperscript{-}trikentrin B (7b) in an enantioselective manner from a stereochemically defined Diels-Alder adduct (42). In all cases, the dimethyl cyclopentindoles were obtained as mixtures of \textit{cis-} and \textit{trans-}diastereomers and were subsequently separated by high performance liquid chromatography (HPLC).

Manipulations beginning with the adduct (42)\textsuperscript{17,18} eventually gave the optically active 3,5$'$-dimethylcyclopentenyl (43) in 7 steps (Scheme 10). Claisen rearrangement\textsuperscript{26} of (43) produced (44) whose lithium salt was reacted with 3-formyl-1-(phenylsulfonyl)pyrrole to give the condensation product (45). Oxidation of the \textit{exo}\textsuperscript{-}methylene group gave the corresponding ketone which was cyclised to an inseparable mixture of indole derivatives. Reduction of the carboxylic ester to the hydroxymethyl group was followed by oxidation to the aldehyde (46). Reaction with propylmagnesium bromide afforded the hydroxybutyl indole isomers (47). The isomers were dehydrated in benzene heated to reflux in the presence of a catalytic amount of \textit{para}\textsuperscript{-}toluenesulfonic acid (\textit{p}-TSA). The resulting \textit{E}\textsuperscript{-}olefin sulfonamides were deprotected using alkaline conditions to give the epimers (6$R$, 8$S$)-\textit{cis}\textsuperscript{-}trikentrin B (6a) and (6$R$, 8$R$)-\textit{trans}\textsuperscript{-}trikentrin B (7b) (in an overall yield of 5\% and 0.9\%, respectively). A comparison of the optical rotation of (7b) with that of the corresponding natural compound established the absolute structure of \textit{trans}\textsuperscript{-}trikentrin B as (6$S$, 8$S$).
Scheme 10

(6R, 8S)-cis-trikentrin B (6a)  (6R, 8R)-trans-trikentrin B (7b)
The natural product cis-trikentrin B had been obtained as an inseparable mixture with iso-trans-trikentrin B. Thus, the intermediate (45) was also used to synthesise iso-trans-trikentrin B (Scheme 11). Oxidation to the ketone was followed by removal of the ethoxycarbonyl group to give (48). Subsequent reaction with phenylsulfonyl-methyllithium followed by oxidative cleavage of the exo-methylene group and subsequent treatment of the resulting ketone with p-TSA and benzylthiol gave a diastereomeric mixture of the trans-isomer (49b) and the cis-isomer (50) (1:3). The trans-isomer (49b) was separated from (50) and selective replacement of the benzylic phenylsulfonyl moiety with an allyl group,
followed by migration of the double bond using a RhCl₃ catalyst and finally alkaline hydrolysis afforded (6R, 8R)-iso-trans-trikentrin B (5b). The compound was synthesised in 18 steps in an overall yield of 2% starting with the known Diels-Alder adduct (42).

In order to determine the absolute configurations of cis-trikentrin B (6a) and iso-trans-trikentrin B (5a) the circular dichroic (CD) spectral curve of the natural inseparable mixture of these trikentrins was compared with curves calculated from different combinations of the measured individual CD curves of the synthesised trikentrins (6a) and (5b) and the mirror image spectra expected for their enantiomers (6b) and (5a). Only the curve obtained from the combination of (6a) and (5a) resembled that of the natural mixture. Therefore, the absolute configurations of natural cis-trikentrin B (6a) and iso-trans-trikentrin B (5a) are 6R, 8S and 6S, 8S respectively (Figure 1).

The aim of this project was to synthesise (±)-iso-trans-trikentrin B (5). There were 3 critical aspects of the synthesis that had to be addressed: (i) development of a method that would selectively give the trans- geometry in 1,3-dimethylindan; (ii) establishing the correct regiochemistry of the substituents on the aryl ring; and (iii) late introduction of the double bond in the butenyl sidechain to avoid styrene-like polymerisation.
CHAPTER 2

SYNTHETIC APPROACHES TO (±)-ISO-TRANS-TRIKENTRIN B (5): MODEL STUDIES ON THE CIS-ANALOGUE

2.1 PREVIOUS APPROACHES

The successful completion of the total synthesis of (±)-cis- and trans-trikentrin A (3) and (4) in this laboratory\(^22\) suggested that a similar approach to the synthesis of (±)-iso-trans-trikentrin B (5) should be feasible and would enhance the general application of the route developed in Chapter 1 (Schemes 1 and 2). While the synthesis of (5) was being carried out, reports appeared in the literature detailing its synthesis as well as that of the enantiomer (6\(^R\),8\(^R\))-iso-trans-trikentrin B (5\(^b\))\(^{17,18}\).

MacLeod and Monahan\(^27\) initially proposed the introduction of a butenyl substituent on the 1,3-dimethylindan ring to give (51) (Scheme 12). It was then envisaged that formylation of (51) should give the formylindan (52). The route to (±)-iso-trans-trikentrin B (5) then follows that used to form the pyrrole ring and complete the synthesis of (±)-cis- and trans-trikentrin A (3) and (4) (see Scheme 2, Chapter 1)\(^{22}\).

Formylation of the model compound cis-1,3-dimethylindan (14) using titanium tetrachloride and dichloromethyl methyl ether\(^27\) gave at best a 49% purified yield of the formylindan (53). The formyl compound (53) was elaborated to the desired \(E\)-butenyl-substituted indan (54) using the conditions described by Schlosser and Christmann\(^{28}\). Attempts to formylate (54) gave a complex mixture probably due to...
the lability of the styryl group. In view of this and the poor yield in the initial formylation step, an alternative approach was attempted.

![Chemical structures](image)

**Scheme 12**

It was proposed to introduce the four-carbon unit by acylation and to modify it to the unsaturated sidechain at a later stage. Friedel-Crafts acylation of cis-1,3-dimethylindan (14) using aluminium trichloride and butyryl chloride gave the desired 5-butyroyl-1,3-dimethylindan (55). Formylation using dichloromethyl methyl ether and titanium tetrachloride (or aluminium trichloride) was attempted on
(55), its dithioethyleneketal, the alcohol (56, R = H) and the dimethyl-iso-propyl silyl protected alcohol (R = DMPS). None of the desired formylated indan was observed.

At this stage the synthesis was taken over by the author.

2.2 CURRENT APPROACHES

The three main criteria to be addressed for the synthesis of (±)-iso-trans-trikentrin B (5) (as given in Chapter 1) were the development of (i) method(s) to selectively give the trans- geometry in 1,3-dimethylindan; (ii) approach(es) to give the correct regiochemistry of the substituents on the aryl ring; and (iii) methodology for the late introduction of the double bond of the butenyl sidechain to remove problems of decomposition.
A method by Jackson et al. that selectively gives trans-1,3-dimethylindan (17) (Scheme 13) addresses the first criterion. Construction of the C₄ sidechain using aluminium trichloride and butyryl chloride on indan (17) provides the correct regiochemistry of the sidechain on the aryl ring. Protection of the ensuing benzylic alcohol ensures that dehydration cannot occur too early in the synthesis, thus preventing styrene-like polymerisation occurring during the subsequent steps. Since Monahan showed that formylation using dichloromethyl methyl ether and a
metal chloride gave poor results, other formylation methods, including indirect methods, would have to be investigated to obtain the aldehyde (57). This intermediate also meets the requirements of the second criterion since the indole ring can be elaborated as described in Chapter 1 (Scheme 2).22 The methodology developed by Moody30 using the aldehyde (57) and ethyl azidoacetate would allow elaboration to the indole (58). Subsequent decarboethoxylation and anti-elimination of the protected alcohol affords the target molecule, (±)-iso-trans-trikentrin B (5) (Scheme 13).

2.3 CIS-1,3-DIMETHYLINDAN (14) AS A STARTING, MODEL COMPOUND

Since it was much more readily accessible, it was considered preferable to establish the synthetic pathway using cis-dimethyl indan (14) while optimising the route for the preparation of the trans-isomer (17).

2.3.1 Synthesis of cis-1,3-Dimethylindan (14)

cis-1,3-Dimethylindan (14) was obtained following the procedure developed by MacLeod and Monahan22 with some modifications. The first step involved the Grignard reaction between 2-bromoacetophenone and allylmagnesium bromide.
Addition of 2-bromoacetophenone to the Grignard reagent formed from allyl bromide and magnesium in diethyl ether gave inconsistent and poor results. Mixtures of the expected bromoarylpentenol (12) and the corresponding debrominated arylpentenol (59) (Scheme 14) were obtained.

Further reaction of the bromoarylpentenol (12) with unreacted magnesium followed by acid hydrolysis was the likely source of the debrominated species (59). To overcome this problem, the Grignard reagent allylmagnesium bromide was prepared in a separate flask and the solution was separated from any unreacted magnesium by decanting it to another reaction vessel. The solution of 2-bromoacetophenone in diethyl ether was then added dropwise to the Grignard reagent and the reaction was allowed to proceed as before. The reaction gave a crude yield of 85% of the required bromoarylpentenol (12) as observed by TLC, \(^1\)H and \(^{13}\)C NMR spectroscopy and mass spectrometry. Due to the lability of the benzylic alcohol, compound (12) was used without further purification.

![Scheme 14](image)
Radical cyclisation of the bromoarylpentenol (12) was carried out in benzene heated to reflux with tributyltin hydride and the initiator azobisisobutyronitrile (AIBN) under the conditions of 'infinite dilution'. This process involves the addition of the hydride to compound (12) over a long period of time so as to minimise the proportion of directly reduced product (59) to the cyclised products (60) and (61). Since the cyclisation reaction for such systems is faster than the hydrogen abstraction reaction\(^3\) (i.e. \(k_{\text{cyc}} > k_H\)), keeping the concentration of tributyltin hydride very low compared to the concentration of the bromopentenol (12) allows only a minimal amount of abstractable hydrogen to be available which permits cyclisation to predominate.

The reaction gave a mixture of trans- and cis-1,3-dimethylindanols (60) and (61) (approximately 2:3) in a 78% yield (Scheme 15). The 1,3-dimethylindanols (60) and (61) obtained are products of 'exo' ring closure\(^3\) and there was no evidence of the six-membered ring which would occur from 'endo' cyclisation.
On one occasion the indanols (60) and (61) (in a ratio of 2 : 3) were separated by careful column chromatography. The less polar compound was obtained as a colourless solid with a melting point at 72°C which was in agreement with that quoted by Plattner and co-workers. The more polar compound was a colourless oil. X-ray crystallographic data collected on the crystalline diastereomer confirmed that it was trans-1,3-dimethylindanol (60) (Figure 2). By deduction, the second fraction was cis-1,3-dimethylindanol (61).
Figure 2. Structural representation of trans-1,3-dimethylindanol (60) by X-ray crystallographic data.
$^1$H and $^{13}$C NMR data was collected on the diastereomers and unambiguous assignments were determined (see Experimental section). There were two major chemical shift differences between the diastereomers. The benzylic proton in trans-1,3-dimethylindanol (60) resonated at 3.07 ppm and the corresponding proton in the cis-compound (61) resonated at 3.34 ppm. Another large difference was observed for the C-1 methyl protons. The protons resonated at 1.34 ppm for the trans-dimethylindanol (60) and at 1.60 ppm for the cis-dimethylindanol (61). Hence, the separable diastereomers could also be easily identified and distinguished from each other by $^1$H NMR spectroscopy.

A catalytic amount of para-toluenesulfonic acid (p-TSA)$^{22}$ was used to eliminate water from the mixture of indanols (60) and (61), to produce 1,3-dimethylindene (13) (Scheme 16). The crude indene was then stirred with 10% palladium on charcoal under an atmosphere of hydrogen at ambient temperature and pressure.$^{22}$ Chromatography gave a colourless oil that contained > 95% cis-1,3-dimethylindan (14), by $^1$H NMR spectroscopy, in an overall yield of 96%. The cis-stereochemistry was assigned on the basis of its $^1$H NMR spectrum which was in agreement with literature data.$^{29}$

\[ \text{(60) and (61)} \rightarrow \text{(13)} \rightarrow \text{(14)} \]

Scheme 16
2.4 CONSTRUCTION OF THE FOUR CARBON UNIT SIDECHAIN

Friedel-Crafts acylation of cis-dimethylindan (14) using aluminium trichloride and butyryl chloride\(^{27}\) gave 5-butyroyl-1,3-dimethylindan (55) in an 82% yield (Scheme 17). The mass spectrum of compound (55) showed a molecular ion at \(m/z\) 216 together with characteristic fragment ions at \(m/z\) 188 (loss of ethene from the sidechain from a McLafferty Rearrangement) and at \(m/z\) 173 (loss of a propyl group). Further evidence for the presence and position of the butyroyl group was provided by the \(^{13}\)C NMR (APT - attached proton test) and \(^1\)H NMR spectra of (55). Evidence that acylation occurred in the 5-position came from the aromatic region in the \(^1\)H NMR spectrum where a singlet was observed at 7.78 ppm, indicative of the proton on C-4. Two doublets were observed at 7.83 and 7.25 ppm, each with a coupling constant of 8.0 Hz which is characteristic of orthocoupling.\(^{34}\) There was no evidence of meta-coupling.

![Scheme 17](image)

Reduction of (55) to the alcohol (56) with sodium borohydride occurred in a high yield (98%). Both the \(^1\)H and \(^{13}\)C NMR spectra showed that more than one
diastereomer was present. It was not necessary to separate and differentiate the
diastereomers since the hydroxyl group would eventually be eliminated to form the
butenyl sidechain.

Previous formylation attempts on the alcohol (56) gave mainly polymeric material
which suggested that the benzylic hydroxyl group could be readily eliminated
allowing subsequent styrene-like polymerisation to occur. Therefore, protection of
the benzylic hydroxyl group was required prior to formylation of the butylindan.

The hydroxyl-protecting group selected would have to withstand Vilsmeier-Haack
or lithiation / formylation conditions (the formylation methods to be used to
introduce the formyl group on (56)). A methoxymethyl ether (MOM) group is
stable towards a wide range of reaction conditions including the proposed
formylation conditions. The MOM group might also prove beneficial if
formylation was attempted using lithiation conditions. It has previously been used
as an ortho-directing group when present as an aromatic ether. Also, several
MOM-deprotection methods are known that give the parent alcohols in high
yields.

The MOM group was incorporated using the standard method of Stork and
Takahashi to give (63) (94% yield) (Scheme 18).
2.5 ATTEMPTED FORMYLATIONS OF THE BUTYL-1,3-DIMETHYL-INDAN COMPOUNDS

2.5.1 A Classical Method

The Vilsmeier-Haack reaction is one of the most common methods for the formylation of aromatic rings. The MOM ether (63) was subjected to the Vilsmeier-Haack reaction conditions of Buu Hoi et al. (Scheme 19). The major product was identified as the deprotected hydroxybutylindan (56) by $^1$H NMR spectroscopy and TLC. A mixture of other compounds was suggested by TLC. The $^1$H and $^{13}$C NMR spectra were complex but signals characteristic of an aromatic aldehyde were not observed. The deprotection of the MOM substituent may have been due to traces of acid that were present with the phosphorus oxychloride (POCl$_3$) reagent, even though the reagent was rigorously purified.
Similar reaction conditions were tried on indan (64). The starting indan was the only compound recovered (96%). It appeared the benzene ring in these indan systems was not sufficiently active for formylation to occur under these conditions.

2.5.2 Lithiation Attempts

The next approach employed to obtain the aldehyde (65) was by the metalation of the MOM ether (63) with an alkyl lithium and subsequent quenching with a formyl-producing reagent such as N,N-dimethylformamide (DMF).
The exchange of a hydrogen atom attached to an sp²-hybridised carbon atom (closest to a heteroatom) by lithium, are lithiations characterised by rate enhancement and a high degree of regioselectivity. For ortho- or beta- lithiations the metalating agent is directed to deprotonate the sp²-carbon atom ortho- to the heteroatom-containing substituent (Figure 3).

![Figure 3: Ortho- or beta- lithiations.](image)

Powerful alkylmetalating reagents are required for the ortho-directed metalation reactions. The choice of the alkylmetalating reagent, solvent and reaction temperature are very important. Alkyllithiums are oligomers of varying complexity in solution and can coordinate with Lewis bases such as amines which cause depolymerisation to some extent. Kinetically these reagents become more basic as...
the aggregate size decreases. For instance, \( n \)-butyllithium (\( n \)-BuLi) in
tetrahydrofuran (THF) is dimeric and is a stronger base than \( n \)-BuLi in a
hydrocarbon solvent which is hexameric. The addition of a compound such as
tetramethylethylenediamine (TMEDA) to a solution of \( n \)-BuLi in a hydrocarbon
solvent, causes the aggregate to break down from a hexamer to the more basic and
more active monomer. \( sec \)-Butyllithium (\( sec \)-BuLi) in a hydrocarbon solvent is of a
similar basic strength as \( n \)-BuLi / TMEDA in a hydrocarbon solvent. The
strongest alkyl lithium base is \( t \)-butyllithium (\( t \)-BuLi).

Another metalating option was to use the \( n \)-BuLi / NaOr-Bu (or KOt-Bu) reagents
which are known as super-bases due to their superior basic strength compared to
\( n \)-and \( sec \)-BuLi.\(^42,43\) Investigations into the applicability of the superbases is an
evolving and developing field.\(^36\) Work has been carried out in order to determine
the exact nature of the metalating mixture and as yet there is no conclusive
answer.\(^42\)

Aromatic substituents containing heteroatoms such as oxygen and nitrogen direct
lithiation \( ortho \)-, and the stronger the directing group, the higher the degree of
lithiation \( ortho \)- to that group. The most powerful groups include amides and
oxazolines. Ethers, alcohols and amines also have quite a good directing
ability.\(^36,41\)

2.5.2.1 Lithiation of the MOM ether (63)

It was anticipated that the MOM group on the benzylic carbon of (63) would direct
metalation \( ortho \)- to the butyl group. Of the two possible \( ortho \)- positions, the
6-position appears the more likely to be metalated since this is the least hindered
site. Numerous metalating reagents were tried on the MOM ether substrate (63)
including \( n\text{-BuLi}, \, n\text{-BuLi} / \text{TMEDA}, \, t\text{-BuLi}, \, \text{NaOt-Bu} / n\text{-BuLi} \) and \( \text{KOT-BuLi} / n\text{-BuLi} \). The reactions were carried out using a variety of different conditions that included varying the solvent (hexane, diethyl ether, heptane); temperature; time; and additives (e.g. TMEDA) (See Table 1 in the Experimental section). The metalation reaction mixtures were quenched with the deuterated electrophile \( \text{D}_1\text{-methanol} \) (\( \text{CH}_3\text{OD} \)) in order to gauge the success of the metalation attempts.

Mass chromatograms from the GC/MS of the product and starting material were used to calculate the percentage incorporation of deuterium. The ions used were those at \( m/z \) 219 and 220. The fragment ion at \( m/z \) 219 in the mass spectrum of the starting material was the base peak arising from the loss of a propyl radical from the molecular ion of the starting material (Figure 4), while that at \( m/z \) 220 was due to its \(^{13}\text{C} \) natural abundance. Any deuterium incorporation in the aromatic ring, as a result of H/D exchange in the product following lithiation, could be readily measured by any shift of \( m/z \) 219 to 220, after correction for \(^{13}\text{C} \) natural abundance. No deuterium incorporation was observed in any instances as deduced by mass spectrometry.

Confirmation that compound (63) was not deuterated came from the \(^1\text{H} \) NMR spectra of the product. There was no relative decrease in any of the intensities of the proton signals. Even when strong bases such as \( n\text{-BuLi} / \text{TMEDA} \) (see Entry 3, Table 1, Experimental section), the super-bases (see Entries 5 - 8) or \( t\text{-BuLi} \) (see Entries 9 and 10) were used, there was no deuteration observed. A high proportion of starting material was recovered in each case.
Steric hindrance from the MOM group was a possible reason for the unsuccessful deprotonation attempts. However, it might be expected that the monomeric n-BuLi with TMEDA (see Entry 3) should be small enough to access the aromatic protons. It appeared that the aromatic protons, in particular that on C-6, were not acidic enough for deprotonation to occur even though strong bases were tried.

2.5.2.2 Lithiation of the Hydroxybutylindan (56)

Metalation of the hydroxybutylindan (56) was attempted next. This would require at least two equivalents of base, one to abstract the hydroxyl proton and the other to deprotonate the aromatic proton. It was hoped that once the readily abstracted hydroxyl proton was removed, the resulting ionic species would not only make the system more activated but would also direct the alkylmetalating reagent ortho- to the butoxide substituent.
Initially, the reactions were quenched with CH$_3$OD. The results of the metalation reactions of the hydroxybutyldindan (56) are shown in Table 2. The reaction conditions were similar to those for the metalation of the MOM ether (63).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method</th>
<th>Metalation Conditions</th>
<th>% Deuteration$^a$</th>
<th>% Recovery$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>sec-BuLi/10%CH$_3$OD</td>
<td>0% (100%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>MTBD/10%CH$_3$OD</td>
<td>0% (100%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>KOt-Bu/10%CH$_3$OD</td>
<td>0% (100%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>t-BuLi/10%CH$_3$OD</td>
<td>20% (80%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>BuLi/10%CH$_3$OD</td>
<td>80% (100%)</td>
<td></td>
</tr>
</tbody>
</table>

The moderate strength bases, sec-BuLi and KOt-Bu / n-BuLi, were initially used but there was no evidence of deuterium incorporation (Entries 1 - 3, Table 2). With t-BuLi, however, high levels of deuterium incorporation were observed. The conditions for deuteration were optimised (Entry 5, Table 2) giving 80% of recovered product. A broad singlet at 7.19 ppm appeared in the $^2$H NMR spectrum indicating that deuteration had occurred in the aromatic ring. In the $^1$H NMR spectrum two sharp singlets at 7.14 and 7.19 ppm, with no observable coupling, suggested that H-6, ortho- to the hydroxybutyl substituent, had been abstracted and replaced by a deuterium atom. Mass spectral data showed there was incorporation of only one deuterium atom in the hydroxybutyldindan.
Table 2. Metalation conditions for cis-1,3-dimethyl-5-(1'-hydroxybutyl)-inden (56) quenched with CH₃OD.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Metalation Conditions&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Deuteration&lt;sup&gt;c&lt;/sup&gt; (%) Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>secBuLi(x4)/hexane/reflux/180min</td>
<td>0% (100%)</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>KOtBu(x1)/nBuLi(x2)/hexane</td>
<td>0% (95%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-60°C/60min</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>KOtBu(x1.5)/nBuLi(x3) hexane</td>
<td>0% (95%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reflux/120min</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>tBuLi(x6)/heptane/reflux/50min</td>
<td>70% (100%)</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>tBuLi(x9)/heptane/reflux/180min</td>
<td>94% (80%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The general methods (A and B) followed for the metalations are detailed in the Experimental section. <sup>b</sup>Molarity of butyllithium solutions were determined according to the procedure by Winkle <i>et al.</i><sup>44</sup> <sup>c</sup>The percentage deuteration was calculated from mass spectral data.

Once again the mass spectral data for the product compared to that of the starting material was used to calculate the percentage incorporation of deuterium. The ions used were the [M<sup>**</sup> – C₃H₇<sup>-</sup>] ion at m/z 175 (base peak) and 176 (Figure 4). The relative abundance of the ion at m/z 176 in the mass spectrum of the deuterated product, corrected for natural abundance of ¹³C of the ion at m/z 175, showed that 94% deuterium had been incorporated.
2.5.3 Formylation and Elaboration of (56)

2.5.3.1 Formylation

The formylation of the lithiated species was investigated and developed now that the lithiation conditions had been optimised. Quenching the aryllithium with DMF gave a mixture of products for which NMR and mass spectral data were consistent with the presence of starting material (56), the lactol (66) and the hydroxyaldehyde (67) (Scheme 20). The typical yield for the mixture of the lactol (66) and the hydroxyaldehyde (67) was between 70 and 85%.

The two products could be separated from the remaining starting material by chromatography. However, the lactol (66) and the hydroxyaldehyde (67) were inseparable and the mixture was obtained as a colourless, viscous oil. In its mass spectrum, a molecular ion at \( m/z \) 246 was observed consistent with structures (66) and (67). Also, fragment ions at \( m/z \) 228 (loss of water), 213 (loss of water and a methyl group) and 203 (loss of a propyl group) were observed. In its \(^1\)H NMR
spectra, two sharp singlets were observed in the aromatic region (resonating at 7.20 and 6.97 ppm). There was a weak signal at 10.09 ppm with an intensity equivalent to less than 0.1H indicating that only a minor amount of aldehyde (67) was present.\textsuperscript{34} Also present was a singlet at 6.44 ppm (\textasciitilde 0.5H) and a doublet at 6.38 ppm (\textasciitilde 0.5H) which may be attributed to the hemiacetal protons of the diastereomeric forms of the lactol (66). The hemiacetal carbons of the diastereomeric mixture were present in the \textsuperscript{13}C NMR at 100.65 and 100.56 ppm. The proton on the other benzylic carbon in the lactol ring in (66) was also split into two signals at 5.38 ppm (\textasciitilde 0.5H) and 5.12 ppm (\textasciitilde 0.5H). Thus, any interconversion between the open hydroxyaldehyde form (67) and the lactol (66) strongly favoured the latter. The spectral data confirmed that lactol (66) was present and thus substitution had occurred in the 6-position.

2.5.3.2 Attempted Formation of the Pyrrole Ring

The molecule must be in the open, hydroxyaldehyde form (67) to permit the elaboration of the pyrrole ring. It has been shown for a similar system\textsuperscript{45} that basic conditions shift the equilibrium to the closed, lactol form (68) while acidic conditions favour the open form (69) (Scheme 21).

\[ \text{H} \xrightarrow{\text{H}_2\text{O}^+} \text{H} \]

\[ \text{OH} \]

\[ \text{H} \rightarrow \text{H} \]

\[ \text{OH} \]

\( (68) \)

\( (69) \)

Scheme 21
The formylated mixture of (66) and (67) was dissolved in a range of solvents with acetic acid or triethylamine added. No change in the proportion of the two forms was observed by $^1$H NMR spectroscopy whether the solution was acidified or basified in increments.

At this stage it was decided to try to continue with the proposed pathway and react the equilibrium mixture of (66) and (67) with sodium ethoxide and ethyl azidoacetate according to the procedure employed by Moody. If sufficient of the aldehyde form (67) was present to condense with the azide reagent to give the required azidocinnamate (70) then it would force the lactol / hydroxyaldehyde equilibrium towards the latter (Scheme 22).

The lactol / hydroxyaldehyde mixture (66) and (67), sodium ethoxide and ethyl azidoacetate were reacted according to the procedure followed by MacLeod and Monahan. The starting material was consumed to give one band by TLC. Upon work-up, a yellow oil was obtained. $^1$H and $^{13}$C NMR spectroscopy and the infrared spectrum of the product indicated the absence of the expected azide and ethyl ester functionalities. The $^1$H NMR spectrum showed that signals consistent with the cis-dimethylindan moiety were still evident but signals for the azidocinnamate (70) were not observed. The compound decomposed before a mass spectrum could be acquired.
The next option was to find a mild method of protecting the aldehyde functionality of (67) (Scheme 23). The free benzylic hydroxyl substituent could then be selectively protected with a group that was capable of withstanding the basic conditions of the subsequent azidoacetate condensation reaction.
It was decided to use the MOM group to protect the hydroxyl group since it was known to be stable under basic conditions. Therefore a suitable aldehyde-protecting group had to be selected that could withstand the conditions required for the formation of the MOM ether (i.e. diisopropylethylamine and methoxymethyl chloride).

Initially, attempts were made to protect the aldehyde functionality of the mixture of (66) / (67) as the ketal (71, X = O). A method reported by Brown et al. using...
adipic acid and ethane diol was followed. Mainly starting material was recovered (81%) which was in the lactol form (66) by $^1$H NMR spectroscopy. The other, minor component was a yellow oil that appeared to be decomposed material by $^1$H NMR spectroscopy. Varying the conditions did not improve the ketalisation reaction.

Thioketalisation was attempted on the equilibrium mixture of (66) and (67) using ethane dithiol and boron trifluoride etherate. The $^1$H NMR spectrum was complex and indicated a mixture of compounds. The chemical shifts of the observed signals did not correspond to the values expected for the thioketal (72, $X = S$).

### 2.5.4 Alternative Approach to the Formylindan Intermediate (73)

It was apparent that the synthesis could not proceed through the lactol (66). Therefore it was proposed that if a halogen, such as bromine, was substituted in the 6-position then the hydroxyl group could be suitably protected (Scheme 24). A metal-halogen exchange could then be carried out and quenching the reaction with a formylating reagent should give the desired carbaldehyde (73).

The hydroxybutylindan (56) was lithiated with $t$-BuLi using the optimised conditions that had been previously established. A variety of brominating reagents were used including bromine ($\text{Br}_2$), pyrrolidone hydrotribromide, N-bromosuccinimide (NBS), 1,2-dibromoethane, 1-bromo-2-chloroethane, 1,2-dibromotetrachloro-ethane (1,2-DBTCE) and carbon tetrabromide ($\text{CBr}_4$) (see Table 3, Experimental section).
Treating the lithiated species with bromine only resulted in decomposition. Since there could have been trace amounts of hydrogen bromide present in the bromine that may have caused the decomposition, one reaction was carried out in the presence of potassium carbonate. Decomposition was again observed. Reaction with pyrrolidone hydrotribromide, considered to be a milder brominating reagent than bromine, gave recovered starting material and another compound.

Comparison of the $^1$H NMR spectrum of the isolated compound with that of R = alcohol protecting group.

Scheme 24

Treating the lithiated species with bromine only resulted in decomposition. Since there could have been trace amounts of hydrogen bromide present in the bromine that may have caused the decomposition, one reaction was carried out in the presence of potassium carbonate. Decomposition was again observed. Reaction with pyrrolidone hydrotribromide, considered to be a milder brominating reagent than bromine, gave recovered starting material and another compound.

Comparison of the $^1$H NMR spectrum of the isolated compound with that of R = alcohol protecting group.
5-butyroyl-1,3-dimethylinden (55) suggested the identity of the product was the indan (55). N-bromosuccinimide (NBS) was also used to quench the reaction mixture and only (55) was produced (Scheme 25). Although NBS is known to brominate benzylic positions\(^{48}\) it is also known to oxidise alcohols to ketones.\(^{49}\)

![Scheme 25](image)

1,2-Dibromoethane and 1-bromo-2-chloroethane were used as brominating reagents with some success (Scheme 26). The ratio of bromoindan (74) to recovered starting material was 1:4 using dibromoethane, and 3:5 using bromochloroethane. Although there were two halogen atoms available for abstraction from both reagents, statistically there were more hydrogens available. Inductive effects due to the halogens rendered the hydrogens quite acidic. Therefore, it was not unexpected when some starting material was recovered.
Confirmation that 6-bromo-5-(1'-hydroxybutyl)-cis-1,3-dimethyl indan (74) was obtained came from analysis of its spectral data. The characteristic 1:1 pattern of the molecular ions at \( m/z \) 298 and 296 indicated that one bromine atom was present. The \(^1\)H NMR spectrum showed a changed aromatic region with only singlets present. One singlet, equivalent to one proton, was observed at 7.35 ppm. Two other sharp singlets were observed at 7.30 ppm and at 7.29 ppm (each ~0.5H) for the diastereomers. This suggested the two aromatic protons were \textit{para-} to each other.

In order to eliminate the possibility of the aromatic carbanion reacting with a proton source, the next brominating reagents selected contained only halogens on the carbon skeleton. Quenching with either 1,2-dibromotetrachloroethane (1,2-DBTCE) or carbon tetrabromide (CBr\(_4\)) gave improved yields of compound (74). Although both reagents gave similar results, CBr\(_4\) was the reagent of choice since it had the advantage of being readily available in large quantities.
Conditions using freshly purified CBr₄ were initially optimised to yield 75% of the isolated 6-bromohydroxybutylin[an (74) and recovered starting material (20%). However, when this reaction was repeated sometime later using the same procedure but a new batch of purified CBr₄, inexplicably the high yield of brominated product could not be obtained. Instead, an approximately 1:1 mixture of the bromoindan (74) to starting material (56) was afforded. This problem was never resolved and since starting material was recovered that could be recycled, it was not considered to be a serious one.

Protection of the hydroxyl group as the MOM ether was carried out as before and gave the MOM ether (75) in very good yields (Scheme 27). The diastereotopic methylene protons of the MOM group were observed in the ¹H NMR spectrum as doublets at 4.55 (4.53)* ppm and 4.52 (4.51) ppm.

Scheme 27

*NMR values in parentheses indicate those belonging to the other diastereomer.
Protection of the hydroxyl group meant that a formyl group in the 6-position could now be introduced without lactol formation. Addition of n-BuLi to the MOM ether (75) followed by the addition of DMF gave an oil (81% isolated yield) whose spectral data was consistent with the structure of aldehyde (76) (Scheme 27). A strong band observed at 1700 cm\(^{-1}\) in the infrared spectrum was characteristic of an aryl-aldehyde carbonyl absorption.\(^{34}\) Its electron impact - mass spectrum (EI-MS) gave an [M - 1]\(^-\) ion at \(m/z\) 289 and its chemical ionisation - mass spectrum (CI-MS) gave an \(MH^+\) ion that indicated the presence of aldehyde (76). Also characteristic of aldehydes were singlets at 10.33 (10.31) ppm that had a cumulative intensity equivalent to one proton. Characteristic of aryl aldehyde groups,\(^{50}\) was the presence of two signals at 192.4 (192.3) ppm in the \(^{13}\)C NMR spectrum. The multiplicity of the signals indicated the presence of two diastereomers.

### 2.6 ANNULATION AND ELABORATION OF THE PYRROLE RING

With one of the major intermediates (76) in hand, albeit by a slightly longer route than initially anticipated, the next step was the construction of the pyrrole ring. Previous work had shown that the condensation of an aromatic aldehyde with a reagent such as ethyl azidoacetate followed by thermolysis produced good yields of the corresponding indoles.\(^{22,30}\)

#### 2.6.1 Formation of the Azidocinnamate (77)

Some modifications were made to the method developed by Moody and co-workers\(^{30}\) to optimise the yield of the azidocinnamate (77) (Scheme 28). The
product (77) was isolated using column chromatography but it was not stable enough to be fully characterised. Nevertheless infrared, NMR and mass spectral data could be obtained on the compound. A strong band at 2130 cm\(^{-1}\) in the infrared spectrum was observed confirming the presence of the azide functionality. Another strong band at 1715 cm\(^{-1}\) indicated the presence of a carbonyl group that was in the expected region for the carbonyl absorbance of an \(\alpha,\beta\)-unsaturated ester.\(^{34}\) In the \(^1\)H NMR spectrum, the ethyl ester methylene group was observed as a quartet at 4.38 ppm (\(J\ 7.1\) Hz) and the methyl group as a triplet at 1.40 ppm (\(J\ 7.1\) Hz). The \(^13\)C NMR spectrum of (77) confirmed the presence of the ethyl azidoacetate group with signals for the ethyl ester group (163.4, 62.1 and 14.1 ppm).

![Chemical structures](image)

\[(76) \xrightarrow{\text{NaOEt, } N_3CH_2CO_2Et, -15\text{ to } -10^\circ\text{C}} (77)\]

\[(78)\]

Scheme 28
An acceptable yield of the purified azidocinnamate (77) was obtained ranging between 55 and 63%. This was lower than that obtained previously\(^\text{22}\) for the azidocinnamate (78), produced from a similar indan system. The lower yield was probably due to the instability of the reagent and the steric hindrance of the MOM group partially shielding the aldehyde from the approaching reagent. There was also the possibility of side reactions occurring, such as the Cannizzaro reaction.

![Scheme 29](image-url)
Cyclisation by heating the azidocinnamate (77) gave two products (Scheme 29). The major compound proved to be the desired indole (79), and the minor compound, isoquinoline (80). The latter arises via nitrene insertion into the benzylic position of the alkoxybutyl sidechain with subsequent loss of the oxygen group.

Moody,30 and Rees and co-workers51 have found evidence that the cyclisation reaction of azidocinnamates, when subjected to high temperatures, occurs via the azirine (81) and vinyl nitrene (82) (Scheme 30). It appears that the azirine (81) is in thermal equilibrium with the vinyl nitrene (82).52 Since this provides a mechanism for Z- / E- isomerisation of the vinyl double bond, the original geometry of the azidocinnamate from the condensation reaction is unimportant. Moody30 has also stated that the mechanism involves the electrocyclisation of the vinyl nitrene (82), followed by a rapid aromatising [1,5]-hydrogen shift, rather than a simple insertion into the aromatic C-H bond.

When an alkyl substituent is ortho- to the azide substituent on the azidocinnamate (e.g. the intermediate (77), Scheme 29), two reaction pathways can occur. The vinyl nitrene can cyclise onto the free ortho-position to give the indole (e.g. indole (79), Scheme 29), or it can interact with the ortho-alkyl substituent to give six- or larger-membered rings (e.g. the isoquinoline (80)).30,51,53
2.6.2 Characterisation of (79) and (80)

A molecular ion at \( m/z \) 373 was present in the mass spectrum of the isolated indole (79). Characteristic fragment ions at \( m/z \) 330 (loss of a propyl group) and 312 (loss of OMOM) were also observed. The infrared spectrum of (79) showed the indole N-H stretch at 3455 cm\(^{-1}\) and the strong carbonyl band shifted from 1715 cm\(^{-1}\) for the azidocinnamate (77) to 1700 cm\(^{-1}\) for the indole (79). 2D NMR spectroscopy (COSY, HETCOR and LRHETCOR) as well as APT and coupling constants in the \(^1\)H NMR spectrum assisted in determining the \(^1\)H and \(^13\)C NMR assignments (Table 4).
Table 4. $^1$H and $^{13}$C NMR assignments$^a$ for indole (79).

<table>
<thead>
<tr>
<th></th>
<th>$^1$H Assignment$^b$</th>
<th>$^{13}$C Assignment$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.73, bs</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>134.7 (134.6)$^c$</td>
</tr>
<tr>
<td>3</td>
<td>7.41 (7.40), d, $J$ 1.9 Hz</td>
<td>108.1 (108.0)</td>
</tr>
<tr>
<td>3a</td>
<td>–</td>
<td>126.4$^c$</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>129.00 (128.97)$^d$</td>
</tr>
<tr>
<td>5</td>
<td>6.97, s</td>
<td>115.4 (115.1)</td>
</tr>
<tr>
<td>5a</td>
<td>–</td>
<td>124.9$^d$</td>
</tr>
<tr>
<td>6</td>
<td>3.45, m</td>
<td>37.1</td>
</tr>
<tr>
<td>7</td>
<td>2.64, 1.35, m</td>
<td>44.1</td>
</tr>
<tr>
<td>8</td>
<td>3.21, m</td>
<td>39.03 (38.97)</td>
</tr>
<tr>
<td>8a</td>
<td>–</td>
<td>146.0</td>
</tr>
<tr>
<td>8b</td>
<td>–</td>
<td>134.1$^c$</td>
</tr>
<tr>
<td>1'</td>
<td>4.94, m</td>
<td>77.1</td>
</tr>
<tr>
<td>2'</td>
<td>2.00 (1.80), m</td>
<td>39.5 (39.4)</td>
</tr>
<tr>
<td>3'</td>
<td>1.51 (1.35) m</td>
<td>19.6</td>
</tr>
<tr>
<td>4'</td>
<td>0.94, t, $J$ 7.3 Hz</td>
<td>13.9</td>
</tr>
<tr>
<td>CO$_2$Et</td>
<td>–</td>
<td>162.1</td>
</tr>
<tr>
<td>CO$_2$CH$_2$CH$_3$</td>
<td>4.41, q, $J$ 7.2 Hz</td>
<td>60.8</td>
</tr>
<tr>
<td>CO$_2$CH$_2$CH$_3$</td>
<td>1.43, t, $J$ 7.2 Hz</td>
<td>14.3</td>
</tr>
<tr>
<td>OCH$_2$O</td>
<td>4.56, m</td>
<td>94.0 (93.9)</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>3.43 (3.41), s</td>
<td>55.5</td>
</tr>
<tr>
<td>6-CH$_3$</td>
<td>1.51, $d$, $J$ 6.9 Hz</td>
<td>20.8</td>
</tr>
<tr>
<td>8-CH$_3$</td>
<td>1.37 (1.36), $d$, $J$ 6.9 Hz</td>
<td>20.63 (20.57)</td>
</tr>
</tbody>
</table>

$^a$ $^1$H measured at 300 MHz; $^{13}$C measured at 75.4 MHz; $\delta$ in ppm from tetramethylsilane. $^b$ The signal of the other diastereomer is listed in parentheses. $^c,d$ The resonance is interchangeable with that with the same superscript.
The HETCOR (heteronuclear correlation spectroscopy) data connected all the protons that were one-bond coupled to their respective carbons (Table 4). The broad singlet at 8.73 ppm was characteristic of the indole N-H. A proton-proton correlation (COSY) with weak intensity (Figure 5) was observed between the N-H signal at 8.73 ppm and that at 7.41 (7.40) ppm, suggesting that the latter proton was on C-3 (Figure 5). H-3 was observed as doublets at 7.41 (7.40) ppm ($J = 1.9$ Hz) for the diastereomers of (79). This coupling pattern has previously been observed for a similarly substituted indole.22 The remaining signal in the aromatic region was a singlet at 6.97 ppm which was assigned as H-5. Assignment of the quaternary carbons in the indole moiety came from LRHETCOR (long range heteronuclear correlation spectroscopy) correlations (Figure 6). These were observed between H-3 at 7.41 (7.40) ppm and the carbon signals at 134.7 (134.6), 134.1 and 126.4 ppm (therefore assigned as C-2, C-3a and C-8b), and between H-5 at 6.97 ppm and carbon signals at 129.00 (128.97) and 124.9 ppm (therefore C-4 and C-5a). The signal at 162.1 ppm was characteristic of the carbonyl carbon of the ester. By elimination, the quaternary carbon signal at 146.0 ppm could be assigned as C-8a. A long range correlation observed between it and an indan methyl group, which was anisochronous at 1.37 (1.36) ppm, defined this as the C-8 methyl substituent.
Figure 5. Observed COSY correlations for the indole (79).

Figure 6. Observed LRHETCOR correlations for the indole (79).
The protons on the methyl group at 1.37 (1.36) ppm (CH\textsubscript{3} on C-8) were connected (LRHETCOR) to the benzylic carbon at 39.03 (38.97) ppm (C-8). This was in turn correlated (HETCOR) to the multiplet at 3.21 ppm which was assigned as H-8. From NMR analysis of other cis-1,3-dimethylindan systems it was known that the methylene carbon resonated at ~44 ppm, and so the signal at 44.1 ppm was assigned as C-7. The HETCOR spectrum indicated that the anisochronous protons at 2.64 and 1.35 ppm were the protons on C-7. A LRHETCOR correlation between the isochronous methyl protons on C-6 at 1.51 ppm and the carbon signal at 37.1 ppm confirmed that it was C-6.

The chemical shift and the coupling pattern (quartet, $J = 7.2$ Hz) for the methylene signal at 4.41 ppm was assigned to the methylene group of the ethyl ester and a triplet at 1.43 ppm, with the same coupling constant, to the methyl group of the ester (COSY). LRHETCOR data showed a correlation between the carbonyl carbon at 162.1 ppm and the methylene protons at 4.41 ppm. These methylene protons in turn showed a two-bond correlation with the carbon signal at 14.3 ppm (methyl group of the ethyl ester).

The $^1$H and $^{13}$C NMR assignments for the MOM-protected butoxide sidechain were readily carried out. The carbon at 55.5 ppm, characteristic of the methoxyl of the MOM group, showed a long range correlation to the protons at 4.56 ppm confirming that these were the methylene protons of the MOM group. These protons also showed a correlation to the carbon at 77.1 ppm on the butyl sidechain, verifying it as C-1'. H-1' at 4.94 ppm showed a long range correlation to the carbon at 115.4 (115.1) ppm confirming that this aromatic carbon was C-5 (Figure 6). The characteristic triplet at 0.94 ppm was assigned as the terminal methyl group (H-4') of the butyl sidechain which showed a LRHETCOR correlation to the carbon at 19.6 ppm (therefore C-3'). A HETCOR correlation
was observed between this carbon and the protons at 1.51 (1.35) ppm (hence C-3' protons). These protons were in turn correlated (COSY) to those at 2.00 (1.80) ppm (therefore C-2' protons). Hence, with the exception of the quaternary aromatic carbons, all the $^1$H and $^{13}$C NMR assignments of indole (79) were determined (Table 4).

NMR and mass spectral techniques aided in the confirmation of the structure of isoquinoline (80) (Scheme 29). Examples in the literature$^{30,51,53}$ show that the formation of the isoquinoline was not unexpected. High resolution accurate mass measurement of the molecular ion of (80) at $m/z$ 311 gave the value 311.1886 corresponding to the formula C$_{20}$H$_{25}$NO$_2$. In its $^1$H NMR spectrum, three protons were present as sharp singlets in the aromatic region (8.39, 7.90 and 7.67 ppm) and thus confirmed the substitution pattern of the aromatic moiety of the isoquinoline (80). The ethyl ester functionality was identified to be present in the compound by $^1$H and $^{13}$C NMR spectroscopy. The $^{13}$C NMR spectrum showed 10 carbons resonating in the region 166.2 to 118.3 ppm of which that at 166.2 ppm was characteristic of and assigned as the carbonyl carbon of the ester. Six of the remaining carbons in that region were quaternary and the other three were methine carbons (APT). The expected signals for the two methyl groups and the fused cyclopentane ring were also observed. The APT also showed two methylene carbons (37.7 and 23.1 ppm) and a methyl group (14.3 ppm) of the propyl sidechain. There were no signals attributable to the MOM ether nor signals for the oxygenated, benzylic methine (multiplet) on the sidechain. This evidence was consistent with the isoquinoline (80) structure.
2.6.3 Optimisation of Cyclisation Conditions

It was found that by rapidly adding the mixture of the azidocinnamate (77) and solvent to a hot bath instead of slowly heating the mixture to reflux, the overall yield of the two products increased and the ratio of indole (79) to isoquinoline (80) increased. This suggested that the 5-membered ring formation was not as energetically favourable as the six-membered ring formation. Reaction temperatures were then varied in an attempt to optimise the overall yield of indole (79) (Table 5). When the reaction was carried out in benzene at reflux, there was a very good overall yield of the two products (96%) of which 53% (isolated) was the required indole (79). In an attempt to improve the proportion of indole (79), the reaction temperature was increased by using toluene as the solvent. A decreased overall yield of the two products was observed but yields between 61 – 81% of the purified indole (79) were obtained. The reaction temperature was further increased by carrying out the reaction in xylene but gave only 26% of the purified indole (79), 15% of the purified isoquinoline (80) and a large amount of decomposition product. The optimum conditions involved the rapid addition of the solution of azidocinnamate (77) and toluene to a preheated bath, then cooling the reaction mixture down as soon as the starting material was consumed.

**Table 5.** Variation in yield of indole (79) and isoquinoline (80) products using increasing reaction temperatures.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Isolated Yield:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indole (79)</td>
</tr>
<tr>
<td>benzene</td>
<td>53%</td>
</tr>
<tr>
<td>toluene</td>
<td>61 - 81%</td>
</tr>
<tr>
<td>xylene</td>
<td>26%</td>
</tr>
</tbody>
</table>
Instead of using purified azidocinnamate (77) for the cyclisation, crude azidocinnamate (77) was used in order to try to minimise losses which occurred during purification. However, it was found that the cyclisation of the purified azidocinnamate (77) to the corresponding indole (79) went in a higher overall yield (58%) from the aldehyde (76) than that of the crude azidocinnamate (77) (41% overall yield). This result suggested that competing side-reactions in the crude azidocinnamate system occurred between the azidocinnamate (77) and the by-products from the previous reaction.

2.7 REMOVAL OF THE ETHYL CARBOXYLATE FUNCTIONALITY FROM (79)

2.7.1 General Approaches

To remove the carboxylic ester group, the most direct approach would be to hydrolyse the ester to the carboxylic acid followed by decarboxylation using one of the classical methods such as copper / quinoline\textsuperscript{54} or flash vacuum pyrolysis (FVP).\textsuperscript{55}

An alternative approach for removing the carboxylic ester group was exploited by Moody and co-workers.\textsuperscript{30,56} This route appears general for the removal of the alkyl carboxylate functionality from indole-2-carboxylates.\textsuperscript{57} It involves initial reduction of the indole-2-carboxylate to the primary alcohol\textsuperscript{30,57} and subsequent oxidation to the aldehyde by reagents such as manganese dioxide (MnO\textsubscript{2})\textsuperscript{30,57} or barium permanganate (BaMnO\textsubscript{4}).\textsuperscript{58} Decarbonylation of the aldehyde can be carried out under mild, neutral, non-oxidising conditions using a rhodium complex.\textsuperscript{30,56,57}
2.7.2 Hydrolysis of (79) and Subsequent Decarboxylation

The indole carboxylate (79) was hydrolysed with sodium carbonate dissolved in a mixture of water/methanol at 50°C to give the corresponding crude acid (83). It was obtained in a high yield with trace amounts of the corresponding transesterified methyl ester (84) present (Scheme 31). On a large scale, the yield of acid (83) was approximately 90%.

\[
\text{OMOM} \quad \text{CO}_2\text{Et} \\
\text{H} \\
\text{CO}_2\text{H} + \text{CO}_2\text{Me}
\]

\[
\text{OMOM} \\
\text{H} \\
\text{H}
\]

\[
\text{Na}_2\text{CO}_3, \text{ water} \\
\text{methanol, } \Delta
\]

Scheme 31
The acid (83) was unstable but could be characterised by mass spectral and $^1$H NMR data. A molecular ion at $m/z$ 345 and an ion at $m/z$ 302 (loss of propyl) both indicated the indole carboxylic acid (83) was present. There was a broad N-H signal (at 8.87 ppm) in the $^1$H NMR spectrum. A broader signal at 8.61 ppm was exchangeable with D$_2$O characteristic of an acidic hydroxyl functionality (CO$_2$H).

The indole-2-carboxylic acid (83) was treated with an excess of copper and quinoline at high temperatures$^{54}$ (Scheme 32), leading to a large amount of decomposition product. However, one product was isolated as a pink oil which was fully characterised as the decarboxylated indole (85). Since the reaction gave less than 10% yield, this was considered not to be a useful pathway to indole (85).

\[
\text{OMOM} \quad \text{OMOM} \\
\text{CO}_2\text{H} \quad \text{copper quinoline, } \Delta \\
\text{(83)} \quad \text{(85)}
\]

Scheme 32

1.7.3 Decarboxylation Method

The acid (83) was unstable but could be characterised by mass spectral and $^1$H NMR data. A molecular ion at $m/z$ 345 and an ion at $m/z$ 302 (loss of propyl) both indicated the indole carboxylic acid (83) was present. There was a broad N-H signal (at 8.87 ppm) in the $^1$H NMR spectrum. A broader signal at 8.61 ppm was exchangeable with D$_2$O characteristic of an acidic hydroxyl functionality (CO$_2$H).

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\[
\text{OMOM} \quad \text{OMOM} \\
\text{CO}_2\text{H} \quad \text{copper quinoline, } \Delta \\
\text{(83)} \quad \text{(85)}
\]

Scheme 32

1.7.3 Decarboxylation Method
It seemed that the conditions of this decarboxylation reaction were too harsh and in the absence of ready access to flash vacuum pyrolysis apparatus, the alternative procedure pioneered by Moody\textsuperscript{30,56} was investigated.

### 2.7.3 Decarboethoxylation Method

If the indole-2-carboxylate (79) could be reduced to the corresponding aldehyde (86) in one step, this would be more direct than going via the alcohol.\textsuperscript{30,56,57} The literature\textsuperscript{59,60} has reported that at low temperatures diisobutylaluminium hydride (DIBALH) reduces various esters of aliphatic and aromatic acids to aldehydes. However, at higher temperatures DIBALH reduces the esters to their corresponding alcohols.\textsuperscript{59} The procedure by Zakharkin and Khorlina\textsuperscript{60} was followed. The substrate (79), DIBALH and toluene were stirred at -74°C for a few hours, with no reaction observed by TLC. The reaction mixture was warmed to -41°C and after 1 h, a trace amount of a more polar material was observed by TLC. Upon work-up, the reaction yielded a yellow oil that contained mainly starting material by \textsuperscript{1}H NMR spectroscopy. There was no signal in the 9–11 ppm region where an aldehydic proton would be expected to resonate. The material was reacted further with DIBALH at room temperature. The reaction was monitored by TLC and showed the starting material was being consumed whilst the amount of the more polar material had increased. After 75 min the reaction was complete. The purified material was a yellow oil that was identified as the corresponding alcohol (87) in 88\% yield (Scheme 33). It appeared that subsequent reduction of the aldehyde to the resultant alcohol (87) at room temperature occurred much too rapidly to allow isolation of the intermediate aldehyde.
Indole and many of its derivatives are readily oxidised by a variety of reagents, commonly at C-3. However, Moody and Meyer and Kruse have found that oxidising indole-2-methanols that have a similar structure to (87) with MnO₂ or BaMnO₄ in hot dichloromethane gives the corresponding aldehyde. Oxidation of the alcohol (87) was successfully achieved by following the procedure by Moody and co-workers. Yields between 60 and 70% of the indole carboxaldehyde (86)
were obtained (Scheme 33). A strong band in the infrared spectrum at 1660 cm⁻¹, a singlet at 9.80 ppm in the ¹H NMR spectrum and a signal at 181.7 ppm in the ¹³C NMR spectrum confirmed that the aldehyde had been formed. The mass spectrum showed ions at \( m/z \) 329 (M⁺⁺) and 286 (loss of propyl) that were further evidence for the indole-2-carboxaldehyde (86).

Doughty and Pignolet,⁶³ and Meyer and Kruse⁵⁷ realised the synthetic importance of the decarbonylation reaction of aliphatic and aromatic aldehydes using catalytic amounts of \( (\text{PPh}_3)_2\text{RhCoCl} \) and 1,3-bis(diphenylphosphino)propane (dppp) in hot solvents at relatively mild temperatures (110 - 180°C). The resulting rhodium complex, \([\text{Rh}(\text{dppp})_2]^+\text{Cl}^-\), has successfully decarbonylated indole-2-carboxaldehyde systems³⁰,⁵⁶,⁵⁷ that are structurally similar to (79).

The catalytic decarbonylation mechanism suggested by Doughty and Pignolet⁶⁴ initially involves a reversible oxidative addition of the aldehyde, RCHO, to \([\text{Rh}(\text{dppp})_2]^+\) (88) to give the rhodium complex (89) (Figure 7). Complex (89) lies in equilibrium with (90) which contains a monodentate dppp ligand. Rearrangement of the acyl moiety followed by migration of the R group to rhodium occurs to afford (91). Subsequent reductive elimination produces RH and the carbonyl rhodium complex (92). Reversible decarbonylation and rearrangement reactions regenerate the rhodium complex (88).
Figure 7. The proposed mechanism\textsuperscript{64} for the decarbonylation of aldehydes using \[ \text{[Rh(P-P)_2]^+} \] as the catalyst where (P-P) denotes dppp.

Aldehyde (86) in mesitylene, heated to reflux, was treated with a catalytic amount of the rhodium complex, \[ \text{[Rh(dppe)_2]^+Cl^-} \] (Scheme 33). The decarbonylation
reaction was usually complete after 30 to 45 min. These relatively fast reaction
times limited the amount of decomposition. A yellow oil was produced that had
spectral data consistent with that of the decarbonylated indole (85). The reaction
reliably gave yields of between 60 and 70%.

2.8 ATTEMPTS TO INTRODUCE THE DOUBLE BOND IN THE C₄
SIDECHAIN OF (85)

With indole (85) in hand, it required only elimination of the benzylic oxygen
function to afford the model cis-analogue of (±)-iso-trans-trikentrin B. Recently,
Natsume and co-workers¹⁷,¹⁸ showed that dehydration of the secondary
alcohol (93), an intermediate in the synthesis of cis- and trans-trikentrin B (6a) and
(7b), could be achieved upon stirring with a catalytic amount of para-
toluenesulfonic acid monohydrate (p-TSA.H₂O, ~0.2 equivalents) in benzene
heated to reflux (Scheme 34). This gave the E-olefin compounds (94) and (95) in
94% and 84% yields, respectively.

2.8.1 Attempted Deprotection and Dehydration

Several attempts at one-pot deprotection and dehydration of (85) to produce (96)
(Scheme 35) using p-TSA under various conditions were tried and were
unsuccessful. The reactions always went a purple colour and TLC showed baseline
material and streaking up the plate. ¹H NMR spectra were complex and no discrete
products could be identified.
Scheme 34

cis- (6a) and trans-trikentrin B (7b)
2.8.2 Protection of the Indole Nitrogen of (79) and (85)

One difference between Natsume's compound (93) and indole (85) was that the nitrogen in (93) had been protected as an N-benzenesulfonamide at an early stage in the synthesis. Due to decomposition occurring when p-TSA was used, it was decided...
to protect the indole nitrogen prior to dehydration. Since Natsume and co-workers\textsuperscript{17,18} had previously worked out the conditions to dehydrate and subsequently deprotect the N-benzenesulfonyl group on the trikentrin B intermediates (Scheme 34), it was decided to protect the indole carboxylate (79) as the N-benzenesulfonate.

Attempts to introduce the benzenesulfonyl group in the indole carboxylate (79) and the indole (85) using a variety of bases (pyridine, diisopropylethylamine, sodium hydride and potassium hydride) were unsuccessful. This was possibly due to steric hindrance from the adjacent methyl group on the indan which could obstruct the incoming, large benzenesulfonyl group. It would be approaching from either above or below the plane of the aromatic system since the electron pair of the nitrogen anion is orthogonal to the \( \pi \)-system.\textsuperscript{61}

It was decided therefore to protect the nitrogen with a smaller group after decarbonylation. An acetyl group was used to protect the indole nitrogen by treating (85) with acetic anhydride (Scheme 36) giving (97) in 60% yield.

\[ \text{OMOM} \quad \begin{array}{c} \text{(85)} \\ \text{1. KH, THF, } \Delta \\ \text{2. Ac}_2\text{O} \end{array} \quad \begin{array}{c} \text{OMOM} \\ \text{(97)} \end{array} \]

Scheme 36
High resolution accurate mass measurement of the molecular ion of (97) at m/z 343 gave the value 343.2146 which corresponded to the expected formula C₂₁H₂₉N₀₃. A strong band at 1720 cm⁻¹ in the infrared spectrum confirmed that a carbonyl group was present. The presence of the acetyl group was confirmed by characteristic signals in the ¹H NMR (singlets at 2.651 (2.648) ppm, NCO-CH₃) and ¹³C NMR (167.5 ppm, NCO-CH₃) spectra.

Again p-TSA was used in an attempt to deprote the MOM group and dehydrate the resulting alcohol. It was anticipated that the N-protected indole (97) would be stable enough to withstand decomposition under the reaction conditions. Nevertheless, only decomposed material was produced when compound (97) was treated with p-TSA in benzene.

2.8.3 Cleavage of the MOM Group from (97)

A variation of the procedure by Guindon et al.⁶⁵ using dimethylboron bromide successfully removed the MOM group to give the corresponding hydroxybutyl indole (98) by ¹H NMR spectroscopy (Scheme 37). It was found that after stirring the mixture of (97) and dimethylboron bromide at -78°C for 15 min, triethylamine had to be added prior to the reaction being warmed, otherwise, residual acid decomposed the product. The crude alcohol (98) was obtained in a 65% yield as characterised by ¹H NMR spectroscopy and mass spectrometry. The mass spectrum showed a molecular ion at m/z 299 and a very intense ion at m/z 281 indicating the loss of water. An ion at m/z 266 was characteristic of the loss of water and a methyl group. In the ¹H NMR spectrum there was an absence of signals characteristic of the methylene and methoxyl groups of the MOM substituent suggesting that the MOM group had been removed. Multiplets at 4.98 and...
4.93 ppm were characteristic of the proton of the diastereomers attached to the benzylic carbon bearing the hydroxyl group.

![Scheme 37](image)

Scheme 37

An attempt to eliminate water from (98) using a catalytic amount of \( p \)-TSA in benzene / THF was unsuccessful. \(^1\)H NMR spectroscopy showed that the major component present corresponded to starting material. A complex set of signals was also observed that was characteristic of decomposed material. There were no characteristic signals in the 6.3 - 7.0 ppm range to show that the \( E \)-olefin in the butyl group had been formed.

At this stage, the synthesis of (\( \pm \))-iso-trans-trikentrin B (5) using trans-dimethyl indan intermediates was in progress. The knowledge gained from the synthetic steps using the cis-dimethylindan diastereomers was invaluable towards the synthesis of (\( \pm \))-iso-trans-trikentrin B (5).
CHAPTER 3

SYNTHESIS OF (±)-ISO-TRANS-TRIKENTRIN B (5)

Following the synthetic studies on the model cis-compounds, methodology was developed which established the correct regiochemistry of the C4 sidechain, the cyclopentane and pyrrole ring systems around the aryl ring, and could readily be applied to the natural trans-series. However, the best procedures for the synthesis of trans-1,3-dimethylindan and for the introduction of the E-double bond to form the butenyl sidechain still remained to be determined.

3.1 SYNTHESIS OF (±)-TRANS-1,3-DIMETHYLINDAN (17)

3.1.1 Background

The key starting compound in the synthesis of (±)-iso-trans-trikentrin B (5) was (±)-trans-1,3-dimethylindan (17) which could be obtained selectively (Scheme 38). All of the trans-trikentrins synthesised to date had been prepared using mixtures of isomers with cis- and trans- stereochemistry where the trans-diastereomer was the minor component.

The approach to (17) followed that employed by Jackson and co-workers who prepared 1,3-dimethylindanol, which they suggested was the trans-diastereomer (60) from 3-methyl indanone (99) and methylmagnesium iodide (Scheme 38). Their suggestion was based on the product, trans-dimethylindan (17), that resulted from the treatment of the indanol with Raney nickel
Chapter 3

(Scheme 38) under conditions which promote retention of configuration during hydrogenolysis.68

\[
\begin{align*}
\text{(99)} & \xrightarrow{\text{MeMgI}} \text{(60)} & \xrightarrow{\text{Ra Ni}} \text{(17)}
\end{align*}
\]

\text{Scheme 38}

3.1.2 Approaches

Initially, three steps were required to obtain 3-methyl indanone (99) (Route 1, Scheme 39). The first was based on a method by Marvel \textit{et al.}69 and involved Friedel-Crafts alkylation of benzene and crotonic acid using aluminium trichloride. It gave 3-phenylbutanoic acid (100) in a yield of 72%. The spectral data was consistent with that expected for the structure of (100).

The corresponding acid chloride was prepared by reacting the butanoic acid (100) with oxalyl chloride. The acid chloride was reacted with aluminium trichloride and intramolecular Friedel-Crafts acylation afforded 3-methyl indanone (99) in an 83% yield from the butanoic acid (100). An overall yield of 60% was obtained from crotonic acid for the three steps (Route 1).

Subsequently, a single step reaction was developed for the conversion of benzene and crotonic acid to 3-methylindanone (99) by the use of 1.5 to 2 equivalents of aluminium trichloride (Route 2, Scheme 39). Not only was the reaction time
reduced but the overall yield was increased to 77%. The procedure was based on the original synthesis of 3-phenylbutanoic acid (100) except that the mixture was heated to reflux and the condensed solvent (benzene) was passed through freshly activated molecular sieves (4A) to remove any water generated, before returning to the reaction vessel.

\[ \text{Route 1} \]
\[ \text{Route 2} \]

The reaction of 3-methylindanone (99) with methylmagnesium iodide gave a mixture of trans- and cis-1,3-dimethylindanol (60) and (61) (89%) typically in a ratio of ~7:1 as determined by \(^1\)H NMR spectroscopy (Scheme 40), although in some instances no cis-dimethylindanol could be detected. The \(^1\)H and \(^13\)C NMR spectra of the products agreed with those previously obtained for the purified trans-indanol (60) and cis-indanol (61) prepared by the radical cyclisation of the corresponding bromoarylpentenol (12). The major compound (60) was
unambiguously assigned the \textit{trans}-geometry using an X-ray crystallographic analysis (Figure 2, Chapter 2).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.5\textwidth]{image.png}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 40}

No attempt was made to separate the \textit{cis-} / \textit{trans}-mixture when minor amounts of \textit{cis}-dimethylindanol were present since the \textit{cis}-compounds could be more easily separated at a later stage.

Although \textit{trans}-dimethylindanol (60) could be obtained stereoselectively, difficulties were encountered in the hydrogenolysis of (60) with retention of configuration to give the \textit{trans}-dimethylindan (17). In many cases, the major product of the Raney nickel reduction was the \textit{cis}-dimethylindan (14). Optimisation of the conditions for the production of (17) necessitated the use of freshly prepared, slightly alkaline Raney nickel (pH of the washings were between 7.5 and 8.5) and distilled, degassed ethanol.
Hydrogenolysis of trans-1,3-dimethylindanol (60) over Raney nickel in ethanol heated to reflux gave trans-dimethylindan (17) as a single product (Scheme 41). Comparison of its $^1$H NMR spectrum with the literature data confirmed the structure of trans-1,3-dimethylindan (17). $^1$H NMR spectral data enabled the trans- and cis-1,3-dimethylindans (17) and (14) to be distinguished from each other. The C-2 methylene protons of cis-1,3-dimethylindan (14) were anisochronous and resonated at 2.48 and 1.15 ppm, while the C-2 protons of the trans-compound (17) were isochronous and were observed as a triplet ($J = 6.9$ Hz) resonating at 1.89 ppm.

![Scheme 41](image-url)
3.2 CONSTRUCTION OF THE FOUR CARBON UNIT SIDECHAIN

The indan (17) was reacted with butyryl chloride to form the corresponding butyroylindan (101) (Scheme 42). The overall yield for the two reactions was 70%, based on the amount of indanol (60) used. The spectral data correlated with that of the isomeric butyroyl-cis-dimethylindan (55) (see Chapter 2). The major difference was the chemical shift of the isochronous protons on C-2 (1.94 ppm) in the trans-diastereomer (101) as compared with the anisochronous C-2 protons at 2.50 and 1.18 ppm for the cis-analogue. The $^{13}$C chemical shift of C-2 was also characteristic. The C-2 carbon of the butyroyl-cis-dimethylindan (55) resonated at 44.7 ppm while the corresponding carbon of the trans-compound (101) was at 42.8 ppm.

Reduction of the butyroylindan (101) to the corresponding hydroxybutylindan (102) using sodium borohydride proceeded in a high yield (95%) (Scheme 42).

![Scheme 42](image-url)
3.3 FORMATION OF THE INDOLE

3.3.1 Approach to the Indole (103)

The approach developed for the synthesis of the corresponding cis- compound would be used to prepare the trans-dimethylindan aldehyde (104) from (102) (Scheme 43). The aldehyde (104) could not be prepared directly from compound (102) since the stable lactol, formed in the corresponding cis- system, precluded any further elaboration of the aldehyde function. Instead, metalation of compound (102) followed by bromination was expected to give the arylbromide (105), followed by protection of the hydroxyl group to give compound (106). A metal-halogen exchange on (106) would provide a metalated species that could be formylated to give the aldehyde (104).

The construction and annulation of the pyrrole ring would then be carried out by the condensation of the aldehyde (104) with ethyl azidoacetate to give the azidocinnamate (107), followed by thermolysis to the indole (103).
3.3.2 Synthesis of the Indole (103)

The hydroxybutyindan (102) was lithiated with t-BuLi and the subsequent aryllithium product was brominated (Scheme 44) according to the procedure previously established for the cis-analogue. The yield of the arylbromide (105) was 52% but starting material (37%) was also recovered. Moderate yields had also been obtained for the preparation of the corresponding cis-aryl bromide (74)
Full characterisation confirmed the structure of the arylbromide (105). A bromine-containing molecular ion at \( m/z \) 298/296 was observed in the mass spectrum. Two sharp singlets in the aromatic region at 7.36 and 7.30 ppm were present in the \(^1\text{H}\) NMR spectrum suggesting that substitution had occurred in the 6-position.

\[
\text{Scheme 44}
\]

An improved procedure\(^\text{70}\) was used for protecting the secondary alcohol of (105) as a MOM ether (Scheme 44) compared to that used for the corresponding \textit{cis}-compound (74). This involved the addition of a catalytic amount of \(N,N\)-dimethylyaminopyridine (DMAP) which increased the rate of the reaction. A yellow oil was isolated and identified as the MOM ether (106) obtained in a 95% yield.

Replacement of the bromine atom with a formyl group was initially attempted using the conditions previously employed for the formylation of the isomeric bromo-\textit{cis}-dimethylindan (75) (see Chapter 2). During lithiation of (75), a precipitate due to the lithiated aryl compound had formed at 40°C.
However, when repeating the conditions for the corresponding bromo-trans-dimethylindan (106) no precipitate was formed and only decomposition product was isolated from the reaction mixture. When the lithiation reaction was carried out at 0°C on the trans-compound (106), a precipitate due to the lithiated species was formed. DMF was added to the stirred solution at -78°C. Upon work-up, aldehyde (104) was obtained (Scheme 45). It appeared therefore, that precipitation of the aryllithium minimised its decomposition during the formylation reaction. Other material recovered included the debrominated MOM ether (108) identified by $^1$H NMR, together with a minor amount of decomposition product. Characteristic of the presence of the aldehyde functionality in (104) was a strong band at 1695 cm$^{-1}$ in the infrared spectrum, a singlet at 10.30 ppm in the $^1$H NMR spectrum as well as the signals at 192.23 (192.20) ppm in the $^{13}$C NMR spectrum for the diastereomers.
Scheme 45
Preparation of the azidocinnamate (107) from the aldehyde (104)* using ethyl azidoacetate (Scheme 45) was carried out under the conditions previously optimised for the corresponding cis-compounds. The purified azidocinnamate (107) was obtained in a 54% yield.

Thermolysis of azidocinnamate (107) in toluene heated to reflux gave a mixture whose $^1$H NMR spectrum showed a major and a minor component (Scheme 45). On the basis of the aromatic singlets in the $^1$H NMR spectrum of the crude reaction mixture, it appeared that the ratio of major product to minor was approximately 10 : 1. Three singlets at 8.38, 7.94 and 7.65 ppm which could be assigned to the minor compound suggested that the isoquinoline (109) had been produced. The chemical shifts of the corresponding protons of the isomeric cis-isoquinoline (80) were at 8.39, 7.90 and 7.67 ppm (Chapter 2). Chromatography of the crude material gave the major product as a yellow oil (79% yield) identified as the ethyl indole-2-carboxylate (103). The minor compound observed in the $^1$H NMR spectrum of the crude mixture was not recovered.

The major compound was fully characterised as the indole (103) by spectral data. The molecular ion at $m/z$ 373 had a measured accurate mass of 373.2253, as required for the expected composition of C$_{22}$H$_{31}$NO$_{4}$. Other characteristic ions in the mass spectrum included those at $m/z$ 330 (loss of propyl) and 312 (loss of OOMOM). Indole (103) gave the expected $^1$H and $^{13}$C NMR spectra. A broad

* From this stage of the synthesis, it was necessary to utilise a ~4 : 1 mixture of the trans- / cis-compound due to losses of the pure trans- compound that occurred during the early attempts to prepare the aldehyde (104). Reference will be made only to the trans-compounds in the following discussion. Quoted yields are from the mixture.
singlet at 8.73 ppm in the $^1$H NMR spectrum indicated the presence of the indole N-H. Two other protons were observed in the aromatic region at 7.42 (7.41) ppm and 6.97 ppm (H-3 and H-5) which compared well with the chemical shifts of the corresponding protons of the cis-indole (79) at 7.41 (7.40) ppm and 6.97 ppm. A quartet at 4.40 ppm ($J$ 7.1 Hz) and a triplet at 1.43 ppm ($J$ 7.1 Hz) in the $^1$H NMR spectrum as well as a signal at 162.2 ppm in the $^{13}$C NMR spectrum confirmed the presence of the ethyl ester group.

### 3.4 REMOVAL OF THE CARBOXYLATE GROUP FROM (103)

The ethyl indole-2-carboxylate (103) was treated with DIBALH to give the corresponding alcohol (110) in a yield of 51% (Scheme 46). Oxidation of the indole alcohol (110) with MnO$_2$ gave the aldehyde (111) in 65% yield. A molecular ion at $m/z$ 329 indicated the presence of the aldehyde (111). The aldehyde functionality was confirmed by spectral data which included a strong band at 1660 cm$^{-1}$ in the infrared spectrum, a singlet at 9.80 ppm in the $^1$H NMR spectrum and a signal at 181.6 ppm in the $^{13}$C NMR spectrum.

Decarbonylation of the indole-2-carboxaldehyde (111) using [Rh(dppp)$_2$]$^+$$\text{Cl}^-$ in mesitylene heated to reflux gave the expected indole (112) in a 64% yield (Scheme 46). Confirmation that the decarbonylated indole (112) was obtained came from high resolution mass spectrometry which showed a strong molecular ion at $m/z$ 301 that had a measured mass of 301.2042 as required for the formula $\text{C}_{19}\text{H}_{21}\text{NO}_2$ (calculated 301.2042). There was an absence of signals in the NMR spectra indicative of the aldehyde functionality suggesting that the decarbonylation was successful. A diastereomeric mixture was indicated by NMR spectral data. In the $^1$H NMR spectrum, the nitrogen proton was present at 8.08 ppm. A singlet at
6.95 ppm indicated the proton on C-5 while the doublet at 6.71 (6.70) ppm ($J$ 5.4 Hz) was assigned as the proton on C-3 and the doublet at 7.17 ppm ($J$ 5.4 Hz) from the proton on C-2.

Scheme 46
3.5 DEPROTECTION OF THE MOM GROUP AND THE INTRODUCTION OF THE DOUBLE BOND IN THE C4 SIDECHAIN OF (112)

3.5.1 Proposed Approach

During the synthetic studies on the model cis-compounds (see Chapter 2), the nitrogen of the cis-indole (85), the isomer of (112), was protected to give (97) in an attempt to prevent decomposition that had occurred in the presence of para-toluene sulfonic acid (p-TSA). It was expected that the p-TSA would deprotect the MOM group from the N-protected cis-indole (97) as well as eliminate water to give the corresponding E-olefin. In the event, decomposition again resulted when the N-protected indole (97) was subjected to p-TSA in benzene (Scheme 47). The MOM group was however successfully removed from (97) on treatment with dimethylboron bromide at -78°C with triethylamine to give (98). Treatment of (98) with p-TSA had also resulted in decomposition. It therefore appeared that N-protection did not prevent decomposition in the presence of p-TSA.

![Scheme 47](image)
It was proposed to use the milder conditions of the dimethylboron bromide reaction on the *trans*-indole (112) to give the parent alcohol (113) (Scheme 48). The hydroxyl group could then be derivatised with a good leaving group that eliminates in a *trans*-fashion to give (±)-*iso-trans*-trikentrin B (5). The ideal group appears to be a benzenesulfonate. It had been shown previously (Chapter 2) that benzenesulfonyl chloride did not react with the indole nitrogen of the *cis*-isomer of (112) and therefore should react selectively with the benzylic hydroxyl group. Treatment of the sulfonate (114) with a reagent such as bis(tetra-*n*-butylammonium) oxalate should ensure that only elimination occurs.\textsuperscript{71}

\[
\text{OMOM} \quad \text{OMOM} \\
\text{N} \quad \text{N} \\
\text{H} \quad \text{H} \\
\text{1. Me}_2\text{BBr, -78°C} \\
\text{2. Et}_3\text{N} \\
\text{3. H}_2\text{O} \\
(\text{112}) \\
\text{OH} \\
\text{1PhSO}_2\text{Cl, base} \\
\text{OSO}_2\text{Ph} \\
\text{e.g. (Bu}_4\text{N}^+)\text{(_2 CO}_2\text{)}\text{2} \\
\text{N} \quad \text{N} \\
\text{H} \quad \text{H} \\
(\text{114}) \\
(\pm)-\text{iso-trans}-\text{trikentrin B (5)} \\
\text{Scheme 48}
\]
3.5.2 Synthesis of (±)-iso-trans-Trikentrin B (5)

A modified method of Guindon et al.\textsuperscript{65} was used in order to deprotect the MOM group. The indole (112) was stirred with dimethylboron bromide in dichloromethane at -78°C (Scheme 49). After a few minutes the solution colour darkened. At this point, excess of triethylamine was added whereupon the solution colour lightened. The reaction was quenched with water and upon work-up an oil was produced. TLC showed a major product which was identified, somewhat surprisingly, as (±)-iso-trans-trikentrin B (5) by \textsuperscript{1}H NMR spectroscopy.\textsuperscript{66}

Repeated flash chromatography under nitrogen removed the minor cis- compound and gave (±)-iso-trans-trikentrin B (5) in a 63% yield.

The data for (5) could not be directly compared with that of the natural iso-trans-trikentrin B (5a)\textsuperscript{14} since the natural product had been isolated as an inseparable mixture with cis-trikentrin B (6a). Instead it was compared with the data acquired by Natsume et al.\textsuperscript{18,66} who had synthesised and purified both (6R, 8R)-iso-trans-trikentrin B (5b) and (±)-iso-trans-trikentrin B (5). Mass spectral data showed a strong molecular ion at \textit{m/z} 239 (89% relative intensity) while the base peak was at \textit{m/z} 224 for the loss of a methyl radical, in agreement with the literature data.\textsuperscript{18,66}

High resolution accurate mass spectrometry of the ion at \textit{m/z} 239 gave a measured mass of 239.1673 corresponding to the formula C\textsubscript{17}H\textsubscript{21}N. The chemical shift values and coupling constants in the \textsuperscript{1}H NMR spectrum matched those reported\textsuperscript{66} and the \textsuperscript{13}C NMR spectral data was also in agreement with the literature data.\textsuperscript{66} A strong band in the infrared spectrum at 3475 cm\textsuperscript{-1} confirmed the presence of the indole N-H.

When the cis-analogue (97) was treated with dimethylboron bromide followed by triethylamine and water (Scheme 47), the reaction gave the expected parent
alcohol (98). These reaction conditions were repeated on the trans-compound (112), which was not N-protected, to give (±)-iso-trans-trikentrin B (5). Therefore, the possible mechanistic scheme involves (115) as a likely intermediate from which the nitrogen proton is abstracted by base to give intermediate (116), followed by aromatisation to produce (±)-iso-trans-trikentrin B (5) (Scheme 49).

Scheme 49
It was therefore an unexpected bonus that the treatment of the MOM ether (112) with dimethylboron bromide had not only cleaved the MOM group but also eliminated the benzylic oxygen to give the target compound, \((\pm)-iso\text{-}trans\text{-}tri$kentrin\ B\ (5)\). The overall yield for the 13 step synthesis of \((\pm)-iso\text{-}trans\text{-}tri$kentrin\ B\ (5)\) was just over 1%.

One method to obtain the natural \((6S, 8S)\)-form of \(iso\text{-}trans\text{-}tri$kentrin\ B\ (5a)\) from the racemic mixture (5) would be to attach a chiral group to the indole. Sulfonamides, from camphor-10-sulfonic acid,\(^{72,73}\) and amides, from tartaric acid,\(^{72,73}\) are common examples of derivatives that have been used to resolve mixtures by employing a variety of techniques (e.g. crystallisation, liquid chromatography). Since the acetamide (97) was successfully synthesised from the \(cis\)-indole (85) but difficulties were experienced trying to prepare the benzenesulfonamide, then it is envisaged that the corresponding amides of the indole (112) from the acid chloride of the smaller (+)- or (-)-tartaric acid could be formed. The amides could be separated by liquid chromatography (e.g. HPLC) and subsequent removal of the acyl group\(^{25}\) would give the separated diastereomers of (112). These could then be individually treated with dimethylboron bromide and triethylamine to afford the natural \((6S, 8S)\)-\(iso\text{-}trans\text{-}tri$kentrin\ B\ (5a)\) as well as \((6R, 8R)\)-\(iso\text{-}trans\text{-}tri$kentrin\ B\ (5b)\).
EXPERIMENTAL

GENERAL PROCEDURES

Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Microanalyses were carried out by the Australian National University Microanalytical Service.

Low resolution electron impact mass spectra (EI-MS) and high resolution accurate mass measurements were recorded on a VG Micromass 7070F double-focussing or a VG ZAB-2SEQ mass spectrometer (low resolution EI-MS only). The molecular ion (M⁺), if present, significant high mass ions and the more intense low mass ions are reported. Chemical ionisation mass spectra (CI-MS) were measured on the VG Micromass 7070F double-focussing mass spectrometer, employing ammonia as the reagent gas.

Infrared spectra were recorded on a Perkin-Elmer 683 or Perkin-Elmer 1800 (FT) spectrophotometer as films (neat) or dichloromethane (CH₂Cl₂) solutions. The following abbreviations were adopted to indicate the intensity and to describe the shape of the band: s (strong), m (medium), w (weak) and b (broad).

¹H NMR spectra were recorded on a Varian Gemini-300 (300 MHz), Varian VXR-300 (300 MHz) or a Varian VXR-500 (500 MHz). Unless otherwise stated the spectrum was recorded at 300 MHz. Tetramethylsilane was used as the internal reference and the signals are quoted in δ values (ppm downfield from tetramethylsilane). The following abbreviations were adopted to indicate the multiplicity and to describe the shape of the signal: s (singlet), d (doublet), t
Experimental

(triplet), q (quartet), quin (quin), m (multiplet), b (broad) and app (apparent). Deuterated chloroform (CDCl₃) with a tetramethylsilane internal standard was used as the solvent unless otherwise stated. The chemical shifts of diastereomers are in parentheses.

¹³C NMR spectra were recorded on a Varian Gemini-300 (75.5 MHz) or a Varian VXR-300 (75.4 MHz). The solvent signal was used as the internal reference (76.9 ppm for chloroform) and the signals are quoted in δ values (ppm downfield from tetramethylsilane). The chemical shifts of diastereomers are in parentheses.

Two dimensional NMR experiments were carried out using a Varian VXR-300. The pulse sequences used were: ¹H-¹H-COSY (correlation between protons on the same carbon or adjacent carbons), ¹H-¹H-DQFCOSY (correlation between protons on the same carbon or adjacent carbons), ¹H-¹³C-HETCOR (one-bond correlation between carbons and protons), ¹H-¹³C-HMQC (one-bond correlation between carbons and protons), ¹H-¹³C-HMBC (multi-bond correlation between carbons and protons), ¹H-¹³C-LRHETCOR (multi-bond correlation between carbons and protons), ¹H-¹H-ROESY (through-space correlation between protons) and ¹H-¹H-TOCSY (correlation between protons in the same spin system).

Ultraviolet-visible (UV-vis) spectra were recorded on a Shimadzu model UV-160 or a Cary 1E spectrophotometer.

Where necessary, solvents and reagents were purified and dried according to procedures of Perrin and Armarego. Anhydrous solvents were distilled from drying agents directly prior to use. Light petroleum refers to the petroleum fraction of b.p. 60 – 80°C. Reactions performed under anhydrous conditions were done under a dry argon atmosphere unless otherwise stated.
Experimental chromatography\textsuperscript{74} was carried out using 230 – 400 mesh silica gel. For thin layer chromatography (TLC), 0.25 mm Merck silica gel 60 F\textsubscript{254} plates were used for analytical purposes. Thin layer chromatograms were visualised under ultraviolet light or by spraying with 13% vanillin in sulfuric acid, followed by heating at 200°C.

The molarity of solutions of butyllithium in various solvents were determined by titration against 2,5-dimethyloxybenzyl alcohol as a self-indicating primary standard according to the method by Winkle \textit{et al.}\textsuperscript{44}

Experimental details of the single crystal X-ray structure analysis are given in the appendix.

**EXPERIMENTAL DETAILS**

\textbf{Chapter 2}

\textbf{2-(2'-Bromophenyl)pent-4-en-2-ol (12)}

The title alcohol was prepared by a modification of the method of MacLeod and Monahan.\textsuperscript{22} Allyl bromide (8.26 g, 68.3 mmol) in dry diethyl ether (20 mL) was added dropwise to a stirred solution of magnesium turnings (0.96 g, 39.5 mmol) in dry diethyl ether (5 mL) at a rate sufficient to maintain gentle reflux under argon. After the addition was complete, the mixture was heated to reflux a further 45 min and was then allowed to cool. The solution was transferred to another flask also under argon. A solution of 2-bromoacetophenone (6.00 g, 30 mmol) in dry diethyl
ether (20 mL) was added dropwise to the stirred solution of Grignard reagent. The mixture was heated to reflux for a further 45 min and then it was cooled. The reaction mixture was poured onto ice water (50 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 30 mL). The combined organic fractions were washed twice with saturated sodium chloride solution (2 x 30 mL), dried (MgSO₄) and the solvent removed in vacuo to give a yellow oil (6.12 g, 85%). ¹H NMR δ 7.71, dd, J 7.9, 1.5 Hz, C-3'H; 7.58, d, J 7.7 Hz, C-6'H; 7.30, dd, J 7.7, 7.1 Hz, C-5'H; 7.09, ddd, J 7.8, 7.1, 1.5 Hz, C 4'H; 5.53, m, =CH; 5.14, m, =CH₂; 3.29, dd, J 14.2, 8.4 Hz, C-3Ha; 2.70, bs, OH; 2.64, dd, J 14.2, 8.4 Hz, C-3Hb; 1.72, s, CH₃. ¹³C NMR δ 145.1 C quat; 135.2, 133.7, 128.6, 128.4, 127.5, 5 x CH; 120.0, C quat-Br; 119.4, =CH₂; 74.5, C quat; 44.8, C-3H₂; 27.1, CH₃. EI-MS: m/z 225 (<1%), 223 (<1), 201 (100), 199 (100), 185 (11), 183 (13). A sample was distilled to give a colourless oil, b.p. 100°C/0.05 mmHg (lit. 118°C/0.06 mmHg).

trans- and cis-1,3-Dimethyldiolan (60) and (61)

A solution of the bromopentenol (12) (1.20 g, 5 mmol) in dry benzene (85 mL) was degassed and kept under an atmosphere of argon. The solution was heated to 70°C and a solution of tributyltin hydride (1.75 g, ~ 6 mmol) and azobisisobutyronitrile (AIBN) (56 mg) in dry, degassed benzene (8 mL) was added slowly dropwise over 2.5 h. The reaction was monitored by thin layer chromatography (TLC) and after 2 h the reaction was complete. The benzene was removed by distillation and the residue was dissolved in diethyl ether (50 mL). Saturated potassium fluoride (30 mL) was added and it was vigorously stirred overnight. The organic layer was separated and the aqueous phase was extracted with diethyl ether (2 x 50 mL). The combined organic fractions were washed with saturated sodium chloride solution (50 mL). The solution was dried (MgSO₄) and
the solvent was removed by distillation. The oil was chromatographed on silica (diethyl ether / hexanes, 1 : 4) to give a colourless oil (0.63 g, 78%). The ratio of (60) to (61) was ~2:3 by $^1$H NMR spectroscopy. The ratio of (60) : (61) was approximately 2 : 3 by $^1$H NMR spectroscopy. EI-MS: m/z 162 (M$^{+}$+, 2%), 147 (100), 144 (9), 129 (44), 115 (7), 105 (7), 91 (14), 77 (16). Repeated column chromatography on another sample gave two fractions. The first fraction contained a colourless solid. It was recrystallised from ethyl acetate / hexane as colourless blocks with m.p. 72°C (lit.33 72°C). $^1$H NMR δ 7.25, m, 4 x ArH; 3.07, m, C-3H; 2.44, dd, J 12.7, 7.2 Hz, C-2Ha; 2.25, bs, OH; 1.71, dd, J 12.7, 8.8 Hz, C-2Hb; 1.34, s, C1-CH3; 1.33, d, J 7.0 Hz, C3-CH3. $^{13}$C NMR δ 148.8, 146.4, 2 x ArC quat: 128.1, 127.0, 123.3, 122.1, 4 x ArCH; 79.9, O-Cquat: 51.9, C-2H2; 35.6, C-3H; 27.6, 19.5, 2 x CH3. See Appendix for X-ray crystallographic data. The second fraction eluted was a colourless oil with b.p. ~70°C/0.005 mmHg. $^1$H NMR δ 7.26, m, 4 x ArH; 3.34, m, C-3H; 2.38, dd, J 13.4, 7.2 Hz, C-2Ha; 2.13, bs, OH; 1.67, dd, J 13.4, 8.0 Hz, C-2Hb; 1.60, s, C1-CH3; 1.28, d, J 7.0 Hz, C3-CH3. $^{13}$C NMR δ 148.3, 147.4, 2 x ArCquat: 128.5, 126.9, 123.7, 122.2, 4 x ArCH; 79.5, O-Cquat: 50.7, C-2H2; 35.5, C-3H; 27.0, 19.2, 2 x CH3.

cis-1,3-Dimethylindan (14)

A mixture of diastereomeric 1,3-dimethylindanol (60) and (61) (5.00 g, 30.82 mmol) in diethyl ether (25 mL) was stirred with para-toluene sulfonic acid monohydrate ($p$-TSA.H2O) (250 mg, 1.31 mmol) for 15 h. TLC (silica, 1 : 4, diethyl ether / hexanes) showed starting material was still present. Another portion of $p$-TSA.H2O (200 mg, 1.05 mmol) was added and the mixture was stirred for another 9 h until all of the starting material had been consumed. The mixture was washed with 0.1% sodium hydroxide solution (3 x 20 mL) and water (3 x 20 mL). The organic fraction was dried (MgSO4) and the solvent was removed by
distillation. The residue was chromatographed on silica (diethyl ether / hexanes, 1 : 4) to give a yellow oil (4.30 g, 97%). The oil was taken up in dichloromethane (150 mL) and stirred with 10% Pd/C (250 mg) under an atmosphere of hydrogen at ambient pressure and temperature. After 38 h the reduction was complete. The mixture was chromatographed on silica (pentanes) and the solvent was distilled to give a colourless oil (4.32 g, 96%) that contained > 95% cis-1,3-dimethylindan (14), the remainder was the trans-isomer (17) by $^1$H NMR spectroscopy (confirmed by the comparison of $^1$H NMR spectrum with literature data).29 This material was used without further purification. $^1$H NMR δ 7.18, s, 4 x ArH; 3.08, dt, J 10.4, 6.9 Hz, C-1H and C-3H; 2.47, dt, J 12.0, 7.1, Hz, C-2Ha; 1.31, d, J 6.8 Hz, 2 x CH3; 1.15, dt, J 12.0, 10.4 Hz, C-2Hb. $^{13}$C NMR δ 148.6, 2 x ArCquat; 126.2, 2 x ArCH; 122.9, 2 x ArCH; 44.8, C-2H2; 37.8, C-1H and C-3H; 19.0, 2 x CH3. EI-MS: m/z 146 (M**, 49%), 131 (100), 115 (34), 91 (59), 77 (24).

5-($\text{I'Oxobutyl}$)-1,3-cis-dimethylindan (55)

Butyryl chloride (4.58 mL, 44.1 mmol) in dry dichloromethane (20 mL) was added dropwise to a vigorously stirred suspension of aluminium trichloride (5.88 g, 44.1 mmol) in dry dichloromethane (20 mL) under argon. The mixture was stirred for 50 min and was then cooled to 0°C. A solution of cis-1,3-dimethylindan (14) (4.30 g, 29.4 mmol) in dry dichloromethane (10 mL) was added dropwise at a rate to maintain the reaction temperature at ~ 2°C. The reaction mixture went an orange colour and after the addition was complete, the mixture was allowed to warm to room temperature. After stirring for 1.5 h, TLC (silica, diethyl ether / hexanes, 1 : 9) showed the starting indan had been consumed and the mixture was poured onto ice (100 g). Dichloromethane (50 mL) was added to the iced reaction mixture and it was stirred, the organic layer was separated and the aqueous layer was
extracted with dichloromethane (3 x 40 mL). The combined organic fractions were washed with saturated sodium hydrogen carbonate solution (3 x 30 mL) and dried (MgSO₄). The dichloromethane and residual butyryl chloride were removed by distillation to give an oil. The oil was chromatographed on silica (diethyl ether / hexanes, 1 : 9) to give a colourless oil (5.20 g, 82%), b.p. 105°C/0.08 mmHg (Found: C, 83.0; H, 9.5; C₁₅H₂₀O requires C, 83.3; H 9.3). ν_max (neat) 1683s (C=O) cm⁻¹. ¹H NMR δ 7.83, d, J 8.0 Hz, C-6H; 7.78, s, C-4H; 7.25, d, J 8.0 Hz, C-7H; 3.12, m, C-1H and C-3H; 2.93, t, J 7.4 Hz, C-2'H₂; 2.50, dt, J 12.1, 7.0 Hz, C-2'Ha; 1.78, sextet, J 7.4 Hz, C-3'H₂; 1.35, d, J 7.4 Hz, CH₃; 1.32, d, J 7.0 Hz, CH₃; 1.18, dt, J 12.1, 10.5 Hz, C-2'Hb; 1.02, t, J 7.4 Hz, C-4'H₃. ¹³C NMR δ 200.9, C=O, 154.3, 149.2, 135.7, 3 x ArCquat; 127.0, 122.8, 122.5, 3 x ArCH; 44.7, C-2'H₂; 40.4, C-2'H₂; 38.0, 37.7, C-1H and C-3H; 18.9, 18.7, 2 x CH₃; 17.7, C-3'H₂; 13.6, C-4'H₃. EI-MS: m/z 216 (M⁺⁺, 11%), 188 (3), 173 (100), 145 (11), 91 (7).

cis-1,3-Dimethyl-5-(1'-hydroxybutyl)indan (56)

5-(1'Oxobutyl)-1,3-dimethylindan (55) (2.50 g, 11.56 mmol) was dissolved in methanol (25 mL) and the solution was cooled to -10°C. Sodium borohydride (0.66 g, 17.36 mmol) was added to the stirred solution in portions at a rate sufficient to maintain the reaction temperature between 0° and 2°C. Once the addition was complete, the mixture was stirred for 50 min as it was warmed to room temperature. The reaction mixture was then poured onto water (40 mL) and the methanol was removed in vacuo. The aqueous residue was extracted with dichloromethane (3 x 30 mL), dried (MgSO₄) and the solvent removed in vacuo to give a colourless oil (2.47 g, 98%), b.p. 154°C/0.08 mmHg (Found: C, 82.2; H, 10.0; C₁₅H₂₂O requires C, 82.5; H, 10.2). ν_max (neat) 3385b (OH) cm⁻¹. ¹H NMR δ 7.15, m, 3 x ArH; 4.68, t, J 6.6 Hz, O-CH; 3.08, m, C-1H and C-3H;
Experimental

2.50, dt, J 12.1, 7.0 Hz, C-2'Ha; 1.95 - 1.20, m, C-2'H2, C-3'H2 and OH; 1.33, d, J 6.8 Hz, CH3; 1.32, d, J 6.8 Hz, CH3; 1.19, dt, J 12.1, 10.5 Hz, C-2'Hb; 0.95, t, J 7.2 Hz, C-4'H3. 13C NMR δ 149.0, (148.9), 148.0, 143.3, 3 x ArCquat. 124.2, (123.9), 122.7, (122.6), 120.4, (120.3), 3 x ArCH; 74.6, (74.5), O-CH; 44.9, C-2'H2; 41.0, C-2'H2; 37.7, 37.5, C-1'H and C-3'H; 18.98, 2 x CH3; 18.98, C-3'H2; 13.6, C-4'H3. EI-MS: m/z 218 (M++*, 3%), 203 (1), 176 (13), 175 (100), 145 (5), 105 (82), 91 (46).

cis-1,3-Dimethyl-5-((1'-methoxymethyl)oxy butyl)indan (63)

The MOM ether (63) was prepared by the method of Stork and Takahashi. To a stirred solution of the alcohol (56) (1.0 g, 4.58 mmol) in dichloromethane (25 mL) at 0°C, methoxymethyl chloride (2.09 mL, 27.48 mmol) was added. Diisopropylethylamine (4.79 mL, 27.48 mmol) was added slowly to the solution. The reaction temperature was warmed to room temperature and it was stirred for further 28 h until the reaction was complete. The reaction mixture was poured onto a mixture of ice and water (30 g) and it was stirred. The aqueous layer was separated and extracted with dichloromethane (3 x 15 mL). The organic layers were combined and washed with 10% hydrochloric acid solution (20 mL), saturated sodium hydrogen carbonate solution (2 x 20 mL) and saturated sodium chloride solution (20 mL). The organic layer was dried (MgSO4) and the solvent was removed by distillation to give a yellow oil (1.13 g, 94%), b.p. 80°C/0.8 mmHg (Found: C, 78.0; H, 9.7; C17H26O2 requires C, 77.8; H, 10.0). \( v_{\text{max}} \) (neat) 1109 (C-O) cm⁻¹. 1H NMR δ 7.11, s, 3 x ArH; 4.53, m, O-CH2-O and O-CH; 3.39, (3.38), s, O-CH3; 3.06, m, C-1'H and C-3'H; 2.48, dt, J 12.0, 7.0 Hz, C-2'Ha; 1.85, m, C-2'Ha; 1.69 - 1.25, bm, C-2'Hb and C-3'H2; 1.30, d, J 6.7 Hz, CH3; 1.29, d, J 6.7 Hz, CH3; 1.16, m, C-2'Hb; 0.93, t, J 7.3 Hz, C-4'H3. 13C NMR δ 148.8, 148.0, 140.4, 3 x ArCquat; 125.1, 122.6, 121.4, (121.2), 3 x
Experimental 104

ArCH; 93.9, O-CH2-O; 77.8, CH-O; 55.3, O-CH3; 45.0, C-2H2; 40.2, C-2' H2; 37.7, 37.6, C-1H and C-3H; 19.0, C-3'H2; 18.9, 2 x CH3; 13.6, C-4'H3. EIMS: m/z 262 (M++, 2%), 247 (1), 220 (15), 219 (70), 201 (10), 175 (21), 145 (27), 105 (52), 91 (100).

**Attempted Formylation of cis-1,3-Dimethyl-5-(1'-methoxymethyloxy butyl)indan**

(63) using Phosphorus Oxychloride

The formylation attempt followed the method of Buu Hoi *et al.*\(^{39}\) Phosphorus oxychloride (49 µL, 530 µmol) was added dropwise to a stirred solution of cis-1,3-dimethyl-5-(1-methoxymethyloxy butyl)indan (63) (105 mg, 400 µmol) in DMF (40 µL, 520 µmol) under argon at 5°C. After the addition of phosphorus oxychloride was complete, the mixture was heated to 100°C for 3.5 h. The reaction mixture was cooled and a solution of concentrated sodium acetate (3 mL) was added and the mixture was heated to reflux for 1 h. The mixture was cooled and taken up in dichloromethane (20 mL). The aqueous layer was extracted with dichloromethane (2 x 10 mL). The organic fractions were collected and washed with dilute hydrochloric acid solution (pH 3, 15 mL), saturated sodium bicarbonate solution (2 x 15 mL) and saturated sodium chloride solution (15 mL). The organic layer was then dried (MgSO\(_4\)) and the solvent was evaporated to give a yellow oil. It was chromatographed on silica (diethyl ether / hexanes, 1 : 4) to yield a yellow oil (89 mg). TLC examination (silica, diethyl ether / hexanes, 1 : 4) indicated a mixture of compounds was present. \(^1\)H NMR spectroscopy showed a major set of signals that were consistent with those of cis-1,3-dimethyl-5-(1'-hydroxybutyl)indan (56) according to the data previously obtained on this compound. A relatively less intense set of signals were present, however there was an absence of signals downfield from 8 ppm.
**Experimental**

*Attempts Formylation of Indan* (64) *using Phosphorus Oxychloride*

Phosphorus oxychloride (513 µL, 5.50 mmol) was added dropwise to a stirred solution of indan (64) (0.50 g, 4.23 mmol) in DMF (0.40 g, 426 µmol). The reaction was carried out similarly to the procedure above. An oil (0.48 g, 96%) was recovered. The data obtained was compared to that of the starting material and it confirmed the recovery of the indan (64).

**Metalation Reactions using Butyllithium Reagents**

**A. General Procedure for Metalation Reactions using Butyllithium Reagents**

To a stirred solution of substrate (MOM ether (63) or alcohol (56)) and an additive (i.e. TMEDA) where applicable, in a dry, distilled solvent under argon at 0°C was added the butyllithium (equivalents shown in Tables). The reaction mixture was warmed to the specified temperature (in Tables) and maintained at this temperature until a colour change occurred (time shown in Tables). The mixture was cooled and quenched with an electrophile (in Tables) and stirred a further 20 min. It was then diluted with diethyl ether and washed with water. The aqueous washings were extracted with diethyl ether. The combined organic fractions were dried (MgSO₄) and the solvent was removed. The product was determined by mass spectrometry and ¹H NMR spectroscopy. Where appropriate ²H and ¹³C NMR spectroscopy were also recorded.

**B. General Procedure for Metalation Reactions using Butoxide / n-BuLi Reagents**

A typical procedure was adapted from that by Lipshutz and Garcia. To a stirred solution of butoxide (NaOri-Bu or KOr-Bu; equivalents shown in Tables) and dry,
distilled solvent (Tables) under argon at 0°C was added n-BuLi (equivalents shown in Tables). It was stirred for 45 min at 0°C. A cold solution of substrate (MOM ether (63) or alcohol (56)), n-BuLi (equivalents shown in the Tables) and dry, distilled solvent was added to the first mixture. It was warmed to the temperature shown in the Tables for the time listed. The mixture was cooled and quenched with an electrophile (Tables) and stirred a further 20 min. It was then diluted with diethyl ether and washed with water. The aqueous washings were extracted with diethyl ether. The combined organic fractions was dried (MgSO4) and the solvent was removed. The product was determined by mass spectrometry and 1H NMR spectroscopy. Where appropriate 2H and 13C NMR spectroscopy was also recorded.

See Table 1, below, and Table 2 in Chapter 2.

cis-1,3-Dimethyl-5-(1'-hydroxybutyl)-6-2H-indan

See Entry 5, Table 2 in Chapter 2. The general lithiation procedure A was followed and produced a colourless oil. 1H NMR δ 7.19, s, ArH; 7.14, s, ArH; 4.68, t, J 6.6 Hz, O-CH; 3.08, m, C-1H and C-3H; 2.50, dt, J 12.1, 7.0 Hz, C-2H; 1.95 - 1.20, m, C-2'H2, C-3'H2 and OH; 1.33, d, J 6.8 Hz, CH3; 1.32, d, J 6.8 Hz, CH3; 1.19, dt, J 12.1, 10.5 Hz, C-2'Hb; 0.95, t, J 7.2 Hz, C-4'H3. 2H NMR δ 7.19, bs, C-62H. EI-MS: m/z 219 (M**, 9%), 218 (1), 201 (12), 186 (20), 177 (15), 176 (100), 175 (9), 106 (57), 92 (20).
Table 1. Metalation reactions for cis-1,3-dimethyl-5-(1'-methoxymethyloxy butyl)indan (63) quenched with CH$_3$OD.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method$^a$</th>
<th>Metalation Conditions$^b$</th>
<th>% Deuteration$^c$ (% recovery of s.m.$^d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>nBuLi(x1)/hexane/rt/60min</td>
<td>0% (97%)</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>nBuLi(x1)/hexane/reflux 60min</td>
<td>0% (96%)</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>nBuLi(x2)/TMEDA/hexane reflux/60min</td>
<td>0% (90%)</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>nBuLi(x1)/ether/rt/60min</td>
<td>0% (96%)</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>NaOtBu(x2.5)/nBuLi(x2.5) heptane/rt/60min</td>
<td>0% (87%)</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>NaOtBu(x3)/nBuLi(x3) heptane/reflux/60min</td>
<td>0% (91%)</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>NaOtBu(x10)/nBuLi(x10) heptane/THF/reflux/60min</td>
<td>0% (90%)</td>
</tr>
<tr>
<td>8</td>
<td>B</td>
<td>KOtBu(x5)/nBuLi(x5) heptane/THF/reflux/150min</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>rBuLi(x3)/heptane/reflux 360min</td>
<td>0% (89%)</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>rBuLi(x6)/heptane/reflux 120min</td>
<td>0% (82%)</td>
</tr>
</tbody>
</table>

$^a$The general methods A and B for the metalations are above. $^b$Molarity of butyllithium solutions were determined according to the procedure by Winkle et al.$^{44}$ $^c$The percentage deuteration was calculated from mass spectral data (see Chapter 2). $^d$Starting material (recovered) denoted by s.m.
cis-4,6-Dimethyl-8-propyl-indano[cfuran-2-(2H, 8H)-ol (66) / cis-1,3-Dimethyl-
6-(1'-hydroxybutyl)indan-5-carbaldehyde (67)

The general metalation procedure A was followed for the lithiation of the
alcohol (56). The amounts of reagents and solvents used were: alcohol (56)
(30 mg, 137 µmol) in dry, distilled heptane (2.5 mL) and 1.4M t-BuLi in pentane
(880 µL, 1.23 mmol). The mixture was heated to reflux for 1 h. It was cooled and
an excess of N,N-dimethylformamide (DMF) (120 µL) was added. The mixture
was stirred for 20 min. Water (5 mL) was added. The aqueous layer was extracted
with diethyl ether (3 x 4 mL), dried (MgSO4) and the solvent was removed in
vacuo. The residue was chromatographed (silica, ethyl acetate / light petroleum,
1 : 4) to afford a colourless oil (29 mg, 85%) b.p. 79 – 80°C/0.8 mmHg (Found:
C, 78.5; H, 8.8; C16H22O2 requires C, 78.0; H, 9.0; Found M**, 246.1619.
C16H22O2 requires 246.1620). \(\nu_{\text{max}}\) (neat) 3380b (OH) cm\(^{-1}\). \(^1\)H NMR \\
\(\delta\) Minor component: 10.09, s, CHO. Major: 7.20, s, ArH; 6.97, s, ArH; 6.44, bs, (6.38,
d, J 4.0 Hz), C-2H; 5.38, m, (5.12, m), C-8H; 3.05, m, C-4H and C-6H; 2.81,
m, OH (exchangeable with D2O); 2.52, m, C-5Ha; 1.8 - 1.2, bm, C-1'H2, C-2'H2
and C-5Hb; 1.33, d, J 6.0 Hz, 2 x CH3; 0.99, t, J 6.0 Hz, (0.97, t, J 5.8 Hz),
Experimental

C-3'H₃. $^{13}$C NMR δ 150.5, 149.05, (148.98), 141.23, (141.17), 137.72, 4 x ArC quat; 117.11, (117.07), 115.43, (115.34), 2 x ArCH; 100.65, (100.56), O-CH-O; 82.96, (82.22), O-CH; 45.35, (45.32), C-5H₂; 37.69, 37.65, C-4H and C-6H; 19.17, 19.11, 2 x CH₃; 18.79, (18.58), C-1'H₂; 18.44, (18.31), C-2'H₂; 14.03, (13.99), C-3'H₃. EI-MS: m/z 246 (M**, 2%), 228 (35), 213 (70), 203 (100), 199 (91), 143 (27), 128 (32), 115 (30).

$^1$H NMR Experiments on cis-4,6-Dimethyl-8-propyl-indano[c]furan-2-(2H, 8H)-ol (66) / cis-1,3-Dimethyl-6-(1'-hydroxybutyl)indan-5-carbaldehyde (67)

Deuterated solvents used in an attempt to shift the equilibrium between the lactol (66) and the hydroxyaldehyde form (67) included: CDCl₃, D₆-acetone, D₆-DMSO and CD₃OD.

The equilibrium mixture of lactol (66) / hydroxyaldehyde (67) (26 mg, 106 µmol) in a ratio of 40 : 3 in CDCl₃ (500 µL) was acidified using 1M CH₃CO₂H. An aliquot (10 µL, 0.1 equiv.) of the acid solution was added and the $^1$H NMR spectrum was recorded. A change in the ratio of (66) : (67) did not significantly change. Further aliquots were added until 3 equivalents of acid had been delivered and $^1$H NMR spectra were recorded throughout. The ratio of (66) : (67) did not significantly change.

The equilibrium mixture of lactol (66) / hydroxyaldehyde (67) (17 mg, 69 µmol) in a ratio of 37 : 7 in CDCl₃ (600 µL) was treated with 0.5M triethylamine. An aliquot (14 µL, 0.1 equiv.) of the basic solution was added and the $^1$H NMR spectrum was recorded. A change in the ratio of (66) : (67) did not significantly change. Further aliquots were added until 3 equivalents of base had been delivered.
and $^1$H NMR spectra were recorded throughout. The ratio of (66) : (67) did not significantly change.

Preparation of Ethyl azidoacetate

Ethyl azidoacetate was prepared according to Forster and Fierz. A mixture of ethyl chloroacetate (11.0 g, 90 mmol), sodium azide (6.7 g, 103 mmol), ethanol (5 mL) and water (20 mL) was heated to reflux for 3 h. The mixture was cooled and extracted with diethyl ether (3 x 40 mL). The organic fraction was dried (MgSO$_4$) and the solvent evaporated to give a colourless oil (10.42 g). $\nu_{\text{max}}$ (neat) 2115s, (N$_3$), 1740s (C=O) cm$^{-1}$. $^1$H NMR $\delta$ 4.28, q, $J$ 7.1 Hz, 2H; 3.88, s, 2H; 1.32, t, $J$ 1.1 Hz, 3H.

Attempted Condensation of cis-4,6-Dimethyl-8-propyl-indan-2-(2H, 8H)-of (66) / cis-1,3-Dimethyl-6-(1'-hydroxybutyl)indan-5-carbaldehyde (67) with Ethyl azidoacetate

The general method of MacLeod and Monahan was followed. To a stirred solution of sodium (19 mg, 830 µmol) in dry, distilled ethanol (1 mL) under argon at 0°C was added a mixture of (66) / (67) (10 mg, 40.4 µmol), ethyl azidoacetate (119 mg, 920 µmol) and ethanol (2 mL). It was stirred at this temperature for 1 h and TLC (silica, ethyl acetate / light petroleum, 1 : 4) showed mainly starting material was present. It was warmed to room temperature over 1 h and stirred a further 1 h. TLC (silica, ethyl acetate / light petroleum, 1 : 4) showed the starting material had been consumed and one band was observed. The mixture was poured onto water (1 mL) and it was extracted with dichloromethane (3 x 2 mL). The combined organic fractions were washed with water (2 mL), dried (MgSO$_4$) and the solvent was evaporated to give a yellow oil (10 mg). $\nu_{\text{max}}$ (neat) 3080bs,
1745 s cm⁻¹. ¹H NMR δ 7.18, s; 7.02, s; 5.28, s; 4.90, t, J 7.5 Hz; 3.07, m; 2.48, m; 2.28, app quin; 1.8 - 0.80, bm; 1.30, d, J 7.0 Hz; 1.28, d, J 7.0 Hz, 1.08, t, J 7.0 Hz. ¹³C NMR δ 120.9, CH; 117.3, CH; 70.6, CH₂; 46.5, 46.4, 46.2, 3 x CH₂; 39.4 - 38.7, 12 x CH; 30.76, CH₂; 20.3 - 19.9, 7 x CH₃; 15.1, CH₃. The sample was left overnight accumulating more scans for the ¹³C NMR experiment. Many more peaks were observed in the ¹³C NMR spectrum the next day and it did not resemble that spectrum from the previous night (listed above). ¹H NMR data also showed a very complex spectrum that was different to that listed above.

Attempted Protection of the Aldehyde Functionality of cis-1,3-Dimethyl-6-(1'-hydroxybutyl)indan-5-carbaldehyde (67)

1. The method followed was that according to Brown et al. To a stirred solution of adipic acid (4 mg, 31 µmol) in benzene (34 mL) was added a solution of the mixture (66) / (67) (27 mg, 110 µmol) in benzene (1 mL) and ethane diol (136 µL, 2.44 mmol) under argon. The mixture was heated to reflux for 39 h. The condensed solvent was passed through activated molecular sieves (4A) before returning to the reaction flask. The reaction was monitored by TLC (silica, ethyl acetate / light petroleum, 1 : 4). The mixture was cooled and washed with sodium bicarbonate solution (3 x 10 mL). The organic layer was dried (MgSO₄) and the solvent was removed to give a yellow oil (29 mg). It was chromatographed (silica, ethyl acetate / light petroleum, 1 : 4) to give two fractions. The first fraction contained the starting material (22 mg, 81%) that was only in the lactol form (66) by ¹H NMR spectroscopy. The second fraction gave a yellow oil (6 mg). ¹H NMR spectroscopy δ 7.62 - 6.80, bm; 5.50 - 4.50, bm; 3.30 - 2.87, bm; 2.62 - 2.22, bm; 2.08 - 0.60, bm; 1.60, s; 1.42, s.
2. To a stirred solution of the mixture of (66) / (67) (43 mg, 175 µmol) and dry dichloromethane (2 mL) under argon at 5°C was added ethane dithiol (22 µL, 263 µmol) and boron trifluoride etherate (24 µL, 193 µmol). The reaction mixture was warmed to room temperature and it was stirred a further 30 min. The mixture was poured onto water (2 mL). The aqueous phase was extracted with dichloromethane (3 x 2 mL). The combined organic fractions were washed with water (2 mL), dried (MgSO₄) and the solvent was removed under a stream of nitrogen. The material was chromatographed using a gradient of solvents (silica, ethyl acetate / light petroleum, 1 : 9, to only ethyl acetate) and gave two fractions. The less polar product was an orange oil (18 mg). ¹H NMR spectroscopy gave a complex spectrum. EI-MS: m/z 276 (0.6 %), 244 (0.8), 229 (54), 201 (9), 187 (10), 169 (5), 159 (15). The second, more polar product was a colourless solid (26 mg). ¹H NMR δ 7.64, s; 7.60, dd, J 7.6, 1.2 Hz; 7.11, m; 7.00, t, J 7.1 Hz; 6.60, d, J 1.2 Hz; 6.03, m; 5.68, m; 5.39, m; 4.40, - 3.47, bm; 3.06, m; 2.95 - 1.75, bm; 2.53 - 2.42, bm; 1.85, m; 1.75 - 1.40, bm; 1.40 - 1.20, bm; 0.99, t, J 7.2 Hz; 0.95, t, J 7.2 Hz; 0.93, t, J 7.1 Hz. EI-MS : m/z 276 (8%), 245 (26), 244 (29), 203 (100), 229 (11), 215 (100).

Bromination Attempts of Lithiated Hydroxybutylindan (56)

For the general metalation procedure see method A (General Procedure for Metalation Reactions using Butyllithium Reagents) above and the specific bromination reaction conditions are listed in Table 3 below.
Table 3. Bromination attempts for lithiated hydroxybutylindan (56)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Alcohol (56)</th>
<th>Brominating Conditionsa</th>
<th>Productb</th>
<th>Recovered Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 mg</td>
<td>Br$_2$ (XS)/0°C - rt (30min)</td>
<td>dec$^c$</td>
<td>19 mg</td>
</tr>
<tr>
<td>2</td>
<td>17 mg</td>
<td>Br$_2$ (XS)/K$_2$CO$_3$/0°C-rt (30min)</td>
<td>dec</td>
<td>14 mg</td>
</tr>
<tr>
<td>3</td>
<td>25 mg</td>
<td>Pyrrolidone hydrotribromide (x20)/-78°C (30min)/-20°C (30min)/0°C (30min)/rt (30min)</td>
<td>s.m.$^d$+(55)$^e$+ dec (~4:3:1)</td>
<td>23 mg</td>
</tr>
<tr>
<td>4</td>
<td>19 mg</td>
<td>NBS$^f$,THF,-78°C (30min)/-20°C (30min)/0°C (30min)</td>
<td>(55)</td>
<td>17 mg</td>
</tr>
<tr>
<td>5</td>
<td>24 mg</td>
<td>Br(CH$_2$)$_2$Br (x10)/0°C-rt (60min)</td>
<td>s.m.+(74)$^g$ (3:1)</td>
<td>24 mg</td>
</tr>
<tr>
<td>6</td>
<td>25 mg</td>
<td>Br(CH$_2$)$_2$Br (XS)/reflux (5min)</td>
<td>s.m.+(74) (4:1)</td>
<td>26 mg</td>
</tr>
<tr>
<td>7</td>
<td>19 mg</td>
<td>Br(CH$_2$)$_2$Cl (x20)/0°C-reflux (30min)</td>
<td>s.m.+(74) (5:3)</td>
<td>21 mg</td>
</tr>
<tr>
<td>8</td>
<td>2.0 g</td>
<td>1,2-DBTCE$^h$ (x2.5),heptane/0°C, 80°C</td>
<td>s.m.+(74) (2:1)</td>
<td>1.93 g</td>
</tr>
<tr>
<td>9</td>
<td>500 mg</td>
<td>CBr$_4$ (x5),heptane/-130°C (15min)/-78°C (15min)/-20°C(15min)</td>
<td>s.m.+(74) (~1:3)</td>
<td>631 mg</td>
</tr>
<tr>
<td>10$^i$</td>
<td>1.0 g</td>
<td>CBr$_4$ (x10),heptane/0°C-rt</td>
<td>s.m.+(74) (~1:1)</td>
<td>&gt; 1.0 g</td>
</tr>
</tbody>
</table>

$^a$The reaction was initially stirred at the temperature for the time listed and if a colour change was not observed the temperature was adjusted to the next temperature shown for the time listed. General procedure A and B above. $^b$Ratio of products determined by $^1$H NMR spectroscopy. $^c$Decomposition denoted by dec. $^d$Starting material denoted by s.m. $^e$5-(1'-Oxobutyl)-1,3-dimethylindan denoted by (55). $^f$N-Bromosuccinimide denoted by NBS. $^g$cis-1,3-Dimethyl-5-(1'-hydroxybutyl)-6-bromoindan denoted by (74). $^h$1,2-Dibromotetrachloroethane denoted by 1,2-DBTCE. $^i$Typical reaction conditions and outcome for the carbon tetrabromide quench after conditions had been optimised.
5-\((1'\text{-Oxobutyl})\)-1,3-cis-dimethylindan (55)

The general lithiation procedure A was followed. See Entry 4, Table 3 for the bromination conditions employed. The amounts of reagents and solvents used were: alcohol (56) (19 mg, 87 µmol) in dry, distilled heptane (2 mL), 1.7M \(t\)-BuLi in pentane (256 µL, 435 µmol) and N-bromosuccinimde (NBS) (155 mg, 870 µmol). The reaction mixture was quenched with water (5 mL) and the aqueous phase was extracted with diethyl ether (3 x 5 mL), dried (MgSO\(_4\)) and the solvent was removed in vacuo to give an orange oil (17 mg, 89%). It was identified as the butyrolylindan (55) by comparison of its \(^1\text{H NMR}\) spectrum with that of the butyrolylindan (55) synthesised previously from cis-1,3-dimethylindan (14) and butyryl chloride.

5-Bromo-cis-1,3-dimethyl-6-(1'-hydroxybutyl)indan (74)

The general lithiation procedure A was followed. See Entry 9 and 10, Table 3 for the bromination conditions used. The residue was chromatographed on silica (ethyl acetate / light petroleum, 1 : 9) to give starting material (56) and a colourless solid, m.p. 80\(^\circ\)C. (Found: C, 59.9; H, 7.3; Br, 26.8; C\(_{15}\)H\(_{21}\)BrO requires C, 60.6; H 7.1; Br, 26.9. Found M**, 296.0787. C\(_{15}\)H\(_{21}\)BrO requires 296.0776). \(^1\text{H NMR}\) \(\delta\) 7.35, s, ArH; 7.30, (7.29), s, ArH; 5.10, m, O-CH; 3.05, m, C-1H and C-3H; 2.47, m, C-2Ha; 1.89, m, OH; 1.71, m, C-2'H\(_2\); 1.57 - 1.34, bm, C-3'H\(_2\); 1.32, d, J 6.7 Hz, CH\(_3\); 1.29, d, J 6.9 Hz, CH\(_3\); 1.25, m, C-2Hb; 0.98, m, C-4'H\(_3\). \(^1^3\text{C NMR}\) \(\delta\) 149.57, (149.46), 148.30, 141.42, (141.34), 3 x ArCquat; 126.91, (126.88), 121.40, (121.35), 2 x ArCH; 119.69, (119.54), Cquat-Br; 72.83, (72.64), O-CH; 45.06, (44.97), C-2'H\(_2\); 39.92, (39.86), C-2'H\(_2\); 37.79, (37.71), 37.69, C-1H and C-3H; 19.11, CH\(_3\); 19.07, (19.01), C-3'H\(_2\); 18.97, CH\(_3\); 13.88,
C-4'H₃. EI-MS: m/z 298 (M⁺⁺, 5%), 296 (M⁺⁺, 5), 255 (91), 253 (100), 185 (25), 183 (26).

5-Bromo-cis-1,3-dimethyl-6-(1'-methoxymethyloxy butyl)indan (75)

The same method was used as that above for protecting the hydroxy group of the alcohol (56) as the MOM ether (63). The reagents and amounts used were: indan (74) (511 mg, 1.72 mmol), diisopropylethylamine (150 µL, 850 µmol) and methoxymethyl chloride (65 µL, 850 µmol). The reaction was complete (by TLC) after 26 h. The crude product was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) to give the starting material (74) (40 mg, 8%) and a colourless oil (532 mg, 91%) b.p. 70°C/1.3 mmHg. (Found: M⁺⁺, 340.1037. C₁₇H₂₅BrO₂ requires 340.1038). ¹H NMR δ 7.27, s, ArH; 7.25, s, ArH; 5.02, m, O-CH; 4.55, d, J 6.5 Hz, (4.53, d, J 6.3 Hz), 4.52, d, J 6.6 Hz, (4.51, d, J 6.6 Hz), O-CH₂-O; 3.41, (3.40), s, O-CH₃; 3.05, m, C-1H and C-3H; 2.46, m, C-2H₂; 1.72 - 1.21, m, C-2'H₂ and C-3'H₂; 1.28, d, J 7.0 Hz, 2 x CH₃; 1.17, dt, J 12.1, 10.8 Hz, C-2Hb; 0.97, m, C-4'H₃. ¹³C NMR δ 149.51, 148.23, 139.35 (139.29), 3 x ArCquat; 126.80, C-4H; 121.85, C-7H; 120.44, Cquat-Br; 94.73, (94.59), O-CH₂-O; 76.63, (76.58), O-CH; 55.72, O-CH₃; 45.08, (44.99), C-2H₂; 39.41, (39.28), C-2'H₂; 37.78, 37.71, C-1H and C-3H; 19.12, C-3'H₂; 19.07, CH₃; 18.99, (18.94), CH₃; 13.79, C-4'H₃. EI-MS: m/z 342 (M⁺⁺, 9%), 340 (M⁺⁺, 9), 299 (92), 297 (90), 269 (19), 267 (21), 253, (95), 251 (100), 239 (87), 237 (91).

cis-1,3-Dimethyl-6-(1'-methoxymethyloxy butyl)indan-5-carbaldehyde (76)

For the general procedure for the lithiation reaction see method A (General Procedure for Metalation Reactions using Butyllithium Reagents) above. The amounts of reagents used were: aryl bromide (75) (532 mg, 1.56 mmol),
1.6M n-BuLi in hexane (1.95 mL, 3.12 mmol) and DMF (1.2 mL, 15.6 mmol). The crude product was chromatographed (silica, ethyl acetate / light petroleum, 1:9) to give a colourless oil (367 mg, 81%) b.p. 62°C/1.0 mmHg. (Found: [M - 1]− 289.1804. C_{18}H_{25}O_{3} requires 289.1804). ν_{max} (neat) 1700s (CHO), 1610m (ArH) cm⁻¹. ¹H NMR δ 10.33, (10.31), s, CHO; 7.64, s, C-4H; 7.40, s, C-7H; 5.44, m, O-CH; 4.57, m, O-CH₂-O; 3.38, (3.37), O-CH₃; 3.12, m, C-1H and C-3H; 2.54, dt, J 12.1, 6.2 Hz, C-2H₆; 1.94 - 1.16, m, C-2'H₂ and C-3'H₂; 1.36, (1.35), d, J 6.3 Hz, CH₃; 1.33, d, J 6.7 Hz, CH₃; 1.22, m, C-2'H₆; 0.95, t, J 7.2 Hz, C-4'H₃. ¹³C NMR δ 192.37, (192.32), CHO; 155.27, 147.97, 144.42, 132.36, 4 x ArCquat; 125.94, (125.81), 121.73, (121.63), 2 x ArCH; 94.85, (94.74), O-CH₂-O; 74.59, (74.33), O-CH; 55.67, O-CH₃; 44.82, (44.76), C-2H₂; 41.09, (41.03), C-2'H₂; 38.55, (38.46), 37.60, C-1H and C-3H; 19.39, C-3'H; 19.02, CH₃; 18.81, (18.78), CH₃; 13.79, C-4'H₃. EI-MS: m/z 289 ([M - 1]−, 1%), 245 (100), 229 (29), 217 (15), 203 (70). CI-MS: m/z 291 (MH⁺, 11%), 289 (12), 259 (20), 245 (30), 229 (100).
Preparation of Ethyl cis-6,8-dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[gl]indole-2-carboxylate (79) (major component) and Ethyl cis-6,8-dimethyl-1-propyl-6,7,8-tetrahydrocyclopent[g]isoquinoline-3-carboxylate (80) via azidocinnamate (77)

1. Preparation of the azidocinnamate (77)

The title indole carboxylate (79) and isoquinoline carboxylate (80) were prepared by a modification of the method of Moody. Initially the azidocinnamate was prepared and thermolysis gave the title compounds. A mixture of formylindan (76) (350 mg, 1.21 mmol), ethyl azidoacetate (2.50 g, 19.4 mmol) and dry, distilled ethanol (1.5 mL) was added dropwise to a stirred solution of sodium (345 mg, 15 mmol) and ethanol (20 mL) under argon at -15°C. The reaction mixture was kept between the temperature range -15 and -10°C over 5.5 h. It was allowed to slowly warm to room temperature over 1 h. Water (20 mL) was added and the solution was extracted with dichloromethane (3 x 10 mL). The combined organic fraction was washed with a phosphate buffer solution (pH 6.8) (2 x 10 mL). It was dried (MgSO₄) and the solvent was removed to give a mixture of compounds by TLC
Experimental

118

(silica, ethyl acetate / light petroleum, 1 : 9). It was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) to give a yellow oil (300 mg, 63%). $\nu_{\text{max}}$ (CH$_2$Cl$_2$) 2130s (N$_3$), 1715s (C=O) cm$^{-1}$. $^1$H NMR $\delta$ 7.63, (7.62), s, C-4H; 7.31, (7.30), s, CH; 7.26, s, CH; 4.85, m, O-CH; 4.59, d, $J$ 6.7 Hz, 4.54, d, $J$ 6.6 Hz, (4.50, d, $J$ 6.9 Hz), O-CH$_2$-O; 4.38, q, $J$ 7.1 Hz, CO$_2$-$\text{CH}_2$-$\text{CH}_3$; 3.39, (3.38), s, O-CH$_3$; 3.06, m, C-1H and C-3H; 2.50, dt, $J$ 11.9, 6.6 Hz, C-2Ha; 1.84 - 1.13, m, C-2’H$_2$, C-3’H$_2$ and C-2Hb, 1.40, t, $J$ 7.1 Hz, CO$_2$CH$_2$-$\text{CH}_3$; 1.37, d, $J$ 6.8 Hz, (1.36, d, $J$ 6.6 Hz), CH$_3$; 1.31, d, $J$ 6.6 Hz, (1.30, d, $J$ 6.8 Hz), CH$_3$; 0.95, t, $J$ 6.9 Hz, (0.94, t, $J$ 7.2 Hz), C-4’H$_3$. $^{13}$C NMR $\delta$ 163.41, CO$_2$-; 150.15, 147.28, 139.84, (139.80), 129.20, (129.14), 125.94, 5 x Cquat; 123.82, (123.72), 123.66, (123.61), 120.85, 3 x CH; 94.15, O-CH$_2$-O; 74.52, (74.49), O-CH; 62.06, CO$_2$-CH$_2$-CH$_3$; 55.50, O-CH$_3$; 44.94, (44.90), C-2H$_2$; 39.95, (39.89), C-2’H$_2$; 38.16, (38.08), 37.74, C-1H and C-3H; 19.19, C-3’H$_2$; 19.07, C-3 HB; 18.97, CH$_3$; 14.05, CO$_2$CH$_2$-CH$_3$; 13.75, (13.64), C-4’H$_3$. EI-MS: $m/z$ 330 (1%), 288 (6), 270 (8), 245 (49).

2a. Thermolysis of azidocinnamate (77) in toluene

A typical procedure for the thermolysis of the azidocinnamate (77) involved a solution of azidocinnamate (77) (300 mg, 750 µmol) and toluene (80 mL) being plunged into a preheated oil bath (140°C). It was heated to reflux for 45 min. The mixture was cooled and the toluene removed in vacuo. The material was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) to yield two fractions. The first was a yellow oil (228 mg, 81%), b.p. 82°C/1.8 mmHg (Found : M**, 373.2253. C$_{22}$H$_{31}$NO$_4$ requires 373.2253). $\nu_{\text{max}}$ (neat) 3455m (N-H), 1700s (C=O) cm$^{-1}$. $^1$H and $^{13}$C NMR data listed in Table 4, Chapter 2. EI-MS: $m/z$ 373 (M**, 11%), 330 (10), 312 (6), 284 (26), 270 (43), 258 (16), 240, (15), 228 (16), 224 (12), 212 (18), 86 (67), 84, (100).
Experimental

The second fraction gave a colourless solid (12 mg, 5%), m.p. 119.5 - 120°C. (Found : M••*, 311.1886. C₂₀H₂₅N0₂ requires 311.1885). ¹H NMR δ 8.39, s, C-4H; 7.90, s, C-9H; 7.67, s, C-5H; 4.50, q, J 7.5 Hz, CO₂-CH₂-CH₃; 3.41, m, H-6 or H-8; 3.36, t, J 7.7 Hz, C-1'H₂; 3.24, m, H-6 or H-8; 2.60, dt, J 12.1, 7.2 Hz, C-7Ha; 1.91, m, C-2'H₂; 1.44, m, 3 x CH₃; 1.32, m, C-7Hb; 1.09, t, J 7.3 Hz, C-3'H₃. ¹³C NMR δ 166.22, CO₂-; 162.07, Pr-C quat·N; 152.64, C quat; 151.62, C quat; 139.90, C quat·CO₂-; 135.41, C quat·8a; 127.84, C quat·4a; 122.57, C-4H; 121.61, C-5H; 118.33, C-9H; 61.34, CO₂-CH₂-CH₃; 45.08, C-7H₂; 38.10, C-6H or C-8H; 37.83, C-8H or C-6H; 37.71, C-1'H₂; 23.12, C-2'H₂; 18.64, CH₃; 18.46, CH₃; 14.30, CH₃; 14.26, CH₃. EI-MS: m/z 311 (M••, 6%), 296 (7), 283 (100), 239 (11), 222 (19), 209 (69), 194 (30).

2b. Thermolysis of azidocinnamate (77) in benzene

The reaction was carried out in the same manner as that for the thermolysis in toluene except : azidocinnamate (77) (24 mg, 60 µmol), benzene (18 mL) and the reaction time was 24 h. The same work-up and purification procedure gave two fractions. The first fraction gave a yellow oil (12 mg, 53%) with spectral data consistent with that for the indole carboxylate (79). The second fraction was a colourless solid (8 mg, 43%) with spectral data consistent with that for the isoquinoline (80).

2c. Thermolysis of azidocinnamate (77) in xylene

The reaction was carried out in the same manner as that for the thermolysis in toluene except : azidocinnamate (77) (17 mg, 42 µmol), xylene (10 mL) and the reaction time was 1.5 h. TLC (silica, ethyl acetate / light petroleum, 1 : 9) showed bands corresponding to the indole carboxylate (79), the isoquinoline (80) and also a
band on the baseline. The same work-up and purification procedure gave two fractions. The first fraction gave a yellow oil (4 mg, 26%) with spectral data consistent with that for the indole carboxylate (79). The second fraction was a colourless solid (2 mg, 15%) with spectral data consistent with that for the isoquinoline (80).

2d. Thermolysis of purified azidocinnamate (77) in toluene

Crude azidocinnamate (77) (72 mg, 179 µmol) was halved. One portion (36 mg, 90 µmol) was thermolysed as before in toluene (10 mL) for 1.5 h until the reaction was complete. The usual work-up and purification procedure gave two fractions. The first fraction gave a yellow oil (17 mg, 41% from the aldehyde (76)) with spectral data consistent with that for the indole carboxylate (79). The second fraction was a colourless solid (1 mg, 3% from (76)) with spectral data consistent with that for the isoquinoline (80).

The second portion of azidocinnamate (36 mg, 90 µmol) was chromatographed, as before, and thermolysed according to the above procedure in toluene (10 mL) for 1.5 h until the reaction was complete. The usual work-up and purification procedure gave two fractions. The first fraction gave a yellow oil (24 mg, 58% from aldehyde (76)) with spectral data consistent with that for the indole carboxylate (79). The second fraction was a colourless solid (3 mg, 9% from (76)) with spectral data consistent with that for the isoquinoline (80).

Hydrolysis of the indole carboxylate (79)

A solution of indole carboxylate (79) (12 mg, 32 µmol) and methanol (2.5 mL) was stirred with sodium carbonate (8 mg, 75 µmol) and water (1.5 mL) for 12 h at
room temperature. TLC showed only starting material was present. Another portion of sodium carbonate (20 mg) was added. After 6 h, TLC (silica, ethyl acetate / light petroleum, 1 : 9) showed only starting material was present. The reaction was heated to 50°C for 12 h and TLC showed that the starting material had been consumed. The mixture was cooled and the methanol was removed. The aqueous layer was extracted with diethyl ether (3 x 3mL). The ethereal layer was dried (MgSO4) and the ether removed to give a yellow oil (1 mg). 1H NMR δ 8.70, bs, N-H; 7.42 (7.41), d, J 1.9 Hz, C-3H; 6.96, s, C-5H; 4.92, m, O-CH; 4.53, m, O-CH2-O; 3.93, s, CO2-CH3; 3.42, (3.41), s, O-CH3; 3.23, m, C-8H; 3.07, m, C-6H; 2.64, m, C-7Ha; 2.01, m, C-2'Ha, 1.80, m, C-2'Hb; 1.5 - 1.2, m, C-3'H2 and C-7Hb; 1.51, d, J 6.5 Hz, CH3; 1.35, d, J 6.5 Hz, CH3; 0.94, t, J 7.2 Hz, C-4'H3. EI-MS: m/z 359 (M**, 2%), 316 (2), 258 (23), 245 (36), 227 (37), 217, (30), 175 (54).

The aqueous layer was acidified with 4M hydrochloric acid solution and extracted with diethyl ether (3 x 3 mL) and dichloromethane (3 mL). The combined organic extracts were dried (MgSO4) and the solvent evaporated to give a colourless solid (12 mg). 1H NMR δ 8.87, s, N-H; 8.61, bs, CO2H (exchangeable with D2O); 7.69 (7.68), d, J 1.8 Hz, C-3H; 7.00, s, C-5H; 4.97, m, O-CH; 4.58, m, O-CH2-O; 3.48, m, C-6H; 3.43, (3.42), s, O-CH3; 3.22, m, C-8H; 2.65, dt, J 6.9, 11.3 Hz, C-7Ha; 2.02, m, C-2'Ha; 1.82, m, C-2'Hb; 1.5 - 1.25, m, C-3'H2 and C-7Hb; 1.52, d J 6.3 Hz, CH3; 1.36, (1.35), d, J 6.4 Hz, CH3; 0.94, t, J 7.0 Hz, C-4'H3. 13C NMR δ 166.15, CO2H; 146.77, 135.06, (135.02), 134.95, 134.73, (134.70), 129.07, (129.02), 125.33, (125.09), 6 x ArCquat; 115.55, (115.24), C-5H; 110.34, (110.23), C-3H; 93.93, O-CH2-O; 77.07, O-CH; 55.44, O-CH3; 44.00, C-7H2; 39.49, (39.38), C-2'H2; 39.09, (39.04), C-8H; 37.04, C-6H; 20.75, CH3; 20.62, (20.55), CH3; 19.50, C-3'H2; 13.83, C-4'H3. EI-MS: m/z 345 (M**, 20%), 302 (23), 283 (25), 242 (100).
Decarboxylation of the cis-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-
tetrahydrocyclopent[g]indole-2-carboxylic acid (83)

The indole-2-carboxylic acid (83) (18 mg, 52 µmol), copper (6 mg, 94 µmol) and quinoline (4 mL) were stirred at 220 - 230°C for 1 h. The mixture was cooled and poured onto an acid / ice slurry (4 mL hydrochloric acid and ~ 10 g ice). It was stirred and extracted with dichloromethane (4 x 5 mL). The organic layer was washed with water (5 mL) and 5% sodium bicarbonate solution until the pH of the washings was 3 - 4. The aqueous layer was further extracted with diethyl ether (4 x 15 mL) and the organic fractions were combined, dried (MgSO₄) and the solvent evaporated to give a red, viscous oil (17 mg). The oil was subjected to repeated flash chromatography under nitrogen (silica, ethyl acetate / light petroleum, 1 : 9 to 3 : 7) to yield a pink oil (1.5 mg, 10%). (Found : M⁺*, 301.2042. C₁₉H₂₇NO₂ requires 301.2042). ¹H NMR δ 8.11, s, N-H; 7.16, m, C-2H; 6.95, s, C-5H; 6.69, m, C-3H; 4.98, m, O-CH; 4.60, d, J 6.7 Hz, (4.59, d, J 6.6 Hz), 4.54, d, J 6.7 Hz, (4.53, d, J 6.6 Hz), O-CH₂-O; 3.46, m, C-6H; 3.44, (3.43), s, O-CH₃; 3.26, m, C-8H; 2.61, dt, J 12.4, 7.5 Hz, C-7Ha; 2.00, m, C-2'Ha; 1.82, m, C-2'Hb; 1.50 - 1.25, bm, C-3'H₂ and C-7Hb; 1.51, d, J 7.0 Hz, CH₃; 1.36, d J 7.0 Hz, (1.35, d, J 6.8 Hz), CH₃; 0.94, t, J 7.4 Hz, C-4'H₃. ¹³C NMR δ 142.62, 132.69, 132.48, 128.39, 125.13, 5 × ArCquat; 122.90, 113.86, (113.57), 101.74, 3 × ArCH; 93.98, O-CH₂-O; 76.89, O-CH; 55.43, O-CH₃; 44.50, C-7H₂; 39.26, C-2'H₂; 38.70, C-8H; 37.13, C-6H; 20.84, CH₃; 20.55, CH₃; 19.56, C-3'H₂; 13.87, C-4'H₃. EI-MS: m/z 301 (M⁺*, 6%), 258 (9), 226 (8), 212 (8), 198 (100).
cis-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]-indole-2-methanol (87)

Adapted from the method of Zakharkin and Khorlina. To a solution of ethyl indole-2-carboxylate (79) (11 mg, 29 µmol) in toluene (2 mL) at -74°C under argon was added 1.5 M DIBALH in toluene (22 µL, 32 µmol). The reaction was monitored by TLC (silica, ethyl acetate / light petroleum, 1 : 4). After 3 h, only starting material was present. The temperature of the reaction was warmed to -41°C. After stirring a further 1 h at this temperature the reaction mixture was quenched with saturated ammonium chloride solution. The mixture was warmed to room temperature and extracted with diethyl ether (3 x 2 mL). The organic layer was washed with water (2 mL) and dried (MgSO4) and the solvent evaporated to yield a yellow oil (9 mg) that was identified as mainly starting material by 1H NMR spectroscopy. Trace amounts of another compound was present. The oil was taken up in toluene (2 mL) and 1.5 M DIBALH in toluene (48 µL, 72 µmol) was added dropwise. The reaction was stirred at room temperature and monitored by TLC. The reaction was complete after 75 min and it was then cooled (ice bath) and saturated ammonium chloride solution (1 mL) was added. It was extracted with diethyl ether (3 x 3 mL) and the organic layer was washed with water (3 mL), dried (MgSO4) and the solvent evaporated. The material was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) to yield a yellow oil (7 mg, 88%). (Found : M**, 331.2146. C20H29NO3 requires 331.2147). νmax (neat) 3460m (N-H), 3500 - 3200b (OH) cm⁻¹. 1H NMR δ 8.41, bs, N-H; 6.91, s, C-5H; 6.56, bs, C-3H; 4.92, m, O-CH; 4.81, s, CH2-0H; 4.57, d, J 6.5 Hz, (4.56, d, J 6.6 Hz), 4.52, d, J 6.7 Hz, (4.50, d J 6.7 Hz), O-CH2-O; 3.42, (3.41), s, O-CH3; 3.42, m, C-6H; 3.19, m, C-8H; 2.60, dt, J 12.3, 7.5 Hz, C-7Ha; 2.01, m, C-2'Ha; 1.82, m, C-2'Hb; 1.5 - 1.25, bm, C-3'H2, C-7Hb and OH; 1.50, d, J 6.8 Hz, CH3; 1.35, (1.34), d, J 6.9 Hz, CH3; 0.93, t, J 7.4 Hz, (0.88, t, J 7.5 Hz), C-4'H3.
Experimental

$^{13}$C NMR $\delta$ 142.89, 136.45, 133.37, 132.30, 128.45, 125.45, 6 x ArCquat; 114.09, (113.79), 99.95, (99.86), 2 x ArCH; 93.87, O-CH$_2$-O; 77.14, O-CH; 58.73, CH$_2$OH; 55.40, O-CH$_3$; 44.44, C-7H$_2$; 39.17, C-2'H$_2$; 38.71, C-8H; 37.09, C-6H; 20.90, (20.81), CH$_3$; 20.55, (20.48), CH$_3$; 19.57, C-3'H$_2$; 13.87, C-4'H$_3$.

EI-MS: m/z 331 (M**, 68%), 270 (36), 256 (57), 228 (100), 226 (100), 198 (74).

cis-6,8-Dimethyl-4-(1'-methoxymethoxy butyl)-1,6,7,8-tetrahydrocyclopent[g]-indole-2-carboxaldehyde (86)

The title aldehyde (86) was prepared with modifications to the procedure by Moody and co-workers. Manganese dioxide (0.25 g, 2.63 mmol) was added to a stirred solution of indole-2-methanol (87) (109 mg, 329 $\mu$mol) and dichloromethane (50 mL). The mixture was heated to reflux. After 30 min, another portion of MnO$_2$ (0.25 g, 2.63 mmol) was added. After 6 h, the reaction mixture was filtered through a pad of celite and the celite was washed with hot toluene (250 mL). The toluene was removed and the residue was chromatographed (florisil, dichloromethane) to yield a yellow solid (71 mg, 65%). (Found: M**, 329.1990. C$_{20}$H$_{27}$N$_{0}$O$_{3}$ requires 329.1991). $\nu_{\text{max}}$ (neat) 1660s (C=O) cm$^{-1}$. $^1$H NMR $\delta$ 9.80, s, CHO; 8.82, bs, N-H; 7.49, (7.48), d, $J$ 1.9 Hz, C-3H; 6.98, s, C-5H; 4.92, m, O-CH; 4.57, 4.56, m, O-CH$_2$-O; 3.44, m, C-6H; 3.42 (3.40), s O-CH$_3$; 3.21, m, C-8H; 2.65, dt, $J$ 12.5, 7.7 Hz, C-7Ha; 2.02, m, C-2'Ha; 1.80, m, C-2'Hb; 1.50 - 1.20, bm, C-3'H$_2$ and C-7Hb; 1.50, d, $J$ 6.9 Hz, CH$_3$; 1.36, d, $J$ 6.9 Hz, (1.35, d, $J$ 6.8 Hz), CH$_3$; 0.95, t, $J$ 7.2 Hz, C-4'H$_3$. $^{13}$C NMR $\delta$ 181.70, CHO; 148.30, 135.74, (135.62), 135.44, 135.26, 129.41, 124.93, 6 x ArCquat; 116.00, (115.70), 114.99, (114.89), 2 x ArCH; 93.97, O-CH$_2$-O; 77.56, O-CH; 55.51, O-CH$_3$; 43.93, C-7H$_2$; 39.50, (39.40), C-2'H$_2$; 39.17, C-8H; 37.01, C-6H; 20.68, CH$_3$; 20.53, (20.48), CH$_3$; 19.52, C-3'H$_2$; 13.85, C-4'H$_3$. 

Based on the procedure by Moody and co-workers. A solution of $\text{HClO}_4$ (1 mL) was added to a solution of the title compound (100 mg) in dichloromethane (5 mL). The solution was filtered through a pad of celite and the eluate was evaporated. The residue was treated with hot toluene (250 mL) and the toluene was removed to yield a yellow solid (62 mg, 50%). (Found: M**, 329.1990. C$_{20}$H$_{27}$N$_{0}$O$_{3}$ requires 329.1991). $\nu_{\text{max}}$ (neat) 1660s (C=O) cm$^{-1}$. $^1$H NMR $\delta$ 9.80, s, CHO; 8.82, bs, N-H; 7.49, (7.48), d, $J$ 1.9 Hz, C-3H; 6.98, s, C-5H; 4.92, m, O-CH; 4.57, 4.56, m, O-CH$_2$-O; 3.44, m, C-6H; 3.42 (3.40), s O-CH$_3$; 3.21, m, C-8H; 2.65, dt, $J$ 12.5, 7.7 Hz, C-7Ha; 2.02, m, C-2'Ha; 1.80, m, C-2'Hb; 1.50 - 1.20, bm, C-3'H$_2$ and C-7Hb; 1.50, d, $J$ 6.9 Hz, CH$_3$; 1.36, d, $J$ 6.9 Hz, (1.35, d, $J$ 6.8 Hz), CH$_3$; 0.95, t, $J$ 7.2 Hz, C-4'H$_3$. $^{13}$C NMR $\delta$ 181.70, CHO; 148.30, 135.74, (135.62), 135.44, 135.26, 129.41, 124.93, 6 x ArCquat; 116.00, (115.70), 114.99, (114.89), 2 x ArCH; 93.97, O-CH$_2$-O; 77.56, O-CH; 55.51, O-CH$_3$; 43.93, C-7H$_2$; 39.50, (39.40), C-2'H$_2$; 39.17, C-8H; 37.01, C-6H; 20.68, CH$_3$; 20.53, (20.48), CH$_3$; 19.52, C-3'H$_2$; 13.85, C-4'H$_3$. 

Based on the procedure by Moody and co-workers.
Experimental

EI-MS: m/z 329 (M**, 12%), 286 (10), 276 (19), 244 (22), 226 (34), 201 (30), 184 (89), 130 (100).

Decarbonylation of cis-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-
tetrahydrocyclopent[g]indole-2-carboxaldehyde (86)

Based on the procedure by Moody and Swann.\textsuperscript{56} A mixture of (PPH\textsubscript{3})\textsubscript{2}RhCOCl (27 mg, 40 µmol) and mesitylene (40 mL) was heated to 85°C under oxygen-free conditions. This temperature was maintained until the rhodium complex had dissolved (20 min). A mixture of 1,3-bis(diphenylphosphino)propane (dppp) (33 mg, 80 µmol) and mesitylene (5 mL) also under oxygen-free conditions, was added to the above rhodium solution. The mixture was stirred at 85°C for a further 20 min. A solution of oxygen-free indole-2-carboxaldehyde (86) (66 mg, 200 µmol) and mesitylene (2 mL) was added to the rhodium complex solution and it was immediately plunged into a Woods metal bath at 200°C. The bath temperature was maintained between 180 - 200°C. After 30 min TLC (silica, ethyl acetate / light petroleum, 1 : 4) showed the starting material had been consumed. The reaction was cooled (ice bath) and the mesitylene was removed by distillation \textit{in vacuo}. The residue was dissolved in dichloromethane (2 mL) and chromatographed (florisil, dichloromethane and then silica, ethyl acetate / light petroleum, 1 : 4) to yield a yellow oil (39 mg, 65%) that had spectral data consistent with that of \textit{cis}-6,8-
dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydro-cyclopent[g]indole (85).
Experimental

**Attempted deprotection of cis-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole (85)**

Based on the procedure by Natsume and co-workers. An aliquot (10 µL) of a solution of p-TSA.H2O (8 mg) and benzene (4 mL) and THF (1 mL) was added to a stirred solution of indole (84) (0.5 mg, 1.7 µmol) and benzene (0.5 mL). After 10 min the reaction went a purple colour. TLC (silica, ethyl acetate / light petroleum, 1 : 4) showed starting material, streaking up the plate and baseline material were present. After 30 min, the solution was a deeper purple colour and TLC showed only baseline material and streaking up the plate. ¹H NMR spectroscopy showed a very complex spectrum. A variety of solvents (benzene, dichloromethane, THF) and various equivalents of p-TSA were tried. However, the same result as that before was obtained for each reaction.

1. **Attempted N-Protection of Ethyl cis-6,8-dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-carboxylate (79)**

   a. using pyridine / PhSO₂Cl

To a stirred solution of indole-2-carboxylate (79) (40 mg, 107 µmol) and dry, distilled dichloromethane (0.5 mL) at 2°C under argon, was added a mixture of pyridine (43 µL, 536 µmol) and benzenesulfonyl chloride (32 µL, 258 µmol). It was warmed to room temperature and stirred for 18 h. Only starting material was observed by TLC (silica, ethyl acetate / light petroleum, 1 : 9). The dichloromethane was removed and a mixture of diisopropylethylamine (1.5 mL) and benzenesulfonyl chloride (0.5 mL) was added at 2°C. The reaction mixture was warmed to room temperature. It was stirred for 9 h and only starting material was observed by TLC.
Experimental

b. *using NaH / THF / PhSO₂Cl*

Based on the procedure by Moody and co-workers. A suspension of sodium hydride (60% dispersion; 6 mg, 290 µmol) in dry THF (1 mL) was cooled to 2°C under argon. A solution of indole-2-carboxylate (79) (36 mg, 96 µmol) and THF (1 mL) was added. It was stirred for 5 min at this temperature and then warmed to room temperature and stirred a further 1 h. Effervescence and a colour change in the reaction mixture were not observed. The mixture was then heated to reflux for 15 min and cooled. Benzenesulfonyl chloride (22 µL, 174 µmol) was added and the mixture was stirred a further 1 h. TLC (silica, ethyl acetate / light petroleum, 1 : 9) showed only starting material was present. It was quenched with water (1 mL) and extracted with dichloromethane (3 x 3 mL). The organic layer was washed with saturated sodium chloride solution (3 mL) and dried (MgSO₄). The solvent was removed to give a yellow oil that was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) to recover starting material.

c. *using KH / THF / PhSO₂Cl*

The same procedure that was used as that for b. (*using NaH / THF / PhSO₂Cl*) above, was employed except the amounts of reagents used were indole-2-carboxylate (79) (30 mg, 80 µmol), potassium hydride (8 mg, 200 µmol) and benzenesulfonyl chloride (28 µL, 220 µmol). Once again only starting material was detected by TLC (silica, ethyl acetate / light petroleum, 1 : 9). After work-up, only starting material was recovered.
d. using KH/DMF/PhSO₂Cl

The same procedure was used except DMF was used as the solvent and the amounts of reagents used were: indole-2-carboxylate (79) (19 mg, 51 µmol), potassium hydride (5 mg, 125 µmol) and benzenesulfonyl chloride (13 µL, 102 µmol). When the solution of indole-2-carboxylate (79) and DMF was added to the potassium hydride and DMF solution at 0°C, effervescence was observed. The solution was warmed and the reaction mixture went from a yellow to an orange colour. The benzenesulfonyl chloride was added to the cooled solution. It was warmed to room temperature and stirred a further 2 h. The colour of the reaction did not change initially and only faded slightly over the 2 h. TLC (silica, ethyl acetate/light petroleum, 1:9) showed only starting material was present. After the reaction was quenched and worked up only starting material was recovered.

2a. Attempted N-Protection of cis-6,8-Dimethyl-4-[(1'-methoxymethyloxy butyl)-1,6,7,8-tetra-hydrocyclopent[g]indole (85) using KH/THF/PhSO₂Cl

The usual deprotonation procedure was followed except the mixture of indole (85) (4 mg, 13 µmol), KH (3 mg, 75 µmol) and THF was heated to reflux for 30 min. Once it turned green and effervescence was observed, it was cooled and benzenesulfonyl chloride (3 µL, 26 µmol) was added. The solution went a pink colour. Triethylamine (10 µL) was added and the reaction was warmed to room temperature. It was stirred for 1 h. TLC showed starting material and baseline material was present. The reaction mixture was worked up in the usual manner. Starting material was recovered.
Experimental

2b. *N*-Protection of cis-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole (85) using KH / THF / Ac₂O

The usual procedure was followed except the mixture of indole (85), KH and THF was heated to reflux for 15 min. It was then cooled prior to the addition of acetic anhydride. The reagents and amounts used were: indole (85) (18 mg, 60 µmol), KH (12 mg, 299 µmol) and acetic anhydride (24 mg, 238 µmol). After the usual work-up procedure and chromatography (silica, ethyl acetate / light petroleum, 1:9), two fractions were yielded. The first fraction gave a yellow oil (3 mg) that was identified as starting material by TLC (silica, ethyl acetate / light petroleum, 1:9) and ¹H NMR spectroscopy. Another fraction gave a yellow oil (12 mg, 60%) (Found: M⁺⁺, 343.2146. C₂₁H₂₉NΟ₃ requires 343.2147). νₘₐₓ (neat) 1720 s (C=O) cm⁻¹. ¹H NMR δ 7.35, (7.34), d, J 3.9 Hz, C-2H; 7.10, s, C-5H; 6.85, (6.84), d, J 3.9 Hz, C-3H; 4.93, m, O-CH; 4.52, m, O-CH₂-O; 4.20, (3.95), m, C-6H; 3.41, s, O-CH₃; 3.30, m, C-8H; 2.70, m, C-7Ha; 2.651, (2.648), s, NCO-CH₃; 2.00, m, C-2'Ha; 1.78, m, C-2'Hb; 1.6 - 1.3, bm, C-3'H₂ and C-7Hb; 1.35, (1.33), d, J 6.7 Hz, CH₃; 1.10, (1.08), d, J 6.8 Hz, CH₃; 0.94, t, J 7.1 Hz, C-4'H₃. ¹³C NMR δ 167.5, NCO; 146.78, (146.28), 134.95, (134.57), 132.56, 128.53, 4 x ArCquat; 125.15, 118.16, (117.83), 2 x ArCH; 110.73, ArCquat; 107.98, ArCH; 93.9, O-CH₂-O; 76.06, O-CH; 55.4, O-CH₃; 42.76, (42.71), C-7H₂; 39.53, (39.35), C-2'H₂; 38.89, 37.21, C-8H and C-6H; 24.52, (24.00), 23.17, (22.09), 21.36, (20.30), 3 x CH₃; 19.32, C-3'H₂; 13.81, C-4'H₃. EI-MS: m/z 343 (M⁺⁺, 45%), 300 (25), 282 (19), 258 (40), 240 (64), 266 (57), 198 (100).
Depréction of the MOM group from 1-Acetyl-cis-6,8-dimethyl-4-(1'-methoxy-methyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole (97)

a. an attempt using p-TSA.H2O

To a stirred solution of acetamide (97) (~ 0.5 mg, ~ 2 µmol) and benzene (1 mL) was added dry p-TSA.H2O (~ 0.5 mg, ~ 2 µmol). After 30 min at room temperature a purple colour appeared. TLC (silica, ethyl acetate / light petroleum, 1 : 4) showed starting material and purple, baseline material. After 1 h, a small amount of starting material and an increase in the amount of baseline material was observed by TLC. The reaction was abandoned.

b. using Me2BBr / NEt3

The method was adapted from that by Guindon et al. To a stirred solution of acetamide (92) (8 mg, 23 µmol) and dry dichloromethane (1 mL) under argon at -78°C, was added an aliquot (200 µL) of a solution of dimethylboron bromide and dichloromethane (43 mg / mL). The solution went green. After stirring for 15 min, triethylamine (30 µL) was added and then water (100 µL) was added dropwise. The reaction mixture was slowly warmed to room temperature. It was extracted with dichloromethane (3 x 3 mL). The organic layer was washed with water (1 mL), saturated sodium chloride solution (1 mL) and dried (MgSO4). The solvent was evaporated to give a colourless solid (15 mg). It was chromatographed (silica, ethyl acetate / light petroleum, 1 : 9) under nitrogen to give a colourless solid (4.5 mg, 65%). 1H NMR δ 7.42, (7.41), d, J 2.1 Hz, C-5H; 7.04, (7.03), d, J 3.7 Hz, C-2H; 6.89, (6.88), d, J 3.7 Hz, C-3H; 4.98, (4.93), m, O-CH; 4.28, (4.18), m, C-6H; 3.38, m, C-8H; 2.63, m, C-7Ha; 2.61, (2.58), s, COCH3; 2.03, m, C-2'Ha; 1.82, m, C-2'Hb; 1.5 - 1.3, bm, C-3'H2, C-7Hb and OH; 1.23, d,
\[ J 7.2 \text{ Hz}, \text{CH}_3; 1.21, \text{d, } J 7.2 \text{ Hz}, \text{CH}_3; 0.89, J 7.0 \text{ Hz}, \text{C-4'H} \\]

EI-MS: \( m/z \) 299 (M\(^{+*}\), 4%), 281 (87), 266 (51), 239 (51), 224 (100).

**Attempted elimination of water from the hydroxybutyl indole (98)**

An aliquot (64 \( \mu \)L) of \( p \)-TSA.\( \text{H}_2\text{O} \) in THF (10 mg in 10 mL) was added to the hydroxybutyl indole (98) (1 mg, 3\( \mu \)mol) in benzene (1 mL) and the mixture was stirred at room temperature, however no reaction was observed by TLC. It was heated to reflux and after a further 2 h the reaction turned a purple colour. Starting material was present by TLC (silica, ethyl acetate / light petroleum, 1 : 9) and other bands appeared. The reaction was cooled and triethylamine (10 \( \mu \)L) was added. Water (1 mL) was then added and the mixture was extracted with dichloromethane (3 x 2 mL). The organic layer was dried (\( \text{MgSO}_4 \)). The solvent was removed to give a yellow oil (1 mg). \( ^1\text{H} \) NMR spectroscopy showed the presence of starting material and a complex set of signals.

**Chapter 3**

**3-Phenylbutanoic acid (100)**

The preparation of the title acid (100) was based on the method by Marvel et al.\(^{69} \) A solution of crotonic acid (10.0 g, 116 mmol) in dry benzene (30 mL) was added slowly to a stirred suspension of aluminium trichloride (26.3 g, 197 mmol) also in dry benzene (15 mL) under nitrogen at 3 - 5°C. After the addition was complete, the mixture was stirred and maintained at this temperature for a further 1 h. The reaction was allowed to come to room temperature and it was stirred vigorously for
48 h. The mixture was then poured onto a mixture of ice (200 g) and hydrochloric acid (10 mL). A white precipitate was formed. Benzene (150 mL) was added and the mixture was stirred vigorously until the precipitate had been dissolved (36 h). The aqueous layer was extracted with benzene (3 x 30 mL). The combined organic fractions was washed with water (3 x 50 mL) and dried (MgSO4). The solvent and excess crotonic acid were removed by distillation. The residue was distilled further (30°C/0.8 mmHg) to give a yellow oil. (13.70 g, 72%) which solidified upon cooling, m.p. 33 - 34°C (uncorrected) (lit.77 37 - 39°C). νmax (CH2Cl2) 3400-2500 (OH), 1708 (C=O) cm⁻¹. 1H NMR δ 11.62, bs, CO2H; 7.28, m, 5 x ArH; 3.27, m, C-3H; 2.63, m, C-2H2; 1.32, d, J 7.0 Hz, CH3. 13C NMR δ 178.8, CO2H; 145.3, ArCquat; 128.5, 2 x ArCH; 126.6, 2 x ArCH, 126.4, ArCH; 42.4, C-2H2; 35.9, C-3H; 21.7, CH3. EI-MS: m/z 164 (M**, 11%), 118 (15), 105 (100).

3-Methylindanone (99)

a. Formation and cyclisation of phenylbutanoic acid chloride

A mixture of the acid (100) (13.7 g, 83.4 mmol) and dry dichloromethane (50 mL) under argon was stirred with oxalyl chloride (10.92 mL, 125.2 mmol) and N,N-dimethylformamide (500 µL). The dichloromethane and excess oxalyl chloride was removed in vacuo. Dichloromethane (20 mL) was added to the residue and it was removed by distillation. The resulting residue was taken up in dry dichloromethane (10 mL) and added dropwise to suspension of aluminium chloride (22.2 g, 166.8 mmol) in dry dichloromethane (40 mL) under argon at 5°C. The mixture was warmed to room temperature and stirred a further 12 h. The mixture was added dropwise to a vigorously stirred mixture of ice (30 g) and concentrated hydrochloric acid (5 mL). The aqueous phase was extracted with
dichloromethane (3 x 50 mL) and the combined organic fractions were washed with water (3 x 30 mL). The organic layer was dried (MgSO₄) and the solvent was removed under vacuum to give a yellow oil (10.2 g, 83%). νₘₚₓ (neat) 1710s (C=O) cm⁻¹. ¹H NMR δ 7.73, d, J 7.6 Hz, ArH; 7.61, t, J 7.6 Hz, ArH; 7.51, d, J 7.6 Hz, ArH; 7.39, t, J 7.6 Hz, ArH; 3.44, m, C-3H; 2.93, dd, J 19.0, 7.4 Hz, C-2Ha; 2.29, dd, J 19.0, 3.3 Hz, C-2Hb; 1.42, d, J 7.1 Hz, CH₃. ¹³C NMR δ 206.6, C=O; 160.1, 136.4, 2 x ArCquat; 134.8, 127.4, 125.3, 123.3, 4 x ArCH; 45.0, C-2H₂; 32.4, C-3H; 20.9, CH₃. EI-MS: m/z 146 (M⁺, 65%), 131 (100), 117 (29), 103 (46), 91 (12), 77 (32).

b. From benzene, crotonic acid and aluminium trichloride

A solution of crotonic acid (17.28 g, 201 mmol) in dry benzene (50 mL) was added over 20 min to a stirred suspension of aluminium trichloride (46.1 g, 345 mmol) in dry benzene (70 mL) that was cooled in an ice bath. The mixture was then heated to reflux for 17 h. The condensed solvent was passed through activated molecular sieves (4A) before returning to the reaction flask. The mixture was poured onto ice (300 g) and stirred. It was extracted with dichloromethane (3 x 150 mL). The combined organic fractions were washed with water (200 mL), dried (MgSO₄) and the solvent was removed. The concentrate was distilled (100 - 105°C/0.8 mmHg) to give a colourless oil (22.7 g, 77%). The spectral data agreed with that reported above for 3-methylindanone (99).

trans-1,3-Dimethylindanol (60)

Iodomethane (17.31 mL, 278 mmol) in dry diethyl ether (30 mL) was added dropwise to a stirred suspension of magnesium turnings (3.38 g, 139 mmol) in dry diethyl ether (100 mL) at a rate sufficient to maintain gentle reflux under argon.
After the addition was complete, the mixture was heated to reflux a further 1 h. It was cooled and the solution was transferred to another flask, also under argon. A solution of 3-methylindanone (99) (10.16 g, 69.5 mmol) in dry diethyl ether (40 mL) was added dropwise to the stirred solution of Grignard reagent. The mixture was heated to reflux for 18 h. The reaction mixture was cooled and slowly poured onto a mixture of iced water (300 g) and hydrochloric acid (5 mL). The mixture was stirred vigorously and the aqueous layer was extracted with diethyl ether (6 x 100 mL). The organic fraction was washed with water (3 x 100 mL), saturated ammonium chloride solution (50 mL) and water (3 x 50 mL). The organic fraction was dried (MgSO₄) and the solvent was removed and the residue was distilled to give a yellow oil (9.98 g, 89%). A mixture of trans- and cis-dimethylindanol (60) and (61) (~7:1) was obtained according to ¹H NMR spectroscopy. The spectral data corresponded well with that previously obtained for trans- and cis-dimethylindanol (60) and (61) prepared from the radical cyclisation of the bromoarylpentenol (12) (above). When this procedure was followed on other occasions, only trans-dimethylindanol (60) was observed by ¹H NMR spectroscopy.

Preparation of slightly alkaline Raney nickel

Raney nickel was prepared by a modification of the method of Pavlic and Adkins. To a stirred solution of sodium hydroxide (32 g) and water (125 mL) at 50°C was added portions of nickel aluminium alloy (25 g, over ~1 h) so as to maintain the temperature at 50 ± 4°C. After the addition was complete, this temperature range was maintained for 1 h. It was cooled, washed with degassed water* thoroughly until the pH of the washings ranged between 7.5 and 8.5. The Raney nickel

* The water was degassed using ultrasonication and argon sparging.
suspension was washed with dried, distilled absolute ethanol (3 x 100 mL) and stored under ethanol.

*Hydrogenolysis of trans-1,3-dimethylindanol (60) with Raney nickel*

A solution of trans-1,3-dimethylindanol (60) (2.50 g, 15 mmol) in distilled, degassed ethanol (100 mL) and a suspension of freshly prepared Raney nickel (~25 g) was heated to reflux at ambient pressure. After 2 h, the spent Raney nickel was collected on a celite pad. The filtrate was distilled to remove the solvent and the oil was collected and identified as trans-1,3-dimethylindan (17) by \(^1\)H NMR spectroscopy.\(^{29}\) A yield of 2.04 g was afforded. \(^1\)H NMR \(\delta\) 7.18, s, 4 x ArH; 3.27, sextet, \(J\) 6.9 Hz, C-1H and C-3H; 1.89, t, \(J\) 6.9 Hz, C-2H; 1.26, d, \(J\) 6.9 Hz, 2 x CH₃.

**5-(1'-Oxobutyl)-1,3-trans-dimethylindan (101)**

The procedure used was according to that followed for the synthesis of 5-(1'-oxobutyl)-1,3-cis-dimethylindan (55) (above). The reagents and amounts used were: trans-1,3-dimethylindan (17) (theoretically 10.3 g, 70 mmol), butyryl chloride (11 mL, 106 mmol) and aluminium trichloride (14.09 g, 106 mmol). The resulting oil was chromatographed on silica (ethyl acetate / light petroleum, 1 : 9) to give a colourless oil (10.6 g, 70%) from trans-1,3-dimethylindanol (60). (Found: M\(^{+}\), 216.1514. C\(_{15}\)H\(_{20}\)O requires 216.1514). \(^1\)H NMR \(\delta\) 7.82, d, \(J\) 8.0 Hz, C-6H; 7.78, s, C-4H; 7.24, d, \(J\) 8.0 Hz, C-7H; 3.31, m, C-1H and C-3H; 2.95, (2.94), t, \(J\) 7.3 Hz, C-2'H; 1.94, t, \(J\) 6.8 Hz, C-2'H; 1.76, sextet, \(J\) 7.3 Hz, C-3'H; 1.27, d, \(J\) 6.9 Hz, CH₃; 1.25, d, \(J\) 6.9 Hz, CH₃; 1.01, t, \(J\) 7.4 Hz, C-4'H. \(^{13}\)C NMR \(\delta\) 200.33, C=O; 153.90, 148.80, 135.84, 3 x ArCquat; 126.88, 123.24, 122.99, 3 x ArCH; 42.76, C-2'H; 40.48, C-2'H; 37.61, 37.28, C-1H.
trans-1,3-Dimethyl-5-(1'-hydroxybutyl)indan (102)

The title alcohol was prepared according to method used for the synthesis of cis-1,3-dimethyl-5-(1'-hydroxybutyl)indan (56) (above). The reagents and amounts used were: butyroylindan (101) (10.6 g, 49 mmol) and sodium borohydride (2.78 g, 74 mmol). A colourless oil (10.13 g, 95%) was afforded, b.p. 130°C/0.02 mmHg. (Found: M**, 218.1670. C_{15}H_{22}O requires 218.1671).

1H NMR δ 7.16, d, J 6.2 Hz, C-6H and C-7H; 7.15, s, C-4H; 4.66, m, O-CH; 3.26, m, C-1H and C-3H; 1.91, t, J 6.7 Hz, C-2H2; 1.77, m, C-2'Ha and OH; 1.69, m, C-2'Hb; 1.50 - 1.30, m, C-3'H2; 1.25, (1.24), d, J 7.0 Hz, CH3; 1.23, d, J 7.0 Hz, CH3; 0.94, t, J 7.3 Hz, C-4'H3.

13C NMR δ 148.57, 147.7, 143.18, 3 x ArCquat; 124.09, 123.16, 120.80, (120.75), 3 x ArCH; 74.55, O-CH; 43.02, C-2H2; 41.16, (41.11), C-2'H2; 37.43, 37.22, C-1H and C-3H; 20.45, 2 x CH3; 19.11, C-3'H2; 13.88, C-4'H3. EI-MS: m/z 218 (M**, 20%), 175 (100), 145 (11), 131 (16), 105 (54).

5-Bromo-trans-1,3-dimethyl-6-(1'-hydroxybutyl)indan (105)

The title arylbromide (105) was prepared according to the same procedure used for 5-bromo-6-(1'-hydroxybutyl)-cis-1,3-dimethylindan (74), above. The reagents and amounts used were: hydroxybutylindan (102) (2.0 g, 9.2 mmol), 1.7M t-BuLi in pentane (27 mL, 46 mmol) and carbon tetrabromide (14.5 g, 44 mmol). The residue was chromatographed on silica (ethyl acetate / light petroleum, 1 : 9) to give starting material (97) (0.74 g, 37%) and a yellow oil (1.44 g, 52%) (Found M**, 296.0776. C_{15}H_{21}BrO requires 296.0776) 1H NMR δ 7.36, s, ArH; 7.30, s,
Experimental

ArH; 5.08, m, O-CH; 3.24, m, C-1H and C-3H; 1.91, t, J 6.8 Hz, C-2H2; 1.80 - 1.30, bm, C-2'H2, C-3'H2 and OH; 1.24, (1.23), d, J 7.4 Hz, CH3; 1.21, d, J 7.2 Hz, CH3; 0.96, t, J 7.3 Hz, C-4'H3. 13C NMR δ 149.51, (149.28) 148.26, (148.19), 141.456, (141.41), 3 x ArCquat: 127.34, 121.92, 2 x ArCH; 119.63, Cquat-Br; 72.62, (72.58), O-CH; 42.99, C-2H2; 39.90, (39.85), C-2'H2; 37.75, (37.65), 37.25, (37.13), C-1H and C-3H; 20.31, (20.22), CH3; 20.18, CH3; 19.08, C-3'H2; 13.82, C-4'H3. EI-MS: m/z 298 (M**, 14%), 296 (M**, 16), 255 (97), 253 (100), 185 (28), 183 (27).

5-Bromo-trans-1,3-dimethyl-6-(1'-methoxymethyloxy butyl)indan (106)

An improved procedure for the MOM-protection of alcohols was used.70 To a stirred solution of bromohydroxybutylindan (105) (0.80 g, 2.7 mmol) and dichloromethane (75 mL) at 0°C was added N,N-dimethylaminopyridine (DMAP) (33 mg, 0.3 mmol), diisopropylethylamine (2.4 mL, 13.5 mmol) and methoxymethyl chloride (1 mL, 13.5 mmol). It was warmed to room temperature and stirred for 17 h. The usual work-up and purification procedure (as for the corresponding cis-compound) gave a yellow oil (0.88 g, 95%). (Found: M**, 340.1037. C17H2579BrO2 requires 340.1038). 1H NMR δ 7.29, s, ArH; 7.26, (7.25), s, ArH; 5.03, m, O-CH; 4.53, m, O-CH2-O; 3.41, (3.39), s, O-CH3; 3.23, m, C-1H and C-3H; 1.90, t, J 6.8 Hz, C-2H2; 1.75 - 1.20, bm, C-2'H2, C-3'H2; 1.23, (1.21), d, J 7.0 Hz, CH3; 1.20, (1.19), d, J 7.0 Hz, CH3; 0.96, t, J 7.4 Hz, C-4'H3. 13C NMR δ 148.18, 139.51, 2 x ArCquat: 127.36, C-4H; 122.46, (122.37), C-7H; 120.45, Cquat-Br; 111.33, Cquat; 94.76, O-CH2-O; 76.09, O-CH; 55.72, O-CH3; 42.98, (42.90), C-2H2; 39.36, (39.26), C-2'H2; 37.70, (37.65), 37.24, (37.21), C-1H and C-3H; 20.32, CH3; 20.27, (20.26), CH3; 19.06, C-3'H2; 13.79, C-4'H3. EI-MS: m/z 342 (M**, 11%), 340 (M**,
trans-1,3-Dimethyl-6-(1'-methoxymethoxy butyl)indan-5-carbaldehyde (104)

To a stirred solution of the arylbromide (106) (0.60 g, 1.8 mmol) and heptane (50 mL) under argon at -78°C was added 1.6M n-BuLi in hexane (2.2 mL, 3.5 mmol) dropwise. It was stirred for 10 min and slowly warmed to 0°C. The reaction mixture went a dark yellow and a precipitate formed. It was stirred a further 15 min and then cooled to -78°C. DMF was added dropwise. The reaction was slowly warmed to room temperature. The usual work-up and purification was employed (as for that used for the corresponding cis-compound) to give two fractions. The first gave a colourless oil (0.33 g, 65%). (Found: [M - 1]+, 289.1804. C_{18}H_{25}O_3 requires 289.1804). ν_{max} (neat) 1695s (CHO), 1610m (Ar-H) cm\(^{-1}\). H NMR* δ 10.30, s, CHO; 7.65, s, C-4H; 7.41, s, C-7H; 5.44, m, O-CH; 4.58, d, J 6.7 Hz, 4.53, d, J 6.6 Hz, O-CH\(_2\)-O, 3.38, (3.36), s, O-CH\(_3\); 3.30, m, C-1H and C-3H; 1.95, t, J 6.8 Hz, C-2H\(_2\); 1.80 - 1.20, bm, C-2'H\(_2\) and C-3'H\(_2\); 1.27, d, J 6.6 Hz, (1.26, d, J 6.4 Hz), CH\(_3\); 1.25, d, J 7.0 Hz, CH\(_3\); 0.95, t, J 7.3 Hz, C-4'H\(_3\). C NMR δ 192.23, (192.20), CHO; 155.07, 147.66, 144.43, 132.33, 4 x ArC\(_{quat}\); 126.66, (126.63), 122.16, 2 x ArCH; 94.71, (94.56), O-CH\(_2\)-O; 74.43, (74.14), O-CH; 55.48, O-CH\(_3\); 42.56, C-2H\(_2\); 40.88, C-2'H\(_2\); 37.86, (37.78), 36.91, C-1H and C-3H; 20.14, CH\(_3\); 19.95, (19.91), CH\(_3\); 19.25, C-3'H\(_2\); 13.67, C-4'H\(_3\). EI-MS: m/z 289 ([M - 1]+, 5%), 259 (7), 245 (100), 229 (34), 217 (25), 203 (58), 201 (56), 199 (57). CI-MS: m/z 291 (MH+, 9%), 259 (27), 245 (57), 229 (100). The second fraction

* The 1H and 13C NMR values quoted for this and subsequent mixtures will only be for the major component (i.e. the trans-diastereomers).
gave a yellow oil (8 mg). The major signals in the $^1$H NMR spectrum corresponded favourably with those for cis-1,3-dimethyl-5-(1'-methoxymethyl butyl) indan (63) except those at 2.48 and 1.16 ppm were absent and a triplet at 1.92 ppm had appeared. Minor signals in the spectrum were complex and appeared to be decomposed material.

Preparation of Ethyl trans-6,8-dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-carboxylate (103) via azidocinnamate (107).

1. Preparation of the azidocinnamate (107)

The azidocinnamate (107) was prepared according to the method used for the corresponding cis-azidocinnamate (77), above. The reagents and amounts used were: dimethylindancarbaldehyde (104) (215 mg, 740 µmol), ethyl azidoacetate (1.75 g, 13.6 mmol) and sodium (218 mg, 9.5 mmol) in ethanol (10 mL). After the usual work-up and purification procedures, a yellow oil (160 mg, 54%) was obtained. $v_{\text{max}}$ (neat) 2110s (N$_3$), 1715s (C=O) cm$^{-1}$. $^1$H NMR $\delta$ 7.67, (7.66), s, C-4H; 7.28, m, 2 x CH; 4.83, m, O-CH; 4.58, m, 4.54, m, O-CH$_2$-O; 4.38, q, J 7.1 Hz, CO$_2$-CH$_2$-CH$_3$; 3.41, (3.38), s, O-CH$_3$; 3.27, m, C-1H and C-3H; 1.92, t, J 6.8 Hz, C-2H$_2$; 1.80 - 1.20, bm, C-2'H$_2$ and C-3'H$_2$; 1.40, t, J 7.1 Hz, CO$_2$CH$_2$-CH$_3$; 1.27, d, J 6.8 Hz, CH$_3$; 1.23, d, J 6.2 Hz, (1.19, d, J 6.6 Hz), CH$_3$; 0.94, t, J 7.2 Hz, (0.92, t, J 7.3 Hz), C-4'H$_3$. $^{13}$C NMR $\delta$ 163.37, CO$_2$; 150.00, (149.97), 147.23, (147.19), 139.98, 129.26, 125.79, 5 x C$_{\text{quat}}$; 124.46, 123.56, 121.61, 3 x CH; 94.20, (94.15), O-CH$_2$-O; 74.81, (74.26), O-CH; 62.00, CO$_2$-CH$_2$-CH$_3$; 55.48, O-CH$_3$; 42.83, C-2H$_2$; 39.89, C-2'H$_2$; 37.51, 37.30, (37.28), C-1H and C-3H; 20.41, (20.37), CH$_3$; 20.17, (20.12), CH$_3$; 19.17, (19.10), C-3'H$_2$; 14.03, CO$_2$CH$_2$-CH$_3$; 13.70, C-4'H$_3$. 
2. Thermolysis of azidocinnamate (107)

A mixture of azidocinnamate (107) (42 mg, 105 µmol) and toluene (20 mL) was plunged into a reaction bath maintained at 135°C. It was heated to reflux for 2 h until TLC (silica, ethyl acetate / light petroleum, 1:9) showed the starting material was consumed. The mixture was cooled and the toluene removed by distillation to yield a yellow oil (43 mg). \(^1\)H NMR spectroscopy showed a complex set of signals. The major signals are given for the purified, major fraction below. A less intense set of signals (by a factor of 10) include: \(^1\)H NMR \(\delta\) 8.38, s, 1H; 7.94, s, 1H; 7.65, s, 1H. The material was chromatographed (silica, ethyl acetate / light petroleum, 1:9) to yield the major fraction as a yellow oil (31 mg, 79%). (Found: M**, 373.2253. \(\text{C}_{22}\text{H}_{31}\text{N}_{\text{O}_4}\) requires 373.2253). \(\nu_{\text{max}}\) (neat) 3455 cm\(^{-1}\). \(^1\)H NMR \(\delta\) 8.73, bs, N-H; 7.42, (7.41), d, \(J = 1.8\) Hz, C-3H; 6.97, s, C-5H; 4.93, m, O-CH; 4.56, m, O-CH\(_2\)-O; 4.40, q, \(J = 7.1\) Hz, \(\text{CO}_2\text{CH}_2\text{CH}_3\); 3.53, m, C-6H; 3.43, (3.42), s, O-CH\(_3\); 3.41, m, C-8H; 2.06, C-7H\(_2\) and C-2'Ha; 1.80, m, C-2'Hb; 1.6 - 1.3, bm, C-3'H\(_2\); 1.43, t, \(J = 7.1\) Hz, \(\text{CO}_2\text{CH}_2\text{CH}_3\); 1.33, d, \(J = 7.1\) Hz, (1.30, d, \(J = 7.0\) Hz), CH\(_3\); 1.29, d, \(J = 6.9\) Hz, CH\(_3\); 0.95, t, \(J = 7.3\) Hz, C-4'H\(_3\). \(^{13}\)C NMR \(\delta\) 162.18, \(\text{CO}_2\text{CH}_2\text{CH}_3\); 145.44, 134.75, (134.67), 133.84, 129.45, 126.51, 124.70, 6 x ArC\text{quat}; 115.36, (115.21), 108.26, (108.23), 2 x ArCH; 93.93, O-CH\(_2\)-O; 77.07, (77.01), O-CH; 60.85, \(\text{CO}_2\text{CH}_2\text{CH}_3\); 55.46, O-CH\(_3\); 43.35, C-7H\(_2\); 39.41, (39.36), C-2'H\(_2\); 37.93, (37.88), 35.91, C-6H and C-8H; 20.30, CH\(_3\); 19.71, (19.67), CH\(_3\); 19.56, C-3'H\(_2\); 14.34, \(\text{CO}_2\text{CH}_2\text{CH}_3\); 13.85, C-4'H\(_3\). El-MS: \(m/z\) 373 (M**, 40%), 330 (49), 312 (27), 284 (61), 270 (100), 240, (24), 224 (17), 212 (22).
Experimental

trans-6,8-Dimethyl-4-(1’-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-methanol (110)

The title alcohol (110) was synthesised according to the procedure used to synthesise cis-6,8-dimethyl-4-(1’-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-methanol (87), above. However, the reaction took twice as long for completion (by TLC). The reagents and amounts used were: ethyl indole-2-carboxylate (103) (160 mg, 428 µmol) and 1.5M DIBALH in toluene (860 µL, 1.29 mmol). The usual work-up and purification steps were employed to yield a yellow oil (73 mg, 51%). (Found: M**, 331.2146. C_{20}H_{29}N_{03} requires 331.2147). \( ^1H \) NMR \( \delta \) 8.42, bs, N-H; 6.91, s, C-5H; 6.56, s, C-3H; 4.94, m, O-CH; 4.81, s, CH\(_2\)-OH; 4.58, d, J 6.8 Hz, (4.57, d, J 6.6 Hz), 4.52, d, J 6.7 Hz, (4.50, d, J 6.7 Hz), O-CH\(_2\)-O; 3.50, m, C-6H; 3.42, (3.41), s, O-CH\(_3\); 3.23, m, C-8H; 2.02, m, C-2'Ha and C-7H\(_2\); 1.83, m, C-2'Hb; 1.60 - 1.20, bm, C-3'H\(_2\) and OH; 1.31, (1.29), d, J 7.0 Hz, CH\(_3\); 1.25, d, J 7.1 Hz, (1.24, d, J 7.0 Hz), CH\(_3\); 0.94, t, J 7.3 Hz, C-4'H\(_3\). \( ^{13}C \) NMR \( \delta \) 142.30, 136.56, 133.05, 132.34, 128.88, 125.09, 6 x ArC\text{quat}; 114.09, (113.98), 100.02, 2 x ArCH; 93.86, O-CH\(_2\)-O; 77.12, O-CH; 58.69, CH\(_2\)OH; 55.41, O-CH\(_3\); 43.61, C-7H\(_2\); 39.18, C-2'H\(_2\); 37.71, (37.33), C-8H; 35.95, C-6H; 20.68, (20.62), CH\(_3\); 20.60, (20.40), CH\(_3\); 19.59, C-3'H\(_2\); 13.91, (13.90), C-4'H\(_3\). EI-MS: \( m/z \) 331 (M**, 67%), 288 (13), 271 (49), 270 (43), 256 (87), 228 (100), 226 (92), 198 (64).

trans-6,8-Dimethyl-4-(1’-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-carboxaldehyde (111)

The title aldehyde (111) was synthesised according to the procedure used to synthesise the corresponding cis-compound. The reagents and amounts used were: indole-2-methanol (110) (123 mg, 371 µmol) and manganese dioxide (0.60 g,
6.2 mmol). The usual work-up and chromatographic procedures were employed to give a yellow oil (79 mg, 65%). (Found: M**, 329.1990. C₂₀H₂₇NO₃ requires 329.1991). νₘₐₓ (neat) 1600s (C=O) cm⁻¹. ¹H NMR δ 9.80, s, CHO; 8.90, (8.87), bs, N-H; 7.49, d, J 2.1 Hz, C-3H; 6.98, s, C-5H; 4.92, m, O-CH; 4.54, m, O-CH₂-O; 3.46, m, C-6H; 3.42, (3.41), s O-CH₃; 3.25, m, C-8H; 2.00, m, C-7H₂ and C-2'Ha; 1.80, m, C-2'Hb; 1.60 - 1.20, bm, C-3'H₂; 1.32, d, J 7.3 Hz, CH₃; 1.28, d, J 7.0 Hz, CH₃; 0.95, t, J 7.3 Hz, C-4'H₃. ¹³C NMR δ 181.64, CHO; 147.69, 135.69, 135.30, 129.78, 124.63, 5 x ArCquat; 115.78, 115.03, 2 x ArCH; 110.73, Cquat; 93.94, O-CH₂-O; 77.04, O-CH; 55.42, O-CH₃; 43.21, C-7H₂; 39.34, C-2'H₂; 38.06, C-8H; 35.83, C-6H; 20.13, CH₃; 19.51, CH₃, 19.43, C-3'H₂; 13.77, C-4'H₃. EI-MS: m/z 329 (M**, 55%), 286 (33), 268 (26), 258 (40), 240 (35), 226 (100), 198 (62).

Decarbonylation of trans-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole-2-carboxaldehyde (111)

trans-6,8-Dimethyl-4-(1'-methoxymethyloxy butyl)-1,6,7,8-tetrahydrocyclopent[g]indole (112) was prepared according to the above procedure used for the synthesis of the corresponding cis-compound (85). The reagents and amounts used were: indole-2-carboxaldehyde (111) (60 mg, 182 µmol), (PPH₃)₂RhClOCl (25 mg, 36 µmol) and 1,3-bis(diphenylphosphino)propane (dppp) (30 mg, 72 µmol). The usual work-up and purification procedures were followed to yield a yellow oil (35 mg, 64%). (Found: M**, 301.2042. C₁₉H₂₇NO₂ requires 301.2042). ¹H NMR δ 8.08, bs, N-H; 7.17, d, J 5.4 Hz, C-2H; 6.95, s, C-5H; 6.71, d, J 5.4 Hz, (6.70, d, J 5.4 Hz), C-3H; 4.98, m, O-CH; 4.61, d, J 6.7 Hz, (4.60, d, J 6.7 Hz), 4.57, d, J 6.6 Hz, (4.55, d, J 6.7 Hz), O-CH₂-O; 3.56, m, C-6H; 3.44, (3.43), s, O-CH₃; 3.40, m, C-8H; 2.00, m, C-7H₂ and C-2'Ha; 1.85, m, C-2'Hb; 1.60 - 1.30, bm, C-3'H₂; 1.33, d, J 7.0 Hz, CH₃; 1.30, d J 6.8
Experimental 143

Hz, (1.29, d, J 6.8 Hz), CH₃; 0.95, t, J 7.3 Hz, C-4’H₃. ¹³C NMR δ 142.11, 132.64, 132.40, 128.78, 124.97, 5 x ArCquat; 122.95, 113.95, (113.81), 101.89, 3 x ArCH; 94.02, O-CH₂-O; 77.11, O-CH; 55.45, O-CH₃; 43.51, C-7H₂; 39.28, C-2’H₂; 37.76, C-8H; 36.00, C-6H; 20.67, CH₃; 19.84, CH₃; 19.60, C-3’H₂; 13.90, C-4’H₃. EI-MS: m/z 301 (M⁺, 49%), 258 (53), 240 (27), 226 (35), 212 (27), 198 (100).

(±)-iso-trans-Trikentrin B (5)

A modification of the method of Guindon et al.⁶⁵ was followed. To a well stirred solution of MOM ether (112) (7 mg, 23 µmol) and dry dichloromethane (2 mL) under argon at -78°C, was added an aliquot (105 µL) of a solution of dimethylboron bromide and dichloromethane (80 mg/mL). The reaction mixture went a brown colour. It was stirred for 4 min and triethylamine (100 µL) was added. The reaction mixture went orange. It was stirred for 5 min and water (1 mL) was added. The mixture was warmed to room temperature and extracted with dichloromethane (3 x 3 mL). The organic layer was washed with water (1 mL), saturated sodium chloride solution (1 mL) and dried (MgSO₄). The solvent was evaporated to give an oil. Repeated flash chromatography under nitrogen (silica, ethyl acetate / light petroleum, 1 : 9) gave a major fraction as a yellow oil identified as (±)-iso-trans-trikentrin B (5)¹⁸.⁶⁶ (5 mg, 63%). (Found : M⁺, 239.1673. C₁₇H₂₁N requires 239.1674). ³¹P NMR δ 8.04, bs, N-H; 7.19, dd, J 2.7, 3.0 Hz, C-2H; 7.09, s, C-5H; 6.80, d, J 15.7 Hz, =C-1’H; 6.75, dd, J 2.3, 3.0 Hz, C-3H; 6.41, dt, J 15.8, 6.6 Hz, =C-2’H; 3.51, m, C-6H; 3.44, m, C-8H; 2.32, m, C-3’H₂; 2.00, m, C-7H₂; 1.33, d, J 7.0 Hz, CH₃; 1.31, d, J 7.2 Hz, CH₃; 1.15, t, J 7.5 Hz, C-4’H₃. ᵃ¹³C NMR δ 142.46, ArCquat; 132.35, CH; 128.99, 128.38, 2 x ArCquat; 127.22, CH; 125.12, ArCquat; 123.17, 112.31, 2 x CH; 110.73, ArCquat; 101.61, CH; 43.59, C-7H₂; 37.68, C-
8H; 35.93, C-6H; 26.40, C-3'H2; 20.68, CH3; 19.82, CH3; 13.88, C-4'H3. EI-MS: m/z 239 (M**, 89%), 224 (100), 198 (19), 182 (23), 154 (9). λmax (MeOH) 309 nm.

Experimental

A colorless block crystal of C_{13}H_{22}O having dimensions of 0.20 x 0.21 x 0.13 mm was mounted on a quartz fibre. All measurements were made on a Rigaku AFC7 diffractometer with graphite monochromated Cu-Kα radiation and a 12KW rotating anode generator. A Li2 refrigeration fluid was used to cool the crystal to avoid sublimation. The data was collected at a temperature of -73±1°C. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 reflections (9.79<2θ<106.12°) corresponded to a primitive triclinic cell with dimensions shown in Table 1. Further crystallographic data is given in Table 1.

The structure was solved by direct methods (SHELXS86) and expanded using Fourier techniques (DIRDIF92). Least-squares refinement was performed using the minimizing function Σw(Fo-Fc)^2 where w = 0.09/Po^2. Maximum and minimum peaks on the final difference Fourier map corresponded to 0.89 and -0.34 eÅ^3. Neutral atom scattering factors were taken from International Tables for X-ray Crystallography. All calculations were performed using the SHELXTL crystallographic software package of Molecular Structure Corporation. Final atomic coordinates for non-H atoms and H atoms are given in Table 2.

The author gratefully acknowledges Dr. A. C. Willett who performed the X-ray crystal structure determination.
APPENDIX

X-RAY ANALYSIS OF TRANS-1,3-DIMETHYLINDANOL (60)*

A colourless block crystal of C₁₁H₁₄O having dimensions of 0.23 x 0.21 x 0.13 mm was mounted on a quartz fibre. All measurements were made on a Rigaku AFC6R diffractometer with graphite monochromated Cu-Kα radiation and a 12kW rotating anode generator. A LN₂ refrigeration fixed tube low temperature system was used to cool the crystal to avoid sublimation. The data was collected at a temperature of -73±1°C. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 reflections (97.79<2θ<109.12°) corresponded to a primitive triclinic cell with dimensions shown in Table 6. Further crystallographic data is given in Table 6.

The structure was solved by direct methods (SHELXS86) and expanded using Fourier techniques (DIRDIF92). Least-squares refinement was performed using the minimising function Σω(\(F_o/ - / F_c/\))^2 where ω = (σ²F₀)^⁻¹. Maximum and minimum peaks on the final difference Fourier map corresponded to 0.89 and -0.34 e/Å³. Neutral atom scattering factors were taken from 'International Tables for X-ray Crystallography'. All calculations were performed using the μScans® crystallographic software package of Molecular Structure Corporation. Final atomic coordinates for non-H atoms and H atoms are given in Table 7.

* The author gratefully acknowledges Dr A. C. Willis who performed the X-ray crystal structure determination.
Table 6. Crystallographic Data for C_{11}H_{14}O.

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Figure 8: X-ray structure numbering of tetramer of C\textsubscript{11}H\textsubscript{14}O.
Table 7. Atomic coordinates and Isotropic Displacement Parameters for C_{11}H_{14}O.

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\[ B_{eq} = \frac{8}{3\pi^2} (U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{12}bb^*cc^*\cos\alpha). \]
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PART TWO

STRUCTURAL STUDIES OF BIOACTIVE NATURAL PRODUCTS
CHAPTER 4

A CHEMICAL INVESTIGATION OF THE CAUSE OF
THE 'DUMB LAMB SYNDROME'

4.1 INTRODUCTION

The 'dumb lamb syndrome' occurs in the Longreach district of Central Queensland causing high mortality rates (~20%) amongst newly born lambs. It is believed\(^1\) that the disorder occurs during gestation when ewes feed on particular plant species. Two species implicated are *Abelmoschus ficulneus* (native rosella) and *Ipomoea lonchophylla* (cowvine). Dr P. Oelrichs, who carried out the initial extractions, could obtain no toxic extract from *A. ficulneus*, but found that one fraction from *I. lonchophylla* was acutely toxic to mice. This fraction is the subject of the present investigation. Unfortunately, no tests were carried out to determine whether this toxic extract contributed to the 'dumb lamb syndrome'.

Many species of *Ipomoea* were and still are used in folk medicine all over the world,\(^2\)-\(^5\) especially as purgatives.\(^6\),\(^7\) Pharmacological studies have reported antimicrobial,\(^8\)-\(^10\) analgesic,\(^11\) spasmogenic,\(^4\) spasmolytic,\(^5,12\) hypotensive,\(^3,13\) insecticidal,\(^14\) psychotomimetic\(^2\) and anticancer,\(^8,15,16,17\) effects. Chemical investigations have shown that indole alkaloids\(^18\) and resin glycosides\(^19\) are the most common, biologically active constituents in the *Ipomoea* plants. Resin glycosides are composed of mono- and dihydroxy C\(_{14}\) and C\(_{16}\) fatty acids glycosidically linked to oligosaccharide chains usually containing 4 to 6 sugar units. The sugars are esterified with varying numbers of C\(_5\) acid moieties.\(^15,20\)
This chapter records the isolation, characterisation and structural elucidation of the resin glycoside from the toxic extract of *Ipomoea lonchophylla*.

### 4.2 ISOLATION, CHARACTERISATION AND STRUCTURAL ELUCIDATION OF THE RESIN GLYCOSIDE

#### 4.2.1 Extraction Procedures and Isolation of the Toxic Extract

Dr P. Oelrichs initially extracted the dried *I. lonchophylla* plant using aqueous methanol. The extract was concentrated and the residue was taken up in water and extracted further with n-butanol. Purification of the concentrate from the butanol extract was achieved by subjecting it to size exclusion chromatography using LH-60 gel. At all stages of purification, the fractions were monitored by toxicity testing with mice. In our laboratory the material from LH-60 supplied by Dr Oelrichs was further purified using LH-20 gel. A colourless solid was obtained, the major component of which was subsequently identified as a resin glycoside having the structure (1) (Figure 1).

High field NMR and mass spectral data was obtained on the solid. The negative FAB mass spectrum indicated that a mixture of high molecular weight compounds were present (Figure 2) with predominant [M - 1]$^-$ ions at m/z 1651, 1551, 1451 and 1351. The ions at m/z 1533, 1433 and 1333 were fragment ions due to the loss of water from the corresponding molecular ions and they may also represent the [M - 1]$^-$ ions for the corresponding macrocyclic lactones which have been previously isolated,21,22,23 where the acid group is esterified to one of the sugar units. The $^1$H NMR spectrum showed multiple signals between 6.42 and 3.18 ppm indicative of a number of sugar units. Signals were also present in the
aliphatic region (1.95 - 0.70 ppm). The $^{13}$C NMR spectrum (CD$_3$OD) exhibited a series of downfield signals at 177.90, 176.90, 176.78, 176.39 and 176.23 ppm. Multiple signals were present around 100 ppm and also between 79.3 and 62.8 ppm confirming the presence of a glycosidic moiety. Signals were also present between 42.4 and 12.1 ppm for the aliphatic residues. This evidence supported the view that the toxic fraction was a mixture of resin glycosides. Unfortunately, from the spectral data obtained on the resin glycoside mixture, it could not be determined if the macrocyclic lactones were present.

Figure 1. Major component (I) of the mixture of resin glycosides.
Figure 2. Negative FAB-MS of the resin glycosides obtained from the toxic extract of *I. lonchophylla*.
More of the toxic material was required to elucidate the structure(s) of the active component(s). Dried *I. lonchophylla* plant material was kindly supplied by Dr P. Oelrichs and was extracted by the author. The extraction and isolation procedure which differs from that used by Oelrichs is shown schematically (Scheme 1). It was based on literature methods\(^6,21,22,24\) used to isolate resin glycosides from other *Ipomoea* species. The dried plant material was steeped in methanol / water (1:1) and kept at 5°C for 3 months. The mixture was mechanically shaken for 7 h and then filtered. The filtrate was concentrated under vacuum. The resulting residue was taken up in water / methanol (5:1) and then extracted with light petroleum spirits. TLC showed the desired mixture of resin glycosides was present in the petroleum extract and the aqueous fraction. Because the resin glycosides contain both hydrophobic and hydrophilic groups, they show partial solubility in both non-polar (e.g. petroleum) and polar solvents (e.g. water).

The extract was concentrated and subjected to size exclusion chromatography using Sephadex LH-20 gel and ethyl acetate / methanol (1:1) as the eluting solvent. The fraction that contained the resin glycosides by TLC was concentrated and the residue was chromatographed using Iatro beads (normal stationary phase gel) and a polarity gradient of eluting solvents including light petroleum, ethyl acetate, and methanol / water. One of the eluted fractions when concentrated gave a colourless solid with similar spectral data (NMR, MS) to the original resin glycosides provided by Dr P. Oelrichs.
Scheme 1. Extraction and isolation of resin glycosides from *I. lonchophylla.*
The combined aqueous fraction and washings from the petrol extract were next extracted with dichloromethane followed by ethyl acetate. TLC indicated that the resin glycosides remained in the aqueous fraction. Extraction of the aqueous fraction with n-butanol gave an organic fraction which contained the resin glycosides by TLC. There were no resin glycosides observed by TLC in the aqueous layer. The n-butanol extract was concentrated in vacuo and was subjected to chromatography using Iatro beads and a gradient of eluting solvents as listed above. The fraction that contained the resin glycosides was concentrated and subjected to size exclusion chromatography using Sephadex LH-20 and methanol. Concentration of one of the fractions from LH-20 gave a colourless solid with similar NMR and mass spectra to the original mixture of resin glycosides and was combined with the above fraction from the petroleum extract.

A mixture of the resin glycosides was further subjected to reversed phase-high performance liquid chromatography (RP-HPLC) on a C-18 column. The chromatograms showed that a large number of components were present which could not be adequately separated into individual pure compounds. From the negative FAB-MS spectral data (namely, 100 mass units difference between [M - 1]⁻) and HPLC chromatograms, it appeared that the mixture of resin glycosides differed mainly with respect to the number of C₅ ester groups on the sugars. Since the compounds in the mixture could not be separated, it was decided to hydrolyse the mixture in order to determine the structures of the individual components which made up the glycosidic and aglycone moieties.
4.2.2 Acid Hydrolysis of the Resin Glycosides from the Toxic Extract

The mixture of resin glycosides was first subjected to acid hydrolysis (Scheme 2). The ether extract of the hydrolysate was silylated and GC/MS showed a major component which had a mass spectrum that corresponded to that expected for the trimethylsilyl (TMS) derivative of 3,11-dihydroxytetradecanoic acid (2) containing the α-cleavage ions shown in Figure 3.15 A minor component was identified as the TMS derivative of 3,11-dihydroxyhexadecanoic acid (3). The smaller C5 acids on the sugars were not detected, as their TMS derivatives eluted with the silylating reagent.

The aqueous phase of the hydrolysate was freeze-dried and silylated. GC/MS analysis of the silylated residue showed the presence of the TMS ethers of glucose (Glc) and the 6-deoxyhexoses quinovose (Qui), fucose (Fuc) and rhamnose (Rha) in the approximate ratio of 2:2:1:1. Authentic samples of these four sugars were subjected to the same conditions both individually and as calibrated mixtures to confirm the assignments and ratios. It was assumed that the sugars were present in their natural forms, i.e. D-glucose, D-quinovose, D-fucose and L-rhamnose.
**Scheme 2.** Acid hydrolysis of the resin glycosides from the toxic extract.

**Figure 3.** Fragmentation pattern observed in the mass spectrum (GC/MS) of the TMS derivative of 3,11-dihydroxytetradecanoic acid (2).

### 4.2.3 Alkaline Hydrolysis of the Resin Glycosides from the Toxic Extract

The resin glycosides were hydrolysed with a sodium hydroxide solution (Scheme 3), and then the mixture was neutralised (pH 7). An aliquot was taken, acidified to pH 1 and extracted with diethyl ether.
Scheme 3. Alkaline hydrolysis of the resin glycosides from the toxic extract.

4.2.3.1 Derivatisation of the Ether Extract

The ether extract was methylated with diazomethane. The GC/MS results (mass spectra and retention times) of the methyl esters compared well with those of the methyl esters of authentic samples of 3-hydroxy-2-methylbutyric acid (HMBA) (4) and 2-methylbutyric acid (MBA) (5) (Figure 4). The sequential differences of 100 mass units observed for the high mass ions \((m/z\ 1651, 1551, 1451, 1351)\) in the negative FAB–MS of the original toxic extract (Figure 2), confirmed the presence of differing numbers of HMBA (4) ester groups.
Figure 4. C₅ acids esterified to the dihydroxytetradecanoic acid oligosaccharide.

4.2.3.2 Isolation and Analysis of the Alkaline Hydrolysed Resin Glycoside

The neutral aqueous phase from the alkaline hydrolysis was freeze-dried and desalted using Sephadex LH-20 and methanol (Scheme 3). Further purification was carried out using reversed phase-high performance liquid chromatography on a C-18 column. One fraction gave a colourless solid (6) which was the major product. The negative FAB-MS of the major component (6) of the alkaline hydrolysate (Figure 5) showed major ions at m/z 1167 [M - 1]⁻, 1021, 859, 567, 405 and 259. The analysis of these ions enabled the sequence of the oligosaccharide to be determined (Figure 6). This technique has proven useful for the determination of other oligosaccharide sequences.⁶,²¹,²³,²⁶

The [M - 1]⁻ ion for compound (6) at m/z 1167 confirmed that the 3,11-dihydroxytetradecanoic acid (2) was glycosidically linked to an oligosaccharide consisting of four deoxyhexose (2 x quinovose, 1 x rhamnose and 1 x fucose) and two hexose (glucose) units as had been determined by the GC/MS results. Since the oligosaccharide was still bonded to the dihydroxytetradecanoic acid (2) after alkaline hydrolysis, this indicated that the oligosaccharide was bonded to one of the hydroxyl groups and not the carboxylate group of the dihydroxy fatty acid.
Figure 5. Negative FAB-MS of the major component (6) of the alkaline hydrolysate.
The fragment ion at \( m/z \) 1021 represents a mass difference of 146 from the \([\text{M} - 1]^-\) ion at \( m/z \) 1167 and indicated the loss of a terminal deoxyhexose \( \text{F} \) or \( \text{D} \) (Figure 6). The next major fragment ion at \( m/z \) 859 was due to the cleavage at the next sugar in the sequence, the glucose \( \text{E} \) unit. Therefore, this ion represented the aglycone, three deoxyhexoses and one glucose. The next most intense ion at \( m/z \) 567 was 292 mass units lower and represented the difference of two deoxyhexose units. This would be expected if one of the deoxyhexose sugars (\( \text{D} \)) was branched as shown in Figure 6. The fragment ion at \( m/z \) 405 represented a mass difference of 162, equivalent to a glucosyl unit, from the ion at \( m/z \) 567. Therefore, the ion at \( m/z \) 405 contains the aglycone and deoxyhexose \( \text{A} \). The \( m/z \) 259 ion in the negative FAB-MS corresponds to the anion of 3,11-dihydroxytetradecanoic acid (2).

Figure 6. The oligosaccharide sequence of (6) (MWt 1168) determined from the fragmentation pattern in the negative FAB-MS.
4.2.4 Determination of the Position of Linkage of the Oligosaccharide to the Dihydroxytetradecanoic acid

The alkaline hydrolysed resin glycoside (6) was permethylated (dimethyl potassium and methyl iodide), acid hydrolysed and then silylated in order to determine to which hydroxyl group on the 3,11-dihydroxytetradecanoic acid (2) the oligosaccharide was linked. GC/MS of the silylation mixture showed a major peak whose mass spectrum is shown in Figure 7. The fragmentation pattern can be rationalised in terms of the structure shown rather than the alternate compound methylated at C-11.

![Mass spectrum and the associated fragmentation pattern observed in the mass spectrum (GC/MS) of the derivatised dihydroxytetradecanoic acid (2).](image)

Figure 7. Mass spectrum and the associated fragmentation pattern observed in the mass spectrum (GC/MS) of the derivatised dihydroxytetradecanoic acid (2).
Similar fragmentation patterns have been observed for related hydroxy fatty acids and their derivatives.\textsuperscript{15,16,24} The results indicated that the 3,11-dihydroxytetradecanoic acid (2) was silylated on the carboxylate and C-11 hydroxyl groups (as indicated by the ion at \textit{m/z} 145) and was methylated on the C-3 hydroxyl group. Therefore, in the alkaline hydrolysed resin glycoside (6) the glycosidic moiety must be bonded through the C-11 hydroxyl group. This was confirmed by NMR studies (Section 4.2.6).

4.2.5 Determination of the Positions of Linkage of the Hexose and Deoxyhexose Units

The Lindberg procedure\textsuperscript{28} is a series of reactions on an oligosaccharide that enables the linkage positions of sugars to be determined. The analysis involves the methylation of all free hydroxyl groups in the oligosaccharide, followed by hydrolytic cleavage with acid of the glycosidic linkages to give the derivatised monosaccharides. Reduction and subsequent acetylation yields methylated alditol acetates that can be analysed by GC/MS. Lindberg has published a complete set of methylated alditol acetate mass spectra\textsuperscript{28} which can be used to identify the products of the procedure and therefore the linkage positions for each sugar.

An adaptation\textsuperscript{27} of the Lindberg procedure was used to determine the number and positions of linkage on each of the two hexose (glucose) and four deoxyhexose (quinovose, fucose, rhamnose) units. The alkaline hydrolysed resin glycoside (6) was treated with dimethyl potassium and then methyl iodide (Scheme 4) to form the corresponding permethylated species. The material was then subjected to acid hydrolysis using trifluoroacetic acid, followed by sodium borohydride reduction and finally acetylation. GC/MS of the product showed the presence of four major
components which corresponded well with literature data\textsuperscript{28} for 1,5-di-\textit{O}-acetyl-6-deoxy-2,3,4-tri-\textit{O}-methylhexitol (7); 1,2,5-tri-\textit{O}-acetyl-6-deoxy-3,4-di-\textit{O}-methylhexitol (8); 1,3,4,5-tetra-\textit{O}-acetyl-6-deoxy-2-\textit{O}-methylhexitol (9) and 1,2,5-tri-\textit{O}-acetyl-3,4,6-tri-\textit{O}-methylhexitol (10).

![Scheme 4](image)

**Scheme 4.** Major products from an adaptation\textsuperscript{27} of the Lindberg procedure on the alkaline hydrolysed resin glycoside (6).

Taken in conjunction with the earlier negative FAB–MS sequence analysis (Section 4.2.3.2), these results permitted the assignment of the positions of linkage between the individual sugar units. The only derivatised hexitol present was (10) showing that both glucose units in the oligosaccharide chain were 1,2-disubstituted. The intensity of the GC signal for this compound was consistent with it representing two glucose units (Figure 8).
Since the diacetylated 6-deoxyhexitol (7) was the only alditol acetate that could be derived from a terminal sugar unit and the tetra-O-acetyl deoxymethylhexitol (9) must be derived from a 1,3,4-trisubstituted deoxyhexose, then this implied that there were two terminal deoxyhexose units as previously suggested by the negative FAB-MS data. The detection of deoxyhexitol (8) confirmed the presence of a 1,2-disubstituted deoxyhexose which must be the sugar linked to the dihydroxy fatty acid (Figure 8).
Therefore, the four 6-deoxyhexose units consisted of one 1-, 3-, 4-trisubstituted unit, one 1-, 2-disubstituted unit and two terminal units. The two glucose units are both 1-, 2-disubstituted. While this further added to the structural assignment, it remained to determine the location of the individual deoxyhexoses and the stereochemistry of the sugar linkages. For this, it was necessary to turn to high field NMR spectroscopy.

4.2.6 NMR Assignments leading to the Structure of (6)

High field NMR techniques ranging from $^1$H (Figure 9) and $^{13}$C NMR experiments to more complex 2D NMR experiments (DQF-COSY, HMQC, HMBC, TOCSY and ROESY) in D$_5$-pyridine enabled the $^1$H and $^{13}$C NMR assignments of the alkaline hydrolysed resin glycoside (6) to be made (Table 1). The $^1$H and TOCSY experiments were recorded on a Varian 750 MHz NMR spectrometer while the other experiments were recorded in-house on a Varian 500 MHz NMR instrument. Initially the $^1$H NMR assignments were determined from $^1$H and DQF-COSY, TOCSY and ROESY experiments, and then $^1$H-$^{13}$C one-bond and multiple-bond correlations from the HMQC and HMBC experiments respectively, allowed the $^{13}$C NMR assignments to be determined.

The assignments of the significant protons of the 3,11-dihydroxytetradecanoic acid (2) were made using $^1$H and DQF-COSY ($^1$H - $^1$H correlations) spectral data. The upfield triplet at 0.88 ppm ($J$ 7.3 Hz) was characteristic of the methyl group of the fatty acid$^{6,21,22,23,26}$ and hence the protons were assigned as those on C-14. A DQF-COSY correlation (Figure 10) was observed between the protons at 0.88 ppm and those resonating at 1.50 ppm and these protons were assigned as those on C-13. Another correlation was observed between the protons on C-13, at
1.50 ppm, and those at 1.32 ppm allowing the methylene protons on C-12 to be assigned. A DQF-COSY correlation between the protons at 1.32 ppm and the one-proton signal at 3.81 ppm indicated that the latter, because of its downfield shift, was on the hydroxylated C-11. A weak signal in the $^{13}$C NMR spectrum at 178.1 ppm was attributed to the carboxylic acid carbon, C-1. The characteristic signal at 2.80 ppm was assigned as the methylene protons on C-2 in the 3,11-dihydroxytetradecanoic acid (2). The assignment was based on literature assignments of the protons on C-2 in similar compounds. A DQF-COSY correlation between these protons and that at 4.40 ppm confirmed that the latter proton was H-3.

Confirmation of the earlier mass spectrometric result that the glycoside was bonded through the hydroxyl group on C-11 and not that on C-3 came from a through-space connectivity observed in the ROESY spectrum (Figure 11) between the proton at 3.81 ppm (H-11 on the fatty acid) and the anomeric proton at 4.84 ppm on deoxyhexose A. A correlation was also observed between C-11 on the fatty acid, 80.2 ppm, and the anomeric proton at 4.84 ppm in the $^1$H-$^{13}$C multiple-bond correlation (HMBC) spectrum (Figure 12) which also confirmed the position of linkage of the oligosaccharide to the fatty acid. There were no correlations in the ROESY nor the HMBC spectra between H-3 on the fatty acid and any of the anomeric protons or carbons on a hexose or deoxyhexose unit.
**Figure 9.** The downfield region of the 750 MHz $^1$H NMR spectrum of the alkaline hydrolysed resin glycoside (6).
Table 1. $^1$H and $^{13}$C NMR assignments\textsuperscript{a} for the alkaline hydrolysed resin glycoside (6) in D$_5$-pyridine.

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<td>1.60, d (5.9)</td>
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\textsuperscript{a} $^1$H measured at 750 MHz; \(\delta\) in ppm from int. std pyridine. Coupling constants (\(J\)) in Hz are given in parentheses. $^{13}$C chemical shifts from HMQC (500 MHz). Agl, aglycone; Fuc, fucose; Glc, glucose; Qui, quinovose; Rha, rhamnose.
Figure 10. Significant $^1$H-$^1$H DQF-COSY correlations for the dihydroxytetradecanoic acid moiety of (6).

The absolute configuration at C-3 and C-11 was not determined but the literature has shown that in all cases investigated to date, these centres have the S-configuration.

Figure 11. Significant $^1$H-$^1$H ROESY correlations of the alkaline hydrolysed resin glycoside (6).
With the aid of the DQF-COSY and TOCSY spectra (Figure 13), the remaining assignments of deoxyhexose A, linked to the hydroxy acid, could be determined (Table 1). The chemical shift (4.84 ppm) of the anomeric proton, its coupling constant ($J = 7.6$ Hz) and the chemical shift of the methyl group of the unit at 1.52 ppm suggested it was a $\beta$-linked fucose$^{6,21,23,26}$ and the other values determined for the sugar unit confirmed it was a $\beta$-fucopyranosyl group. A ROESY correlation between H-2 on fucose A, 4.18 ppm, and the anomeric proton of another sugar at 5.76 ppm was observed (Figure 11). There was also an HMBC correlation observed between the corresponding anomic carbon (102.1 ppm) and H-2 on fucose A (Figure 12). This confirmed that fucose A was 1-,2-disubstituted.

![Figure 12. Significant $^1$H-$^{13}$C HMBC correlations for the alkaline hydrolysed resin glycoside (6).](image-url)
Figure 13. The TOCSY spectrum of the alkaline hydrolysed resin glycoside (6).
From previous mass spectral data, the second hexose unit B was determined to be glucose. The chemical shift (5.76 ppm) and coupling constant of its anomeric proton ($J=7.6$ Hz) confirmed that it was $\beta$-linked to fucose A. The other $^1\text{H}$ assignments made for glucose B shown in Table 1 were obtained from correlations observed in the DQF-COSY and TOCSY (Figure 13) spectra. These assignments were characteristic of a $\beta$-glucopyranosyl unit when compared with those $\beta$-glucopyranosyl residues in oligosaccharides given in the literature. Correlations were observed between H-2 of glucose B at 4.27 ppm, and the anomeric proton at 5.86 ppm (ROESY) on sugar C, as well as with the corresponding anomeric carbon at 102.4 ppm (HMBC) (Figures 11 and 12).

Negative FAB and Lindberg mass spectral data had suggested that the sugar with the anomeric proton at 5.86 ppm ($J=7.8$ Hz) $\beta$-linked to glucose B was a trisubstituted deoxyhexose. Correlations in both the TOCSY and DQF-COSY spectra allowed complete $^1\text{H}$ assignments to be made (Table 1). It was confirmed as a $\beta$-quinovopyranosyl unit by comparison of its NMR data with that of $\beta$-quinovopyranosyl units in oligosaccharides given in the literature and therefore could be assigned as quinovose C. The downfield chemical shift of the anomeric proton of the quinovosyl unit, at 5.86 ppm, was characteristic of and confirmed the presence of a trisubstituted quinovosyl group. A ROESY correlation was observed between H-3 on quinovose C, 4.28 ppm, and an anomeric proton at 6.21 ppm (Figure 11).

A combination of previous mass spectral and NMR data indicated that the unit attached to quinovose C was glucose E and was $\beta$-linked to the C-3 hydroxyl group of quinovose C. Once again TOCSY (Figure 13) and DQF-COSY correlations, as well as coupling constants, enabled $^1\text{H}$ assignments of glucose E to
be determined as shown in Table 1. Most of the values corresponded well to literature values for similarly substituted β-glucopyranosyl units.\(^6\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\)

However, it is unusual for the chemical shift of the anomeric proton of a 1,2-disubstituted glucose to be as far downfield as 6.21 ppm. Usually this proton is found resonating between 4.90 and 5.95 ppm.\(^6\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\) The sugar on the adjacent 4-position of quinovose C (i.e. rhamnose D) is within close proximity and this may be responsible for the deshielding of the anomeric proton. In support of this, through-space ROESY correlations were observed between H-1 on glucose E and H-3 and H-4 of rhamnose D. The C-1 of glucose E, at 100.7 ppm, was within the expected chemical shift range.\(^6\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\) A ROESY correlation between H-2 on glucose E at 4.03 ppm, and the anomeric proton at 5.08 ppm (Figure 11) as well as an HMBC correlation (Figure 12) between H-2 on glucose E and the anomeric carbon of quinovose F (104.8 ppm, one-bond coupled to the proton at 5.08 ppm) were observed.

The chemical shift (5.08 ppm) and coupling constant (\(J \approx 8.0\) Hz) of this sugar suggested that it was the other quinovose unit β-linked to glucose E.\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\)

Assignments for the sugar (Table 1) were determined from the correlations present in the DQF-COSY and TOCSY spectra (Figure 13) and the assigned values corresponded well to the literature values for a β-quinovopyranosyl unit.\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\) This was one of the terminal deoxy hexose units and was assigned as quinovose F.

Previous mass spectral results had shown that another terminal deoxyhexose D was present and bonded to the 4-position of quinovose C. The distinctive anomeric proton of deoxyhexose D had a chemical shift of 6.41 ppm and was a singlet which was characteristic of an α- or β-linked rhamnose.\(^6\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\),\(^2\) TOCSY, DQF-COSY and HMBC correlations allowed the \(^1\)H and \(^13\)C NMR assignments for the sugar to be determined. The coupling constant values as well as the chemical shift
values confirmed it as an α- or β-rhamnopyranosyl group (Table 1).6,21,22,23,24,26,31 There were no correlations observed between the anomeric CH of rhamnose D and any other proton or carbon on another sugar.

This completed the structural assignment of the alkaline hydrolysis product (6) of the resin glycoside mixture. All that remained was to attempt to determine where the C₅ ester groups were located on the oligosaccharide in the mixture of resin glycosides.

4.2.7 Determination of the location of the C₅ acids

The number and type of ester groups on each sugar could be determined by comparison of the negative FAB fragment ion sequence pattern in the alkaline hydrolysed resin glycoside (6) (Figure 5) with that of the intact resin glycoside mixture (Figure 2). A similar analysis had been carried out by Miyahara and co-workers22 for a related resin glycoside. No information was obtainable from this study on the actual position of attachment of the ester groups on the sugars.

The major ion at m/z 1551 in the negative FAB-MS of the resin glycoside (Figure 2) represents the [M - 1]⁻ ion of (1) containing the dihydroxytetradecanoic acid (2) glycosidically linked to the oligosaccharide unit containing one MBA (5) and three HMBA (4) ester units (i.e. the alkaline hydrolysed product (6) of molecular weight 1168 + (1 x 84) + (3 x 100) = 1552). Other [M - 1]⁻ ions at m/z 1651, 1451 and 1351 correspond to the presence of the dihydroxytetradecanoic acid (2) linked to the hexasaccharide unit containing one MBA ester plus four, two and one HMBA ester groups, respectively.
As can be seen in Scheme 5, the ion at $m/z$ 405 (Figure 2), which contains the aglycone and fucose A, has no ester groups (Figure 14). The next highest ion at $m/z$ 667, representing the addition of the glucose B unit of (1), possesses one HMBA ester (i.e. plus 100 mass units) (Scheme 5). The two sugar units consisting of quinovose C and rhamnose D are contained in the next highest ion of (1) at $m/z$ 1143. This disaccharide unit must contain one HMBA and one MBA ester although it was not possible to tell which ester was on which of the two sugars. Since quinovose C has only one free hydroxyl group, however, it seems probable that both esters are on rhamnose D. The next highest fragment ion at $m/z$ 1305 of (1), which represents the addition of the glucose E unit, contains the same number of ester groups as the $m/z$ 1143 ion and therefore it can be concluded that the glucosyl moiety E is not esterified. Hence, the terminal quinovose F sugar of (1) must contain the remaining one HMBA ester group (Scheme 5). Other ions present in the mass spectrum of the resin glycosides, namely, $m/z$ 567, 943, 1043 and 1205 represent cleavage ions from the less substituted components of the mixture.

**Fragment ions of:**

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<th>405</th>
<th>567</th>
<th>859</th>
<th>1021</th>
<th>1167</th>
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<td>2xHMBA</td>
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<td>(1) $m/z$:</td>
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<td>405</td>
<td>667</td>
<td>1143</td>
<td>1305</td>
<td>1551</td>
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**Scheme 5.** Negative FAB–MS fragmentation pattern of the major compound (1) of the resin glycoside mixture and alkaline hydrolysed product (6).

Five carbon signals at 177.90, 176.90, 176.78, 176.39 and 176.23 ppm were present in the $^{13}$C NMR and represented C-1 in the dihydroxytetradecanoic acid moiety$^{21,23}$ together with four different ester groups on the sugars.$^{21,22,23}$ The
carboxylic acid carbon signal resonates downfield to the HMBA and MBA ester carbons,\textsuperscript{21,23} hence it was assigned as that at 177.9 ppm. Due to the complexity of the NMR spectra of the resin glycoside mixture, the exact positions of the MBA and HMBA ester units on the glucose and deoxyhexose units could not be ascertained. With that limitation, the structure of the major component of the toxic extract from \textit{Ipomoea lonchophylla} could be assigned as the resin glycoside (1) shown in Figure 14.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structure.png}
\caption{The structure of the major component (1) from the mixture of the resin glycosides from the toxic extract and the fragmentation pattern from the negative FAB-MS.}
\end{figure}
EXPERIMENTAL

GENERAL PROCEDURES

See also General Procedures in Experimental, Part 1.

Size exclusion chromatography was carried out using Sephadex LH-20 and LH-60 gel with Pharmacia SR columns. Iatro beads (Iatron Laboratories, Inc.) were used for normal phase chromatography. High performance liquid chromatography (HPLC) was performed using a Maxima 820 chromatography workstation with a Waters 510 HPLC pump and either a Waters Lambda-Max 481 AZ LC spectrophotometer or a Waters differential refractometer R401 as detector. Waters Prep Novapak HR C18 column (60A, 6 µm, 7.8 x 300 mm) was used for reversed phase-HPLC (RP-HPLC).

Fast Atom Bombardment (FAB) mass spectra were obtained using a VG ZAB-2SEQ mass spectrometer using either glycerol or triethanolamine as the matrix.

Gas chromatography - mass spectra (GC/MS) were recorded on a Hewlett Packard model 5970B system.

NMR experiments were recorded on a Varian 500 MHz NMR spectrometer (DQF-COSY, ROESY, HMQC and HMBC). ¹H and TOCSY NMR experiments were recorded on a Varian 750 MHz NMR instrument by P. A. Keifer of Varian Associates, Palo Alto, California. The sample was dissolved in D₅-pyridine.
A double quantum filtered $^1$H-$^1$H COSY (DQF-COSY) spectrum was recorded in the phase-sensitive mode. The parameters included: a spectral width of 5800 Hz; a relaxation delay of 3.0 s; the number of transients = 32 and the number of increments = 360. A shifted sinebell function (0.031) was used to process the data and the data matrix was expanded to a 4k x 4k data file before Fourier transformation.

Parameters for the $^1$H-$^1$H rotating frame NOE (ROESY) experiment included: a spectral width of 6000 Hz, a relaxation delay of 4.7s; the number of transients = 16 and the number of increments = 350. A shifted sinebell function (0.088) was used to process the data and the data matrix was a 2k x 2k data file.

A $^1$H-detected heteronuclear multiple quantum coherence (HMQC) experiment was recorded with parameters which included: spectral width of 4500 Hz ($^1$H) and 30000 Hz ($^{13}$C); a relaxation delay of 3.0s; a $J$ value of 125.0 Hz; the number of transients = 80 and the number of increments = 200. A sinebell function (sb = 0.114, sb1 = 0.003) was used to process the data and the data matrix was expanded to a 2k x 2k data file before Fourier transformation.

Parameters for a heteronuclear multiple bond correlation (HMBC) spectrum included: a spectral width of 4500 Hz ($^1$H) and 30000 Hz ($^{13}$C); a relaxation delay of 3.0s; a $J$ value of 125.0 Hz and a tau (mb) value of 0.06; the number of transients = 80 and the number of increments = 200. A Gaussian function (gf = 0.093, gf1 = 0.004) was used to process the data and the data matrix was expanded to 2k x 2k before Fourier transformation.

The $^1$H-$^1$H total correlation (TOCSY) experiment was recorded and some of the parameters included: a spectral width of 7500 Hz; a relaxation delay of 0.001s; the
number of transients = 8 and the number of increments = 256. A Gaussian function (gf = 0.194, gfs = 0.064) and a sinebell and shifted sinebell functions (sbl = -0.04, sbsl = -0.035) were used to process the data. The data matrix was expanded to 4k x 2k before Fourier transformation.

EXPERIMENTAL DETAILS

Extraction of the Resin Glycosides (1) - Procedure 1*

The milled dried plant material (100 g) was extracted with methanol / water (1:1) at 100°C. It was concentrated to 200 mL and extracted with n-butanol. The n-butanol extract was concentrated to dryness and 0.1 M ammonium hydroxide (100 mL) was added. The material was centrifuged and the supernatant was collected and the evaporation of the aqueous solution removed any ammonia. Sulfuric acid (2 N) was added and it was left to stand overnight. The solution was centrifuged. The pellet was washed with water until the washings measured pH 7.0. The residue was dissolved in methanol and concentrated to give a pale yellow solid (0.25 g). The solid was dissolved in methanol and subjected to size exclusion chromatography (LH-60 and LH-20) to give a colourless powder identified as resin glycosides (205 mg). $^1$H NMR (D$_5$-pyridine) $\delta$ 6.42, s; 6.20, d, $J$ 7.0 Hz; 5.83, bs, 5.75, bs; 5.50 - 5.30, m; 5.30 - 3.18, bm; 2.82 - 2.47, m; 2.45 - 2.30, m; 1.95 - 0.90, bm; 0.85, t, $J$ 7.2 Hz; 0.83 - 0.70, m. $^{13}$C NMR (CD$_3$OD) $\delta$ 177.90, 176.90, 176.78, 176.39, 176.23, 5 x C$_{quat}$; 106.91, 103.18, 103.04, 102.76,

* The extraction of resin glycosides using procedure 1 was carried out by Dr P. Oelrichs.

**Extraction of the Resin Glycosides - Procedure 2**

The extraction and isolation procedure was based on literature methods. Dried plant material of *Ipomoea lonchophylla* (1 kg) was steeped in methanol / water (1:1, 3 x 100 mL) and kept at 5°C for three months (see Scheme 1). It was mechanically shaken for 7 h. After filtration, the concentrated extract was dissolved in water / methanol (5:1, 600 mL). The aqueous fraction was extracted with light petroleum spirits (3 x 400 mL). The petrol fraction was concentrated and chromatographed (LH-20, ethyl acetate / methanol, 1:1) to give 7 fractions. The first fraction was subjected to repeated chromatography using Iatro beads and a gradient of mobile phase (from an increasing ratio of ethyl acetate / light petroleum to methanol / ethyl acetate to methanol / water, 85:15). One of the fractions gave a colourless powder (38 mg) identified as the required resin glycosides.

The aqueous layer and washings from the petrol extract were combined and extracted with dichloromethane. There were no resin glycosides present in the

* Provided by Dr P. Oelrichs.
dichloromethane fraction by TLC. The combined aqueous layer and washings were extracted with ethyl acetate. TLC showed that there were no resin glycosides present in the ethyl acetate fraction.

The aqueous layer and washings were combined and extracted with n-butanol. It was concentrated and the residue was chromatographed using iatro beads and a gradient of mobile phase (from light petroleum / ethyl acetate to methanol / ethyl acetate to water / methanol, 1:5). A fraction was collected and subjected to size exclusion chromatography (LH-20, methanol) to give a colourless powder (80 mg) identified as the required resin glycosides.

**Acid Hydrolysis of the Resin Glycosides**

A mixture of the resin glycosides (2 mg) and 2M HCl (1 mL) was heated at 90°C for 2.5 h. It was cooled and water (1 mL) was added. The aqueous mixture was extracted with diethyl ether (4 x 1 mL). The combined ethereal extracts were washed with water (4 x 1 mL), dried (Na2SO4) and the solvent was evaporated by blowing a gentle stream of nitrogen over the top of the mixture to give a yellow residue (0.7 mg).

**Silylation of the Ether Extract**

The residue was taken up in dry pyridine (50 µL) and treated with Trisil (1.5 meq / mL N-trimethylsilylimidazole in pyridine) (100 µL) at 70°C for 30 min. The silylated hydrolysis products were analysed by GC/MS (50 - 250°C, Δ 10°C/min). A major and minor product were identified as the TMS derivatives of C14 and C16 dihydroxy fatty acids (2) and (3) respectively, by comparison of the
mass spectral data with that for a similar silylated hydroxy fatty acid.\textsuperscript{15} TMS-(2): $t_R = 18.65\ \text{min}$; EI-MS: $m/z$ 461 ([M$^{+•}$ − CH$_3$], 18%), 433 (48), 305 (43), 233 (23), 145 (50), 73 (100). TMS-(3): $t_R = 20.0\ \text{min}$; EI-MS: $m/z$ 489 ([M$^{+•}$ − CH$_3$] 16%), 433 (100), 305 (100), 233 (59), 173 (100), 147 (61), 73 (82).

**Silylation of the Aqueous Phase**

The combined aqueous phase and washings from the ether extract of the acid hydrolysis was neutralised with 2M sodium hydroxide solution and freeze-dried to give a colourless powder. The residue was taken up in dry pyridine (50 µL) and treated with Regisil (10% trimethylchlorosilane and bis(trimethylsilyl)-trifluoroacetamide) (100 µL) at 70°C for 20 min. The silylated hydrolysis products were analysed by GC/MS (100 - 250°C, Δ 10°C/min). Glucose: $t_R = 12.52$ and 13.47 min, area 38%; quinovose: $t_R = 10.84$ and 11.49 min, area 38%; rhamnose: $t_R = 9.54$ and 10.32 min, area 9%; fucose: $t_R = 10.04$ and 10.47 min, area 15%.

The peaks corresponded to the TMS ethers derived from quinovose, glucose, rhamnose and fucose (2:2:1:1). Identification was made by the comparison of the retention times and mass spectral data with those for the silylated authentic samples recorded under the same conditions. Authentic samples of the four sugars were individually silylated and analysed by GC/MS and also a mixture of glucose, quinovose, rhamnose and fucose (authentic samples) in a molar ratio of 2:2:1:1 was silylated and analysed in order to identify and determine the ratio of derivatised sugars in the acid hydrolysate. A mixture of authentic glucose (4 mg, 22 µmol), quinovose (4 mg, 24 µmol), rhamnose (2 mg, 12 µmol) and fucose (2 mg, 12 µmol) was taken up dry pyridine (100 µL) and treated with Regisil (150 µL) at 70°C for 20 min. The products were analysed by GC/MS (same conditions as above). Glucose: $t_R = 12.52$ and 13.47 min, area 37%; quinovose: $t_R = 10.84$...
and 11.49 min, area 40%; rhamnose: $t_R = 9.54$ and 10.33 min, area 10%; fucose: $t_R = 10.03$ and 10.47 min, area 13%.

**Alkaline Hydrolysis of the Resin Glycosides**

The mixture of resin glycosides (40 mg) was stirred with sodium hydroxide solution (5 mL of 0.65 g in 10 mL). After 6 h it was neutralised with 4M HCl. An aliquot of the aqueous layer was acidified to pH 1 and extracted with diethyl ether (4 x 2 mL). The ether extract was washed with water (3 x 1 mL) and dried (Na$_2$SO$_4$). The residue was dissolved in diethyl ether.

**Methyl esterification of the Ether Extract**

To the ether extract, was added an aliquot of diazomethane (generated from aqueous sodium hydroxide and N-methyl-N-nitroso-4-toluenesulfonamide (Diazald)) in diethyl ether until a yellow colour in the reaction mixture persisted. The solution was analysed by GC/MS (50 - 250°C, $\Delta 10^\circ$C/min). The products were identified as methyl esterified 3-hydroxy-2-methylbutyric acid (HMBA) (4) (see below for preparation of an authentic sample) and 2-methylbutyric acid (MBA) (5) when the retention times and mass spectral data were compared with that for authentic samples recorded under the same conditions. Methyl esterified (4): $t_R = 4.45$ min; EI-MS: $m/z$ 117 ([M$^{+*}$ - CH$_3$], 9%), 101 (19), 88 (100), 57 (77). Methyl esterified (5): $t_R = 3.87$ min; EI-MS: $m/z$ 101 ([M$^{+*}$ - CH$_3$], 20%), 88 (100), 85 (21), 59 (28), 57 (80).
Preparation of 3-hydroxy-2-methylbutyric acid (HMBA) (4)

The method by Maskens and Polgar was followed. To a solution of mercuric acetate (703 mg, 2.21 mmol) and water (1 mL) was added tiglic acid (221 mg, 2.21 mmol). After 3 days a thick, colourless suspension had formed. The suspension was collected and washed with water (2 x 1 mL) and it was allowed to air-dry overnight. The solid was dissolved in 2M sodium hydroxide solution (1.5 mL) and stirred while sodium borohydride (35 mg) in 2M sodium hydroxide solution (1 mL) was added. A precipitate formed and it was acidified to pH 2 with 3M HCl. The solution was stirred with a saturated sodium chloride solution (3 mL) and then extracted with diethyl ether (4 x 3 mL). The ethereal extract was washed with saturated sodium chloride solution (3 x 3 mL), dried (Na₂SO₄) and the solvent evaporated by blowing a gentle stream of nitrogen over the top of the mixture to afford a yellow residue (98 mg). The spectral data agreed with that in the literature.

Isolation and Analysis of the Aqueous Phase

The neutral aqueous phase from the ether extraction was freeze-dried and the solid was chromatographed using LH-20 gel and methanol to desalt the mixture. One fraction gave a colourless solid (31 mg). Further purification was carried out by using reversed phase-high performance liquid chromatography (RP-HPLC). The mobile phase was a mixture of methanol / water, 55:45. The final, major fraction gave a colourless solid (6) (12 mg). Negative FAB-MS: m/z 1167 ([M – 1]⁻, 100%), 1021 (11), 859 (21), 567 (19), 405 (20), 259 (8) (see Figure 5). Prior to NMR analysis, the solid was taken up in D₂O (0.5 mL), mixed and freeze-dried. This procedure was repeated twice. ¹H and ¹³C NMR spectral data given in Table 1.
Permethylation, Acid Hydrolysis and Silylation of the Alkaline Hydrolysed Resin Glycoside (6)

The permethylation and acid hydrolysis procedure by Harris et al.\textsuperscript{27} was followed. The alkaline hydrolysed resin glycoside (6) was dried over phosphorus pentoxide (P\textsubscript{2}O\textsubscript{5}) under vacuum overnight. It was dissolved in dimethyl sulfoxide (DMSO) (200 µL) under argon, and a dimsyl potassium solution\textsuperscript{*} (100 µL) was added. It was stirred (15 min) and cooled until the mixture was just solidifying (ice-bath). Cold iodomethane (10 mL) was added to the reaction mixture and it was warmed slowly to room temperature with stirring. It was stirred a further 1.5 h and the solvent was then evaporated to give a yellow residue. The residue was dissolved in a mixture of dichloromethane and methanol (2:1, 3 mL) and the pH was adjusted to 2 using 2M HCl. The mixture was stirred and the aqueous phase was extracted with dichloromethane (2 x 1 mL). It was combined with the organic phase and washed with water (2 x 1 mL), dried (MgSO\textsubscript{4}) and the solvent was removed to give a residue. It was taken up in 2M trifluoroacetic acid (0.3 mL) and heated at 110°C in a sealed vessel for 1 h. The reaction mixture was cooled and water was added (1 mL). It was extracted with dichloromethane (3 x 1 mL), dried (MgSO\textsubscript{4}) and the solvent and any residual acid were removed by evaporation. The sample was dissolved in dry pyridine (50 µL) and Regisil (50 µL) was added. The mixture was heated at 75°C for 25 min. The reaction mixture was analysed by GC/MS (100 - 250°C, Δ 10°/min) and a product was identified as the derivatised 3,11-dihydroxytetradecanoic acid (2) with TMS groups on the carboxylate and

\textsuperscript{*} The author wishes to thank Dr L. Shäffeler for the preparation of dimsyl potassium according to the procedure by Harris et al.\textsuperscript{27}
C-11 hydroxyl groups and a methyl ether at C-3 (see Figure 7): \( t_R = 14.67 \) min; EI-MS: \( m/z \) 403 ([M•• - CH₃], 2%), 375 (70), 247 (56), 145 (100), 73 (95).

**An Adaptation of the Lindberg Procedure on Alkaline Hydrolysed Resin Glycoside (6)**

The method followed was that by Harris et al. The permethylation and acid hydrolysis procedure was carried out according to that in the above procedure. The solvent and any residual trifluoroacetic acid were removed by heating the reaction vessel to 40°C and blowing a gentle stream of nitrogen over the top of the mixture. A solution of 0.5 M sodium borohydride in 2M ammonium hydroxide solution (750 µL) was added to the residue and it was heated at 60°C for 60 min. The mixture was cooled and acetone was added. The solvent was removed by heating the reaction vessel to 40°C and blowing a gentle stream of nitrogen over the top of the mixture. The yellow residue was stirred with 18M acetic acid (200 µL) and ethyl acetate (1 µL). Acetic anhydride (3 mL) and perchloric acid (100 µL) were added to the solution and it was stirred a further 15 min. The mixture was cooled (ice-bath), water (7 mL) was added and the mixture was stirred. The mixture was extracted with dichloromethane (2 mL) and dried (MgSO₄). The sample was analysed by GC/MS (100 - 250°C, \( \Delta \) 5°C/min) and four major compounds were identified. By comparison of their mass spectral data with that in the literature they were identified as: 1,5-di-O-acetyl-6-deoxy-2,3,4-tri-O-methylhexitol (7): \( t_R = 12.1 \) min; 1,2,5-tri-O-acetyl-6-deoxy-3,4-di-O-methylhexitol (8): \( t_R = 14.2 \) min; 1,3,4,5-tetra-O-acetyl-6-deoxy-2-O-methylhexitol (9): \( t_R = 15.6 \) min; and 1,2,5-tri-O-acetyl-3,4,6-tri-O-methylhexitol (10): \( t_R = 16.9 \) min.
REFERENCES


CHAPTER 5
BIOLOGICALLY ACTIVE COMPONENTS OF *PERSEA AMERICANA*

5.1 INTRODUCTION

The avocado tree (*Persea americana*) is widely cultivated in many countries for its highly nutritious fruit which has a high oil content. The two varieties grown commercially are named after their country of origin, namely, Guatemala and Mexico.

Toxicity of the leaves, fruit, bark and seeds of the *P. americana* plant has been reported. Lactating cattle, horses, goats, and mice develop non-infectious mastitis and a reduction in milk flow after eating the leaves of *P. americana* var Guatemalan. This syndrome was not observed with the Mexican variety. Ingestion of the avocado leaves is also cardiotoxic for livestock, ostriches and laboratory animals.

Seawright and co-workers have investigated the effects of the *P. americana* var Guatemalan leaves and extracts thereof when administered to lactating goats and mice. According to Seawright, the mastitis is associated with extensive coagulative necrosis of the secretory acinar epithelium and interstitial oedema, congestion and haemorrhage. In addition, an extract of the seeds that was cardiotoxic to mice was also isolated. It was first thought that the cardiotoxin and the component which affects the mammary glands were the same and hence initial studies were carried out on the seed extract. As part of an on-going collaborative research with researchers at the National Research Centre for Environmental...
Toxicology, samples of the active components were provided by Dr P. Oelrichs to the author for structural identification.

5.2 TOXIC EXTRACT OF *P. AMERICANA* SEEDS

A fraction from an aqueous methanolic extract of *P. americana* seeds was isolated by Oelrichs, which had a cardiotoxic effect on mice. The sample was received by the author as a brown suspension in methanol.

A brown pellet was obtained after centrifuging the suspension which was sparingly soluble in warm methanol. Negative and positive FAB mass spectral data of the pellet showed that the major component had a molecular weight of 864 mass units.

Further purification (recrystallisation and chromatography) of the solid was attempted but was unsuccessful. Elemental analysis was carried out and it showed the presence of carbon, hydrogen and oxygen but no nitrogen.

The $^1\text{H}$ NMR spectrum showed the presence of aromatic resonances (7.4 - 6.7 ppm). Signals between 6.2 and 3.8 ppm in the $^1\text{H}$ NMR spectrum were present and a multiplet was observed at 2.82 ppm. $^{13}\text{C}$ NMR data confirmed the presence of aromatics with multiple signals between 158 and 115 ppm. Quaternary carbons resonating around 100 ppm suggested the presence of hemiacetal functionalities. Methine carbons were observed between 80 and 67 ppm as well as around 30 ppm. Since the brown pellet was insoluble in most solvents and only sparingly soluble in warm methanol, 2D NMR data could not be obtained.

Methylation of the solid using a procedure by Vyas and Shah gave a product whose positive FAB-MS showed an intense MH$^+$ ion at $m/z$ 1019 and other, less
intense MH$^+$ ions at $m/z$ 1033 and 1047. This equated to the addition of 11 methyl groups to the major component which had a molecular weight of 864 mass units.

Proanthocyanidins, which are polymeric hydroxyflavanoids, have been isolated from the leaves, stems, fruit and seeds of various plants. Compounds of this class have been shown to possess cytotoxic activity against human tumour cell lines. A trimeric proanthocyanidin with a molecular weight of 864 (C$_{45}$H$_{36}$O$_{18}$) has previously been isolated from the seeds of *Persea gratissima* and was shown to have structure (1) (Figure 1). The molecular weight of (1) would be expected to shift to 1018 when methylated under the above conditions, as reported in the literature for compound (1), which would methylate only the phenolic hydroxyl groups. No NMR data was reported for the proanthocyanidin (1).

The NMR data for the toxic extract from *P. americana* seeds, when compared to that reported for other trimeric proanthocyanidins, indicated that the major active component has the same or a similar structure to (1).

While the above work was in progress, a bioassay was established which monitored the effect of plant extracts on mammary cells in lactating mice. It was found that the trimeric proanthocyanidin from the seeds of *P. americana* did not cause mastitis and reduction in milk flow in lactating mice. By this time, two fractions from the leaves of *P. americana* had been isolated which showed the requisite biological activity in the lactating mice assay and these were supplied to the author. No further structural studies were undertaken on the trimeric proanthocyanidin as the emphasis was placed on the leaf extracts.
5.3 BIOLOGICALLY ACTIVE FRACTIONS OF *P. AMERICANA* LEAVES

Two biologically active fractions were isolated from the chloroform extract of the leaves of *P. americana* by Dr P. Oelrichs.9 One of the fractions caused milk reduction in lactating mice and at higher dosages the heart was affected. The other fraction caused damage specifically to mammary cell tissue in lactating mice.

5.3.1 Fraction 1

A yellow, viscous oil was received and further purification was carried out using normal phase high performance liquid chromatography. The major fraction was collected as a pale yellow, viscous oil.
Infrared, NMR and mass spectral data collected on the oil suggested it was a mixture of polyisoprenols. The different types of plant polyisoprenols (2) and (3) are shown in Figure 2. The natural mixtures of polyisoprenols differ with respect to the type and number of components. The tri-trans-poly-cis-prenols (2) occur only in angiosperms while both angiosperms and gymnosperms are good sources of the di-trans-poly-cis-prenols (3). A literature survey shows that this class of compound has been found in the leaves of many different plant genera but as yet there have been no reports of polyisoprenols in *Persea* leaves.

The infrared spectrum showed a broad band between 3600 – 3200 cm\(^{-1}\) characteristic of hydroxyl functionality. Bands between 2960 and 2850 cm\(^{-1}\) showed aliphatics were present and those at 3040 and 1660 cm\(^{-1}\) indicated the presence of olefinic group(s). The data agreed with that obtained for a mixture of polyisoprenols isolated by Wellburn \textit{et al.} \textsuperscript{20}

![Figure 2. Polyisoprenols found in plants.](image-url)
The $^1$H NMR spectrum (Figure 3) was not a complex one and contained signals characteristic of polyisoprenols.\textsuperscript{17-21} Based on $^1$H, $^{13}$C (including APT - attached proton test) and 2D NMR data, together with published literature values,\textsuperscript{17-21} $^1$H and $^{13}$C NMR assignments were determined (Table 1).

The characteristic doublet at 4.09 ppm in the $^1$H NMR spectrum was assigned as the protons on C-1 of the cis(\(\alpha\))-unit (Figure 2) based on the literature data.\textsuperscript{17-21} A one-bond correlation in the HETCOR spectrum showed that C-1 gave a signal at 58.9 ppm in the $^{13}$C NMR spectrum, which compared well with the literature value (59.0 ppm).\textsuperscript{18} A proton-proton correlation (COSY) between the doublet at 4.09 ppm and the triplet at 5.45 ppm as well as a shared coupling constant value (J 7.0 Hz), confirmed the latter protons were those on C-2. This was the expected value for the C-2 protons of the cis(\(\alpha\))-unit.\textsuperscript{17-21} The characteristic signal at 139.8 ppm in the $^{13}$C NMR spectrum was assigned as C-3 in this unit.\textsuperscript{18} The remaining values of the cis(\(\alpha\))-isoprene unit (Table 1) were based on literature data\textsuperscript{17-21} as well as observed HETCOR correlations.

Similarly, literature values\textsuperscript{17-21} together with observed HETCOR and COSY correlations were used to determine the assignments of the cis-, trans- and \(\omega\)-units listed in Table 1.
Figure 3. $^1$H NMR spectrum of the oil from the first biologically active fraction of the chloroform extract of *P. americana* leaves.
Table 1. $^1$H and $^{13}$C NMR assignments$^a$ for polyisoprenols found in an active fraction of the chloroform extract of *P. americana* leaves.

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$^a$ $^1$H measured at 300 MHz; $^{13}$C measured at 75.5 MHz; $\delta$ in ppm from tetramethylsilane (coupling constants ($J$) in Hz are given in parentheses).

$^b,c,d$ Values are interchangeable with those with the same superscript.
Significant ions in the electron impact - mass spectrum (EI-MS) of the avocado leaf extract were present at $m/z$ 816, 748, 680, 611, 543, 475, 407, 339, 271, 203 and 135 (Figure 4). The same ions were observed in a $C_{50}$, $C_{55}$ and $C_{60}$ polyisoprenol mixture that Wellburn \textit{et al.}\textsuperscript{20} investigated, corresponding to the group of compounds (2) where $n = 5 - 7$ (Figure 2).

The intense ions at $m/z$ 816, 748 and 680 represent the loss of water from the molecular ions of the $C_{60}$, $C_{55}$ and $C_{50}$ polyisoprenols, respectively (Figure 5). This is followed by a series of allylic cleavages from the $\omega$-terminus to give the ions differing by 68 mass units ($C_5H_8$) shown schematically in Figure 5.

In order to confirm the presence of these three major polyisoprenols, the mixture was separated by RP-HPLC using a convex gradient system of mobile phase.\textsuperscript{17} The method resolved a dilute solution of the polyisoprenol mixture into an initial set of three minor peaks and a later eluting set of four, major peaks. Repeated RP-HPLC allowed sufficient sample of each fraction to be collected for EI-MS studies only.

In their EI-MS, the initial set of three minor components of the mixture showed ions at $m/z$ 694 and 696 in one fraction, $m/z$ 762 and 764 in the second fraction and $m/z$ 830 and 832 in the final fraction. The components appear to consist of $C_{50}$, $C_{55}$ and $C_{60}$ compounds with one and two degrees of unsaturation more than the corresponding polyisoprenols.
Figure 4. EI-MS of the mixture of polyisoprenols from the first fraction of the chloroform extract of *P. americana* leaves.
Figure 5. Assignments of some of the significant peaks in the EI-MS of the polyisoprenol mixture.

The second set of four major components in the ratio of 6:28:19:2 had molecular weights by EI-MS of 698, 766, 834 and 902 mass units. This confirmed that the major components of the extract that caused milk reduction in mice were C55 and C60 polyisoprenols together with minor amounts of the C50 and C65 compounds. Therefore, with these molecular weights the compounds were the polyisoprenols (2) where \( n = 5 - 8 \) in Figure 2.

5.3.2 Fraction 2

The second fraction of the chloroform extract of *P. americana* leaves was shown to cause necrosis of the mammary tissue in mice.\(^3\),\(^8\) A yellow oil was provided by Dr P. Oelrichs which solidified upon cooling (at approximately 4°C). No further
purification was required. The oil was examined by infrared, ultraviolet and NMR spectroscopy as well as mass spectrometry and identified as (12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate (4) (Figure 6).

![Structure of compound 4](image)

**Figure 6.** The structure of the active component of the second fraction of the chloroform extract of the leaves of *P. americana.*

Dienone (4) has previously been isolated from the *P. americana* plant.\textsuperscript{22,23} Chang \textit{et al.}\textsuperscript{22} found (4) in the leaves and biological studies showed that it inhibited the growth of silkworm larvae. Prusky \textit{et al.}\textsuperscript{23} isolated compound (4) from the peels of unripe *P. americana* fruit. It was found to be a naturally occurring antifungal agent which inhibits vegetative growth of *Colletotrichum gleosporioides,* the cause of the most important fruit-rotting disease of avocado known as 'avocado anthracnose'.

The optical rotation of the active compound (4) ([α]\textsubscript{D}\textsuperscript{22} + 11.9) corresponded well with the value previously published ([α]\textsubscript{D}\textsuperscript{24} + 11.3).\textsuperscript{22} The infrared spectrum of the dienone (4) contained bands with frequencies similar to those observed by Chang \textit{et al.}\textsuperscript{22} A broad band at 3460 cm\textsuperscript{-1} showed that a hydroxyl group was present. Characteristic of an ester carbonyl group was a strong band at 1740 cm\textsuperscript{-1}. Another strong band at 1720 cm\textsuperscript{-1} indicated the presence of ketone functionality.

In the ammonia chemical ionisation mass spectrum (CI-MS), an [M + NH₄]\textsuperscript{+} ion at \textit{m/z} 398 was observed and confirmed that compound (4) had a molecular weight of 380. The EI-MS showed significant ions at \textit{m/z} 362 [M\textsuperscript{++} - H₂O], 320
[M⁺⁺ - CH₃CO₂H] and 302 [M⁺⁺ – (H₂O + CH₃CO₂H)]. These ions have also been reported by Prusky et al. High resolution accurate mass measurement of the m/z 362 ion gave the value 362.2819 which corresponded to the composition C₂₃H₃₈O₃, confirming the molecular formula as C₂₃H₄₀O₆.

Inspection and comparison of the ¹H (Figure 7), ¹³C and ²⁰NMR spectral data with that in the literature showed the presence of two olefinic bonds separated by a methylene group (see discussion below). In order to determine the positions of the double bonds, ozonolysis with oxidative work-up was carried out on compound (4). The ozonolysis product was taken up in diethyl ether and treated with a solution of diazomethane in diethyl ether. GC/MS of the methylated product showed a major peak whose retention time and mass spectrum were identical to those of an authentic sample of methyl hexanoate. This must arise from the terminus of the aliphatic moiety, confirming that one double bond was at C-15/16. Since NMR analysis showed that there was a methylene group between it and the other double bond, it could be deduced that the other double bond in (4) was at C-12/13.

¹H and ¹³C NMR assignments for (4) (Table 2) were determined from the 1D and 2D NMR spectroscopic techniques that included HETCOR, COSY and HMBC. Where reported, assignments were confirmed by comparison of the literature data. Further assignments were made using high field 2D NMR techniques.
Figure 7. $^1$H NMR spectrum of the active compound (4).
**Table 2.** $^1$H and $^{13}$C NMR assignments for compound (4).\(^a\)

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<td>2.01, dd (7.3, 7.0)</td>
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<tr>
<td>12</td>
<td>5.32, m</td>
<td>130.0(^c)</td>
</tr>
<tr>
<td>13</td>
<td>5.32, m</td>
<td>127.9(^d)</td>
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<td>29.4(^b)</td>
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<td>19</td>
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<tr>
<td>20</td>
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<tr>
<td>OH</td>
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\(^a^1\)H measured at 500 MHz; $^{13}$C measured at 75.4 MHz; δ in ppm from chloroform (\(^{13}\)C) or tetramethylsilane (\(^1\)H) (coupling constants (\(J\)) in Hz are given in parentheses). \(^b,c,d\) Values are interchangeable with those with the same superscript.
Evidence from the infrared and mass spectra of compound (4) indicated that a hydroxyl group, an ester group and ketone functionality were present. A broad singlet at 3.24 ppm in the $^1$H NMR spectrum (exchangeable with D$_2$O) confirmed the presence of the hydroxyl group. The ester was identified as an acetate group by the HMBC correlation observed between the ester carbon at 170.8 ppm and the characteristic protons of the acetate methyl singlet at 2.06 ppm (Figure 8).

Figure 8. HMBC spectrum and observed correlations for compound (4).

The acetate group was connected to a methylene group as indicated by the HMBC correlation between the ester carbon at 170.8 ppm and two protons that resonated at...
4.07 and 4.02 ppm (Figure 8). The proton at 4.07 ppm was a doublet of doublets (\(J = 11.5, 4.2\) Hz) as was the other at 4.02 ppm (\(J = 11.5, 6.2\) Hz) and could be assigned as the protons on C-1 in agreement with the literature values.\(^{23}\)

A proton-proton correlation (Figure 9) was observed between the protons on C-1 and the multiplet at 4.27 ppm that was assigned as the proton on C-2. The one-bond proton-carbon (HETCOR) correlation showed that C-2 resonated at 65.8 ppm. The chemical shifts of C-2 and its proton were characteristic of the hydroxylated CH.\(^{23}\)

![Diagram of compound (4)](image)

**Figure 9.** Observed COSY correlations for compound (4).

A COSY correlation (Figure 9) between H-2 at 4.27 ppm and a two-proton multiplet at 2.58 ppm indicated the latter were on C-3. The protons at 2.58 ppm were HMBC correlated to the ketone carbon at 210.7 ppm which therefore was assigned as C-4. Another HMBC correlation (Figure 8) was observed between C-4 and the protons at 2.41 ppm that were assigned as the protons on C-5, in agreement with the literature.\(^{23}\) A proton-proton correlation (COSY) was present between the protons at 2.41 ppm (\(J = 7.3\) Hz) and those at 1.54 ppm (\(J = 7.3\) Hz) that indicated the latter protons were on C-6, and in turn, the protons at 1.54 ppm were connected (COSY) to the 14-proton signal at 1.26 ppm which therefore contained the protons on C-7 (Figure 9). An HMBC correlation was observed between the protons (H-6) at 1.54 ppm and the carbon (C-7) at 29.2 ppm.
A characteristic triplet in the $^1$H NMR spectrum at 0.85 ppm was assigned as the terminal C-21 methyl group.\textsuperscript{22,23} A COSY correlation between this methyl group and the 14-proton multiplet at 1.26 ppm indicated that this contained the protons on C-20 (Figure 9). An HMBC correlation between the protons at 0.85 ppm and the carbon at 22.4 ppm allowed this carbon to be assigned as C-20. A less intense HMBC correlation was observed between the C-21 protons at 0.85 ppm and the carbon at 31.3 ppm. Therefore, this carbon was assigned as C-19. Its chemical shift was in keeping with that observed for a methylene group in similar systems.\textsuperscript{24}

A carbon-proton (HETCOR) correlation was present between C-19 at 31.3 ppm and the 14-proton multiplet at 1.26 ppm that showed that this latter signal contained the protons on C-19. The other protons contained in the multiplet (14H) at 1.26 ppm were assigned as those on C-8, C-9, C-10 and C-18.

A characteristic triplet in the $^1$H NMR spectrum at 2.73 ppm was assigned as the bridging methylene protons between the two double bonds (H-14). The chemical shift compared favourably with literature values at 2.72 ppm\textsuperscript{24} and 2.78 ppm.\textsuperscript{22,23} A COSY correlation (Figure 9) was observed between the protons at 2.73 ppm and the multiplet at 5.32 ppm (equivalent to 4H) which had a chemical shift characteristic of the olefinic protons in (4).\textsuperscript{22,23} Based on this data and on the ozonolysis results, the olefinic protons were assigned as those on C-12/13 and C-15/16. These protons were also correlated (COSY) to the doublet of doublets at 2.01 ppm (4H) which could be assigned as the protons on C-11 and C-17. These assignments were confirmed by correlations observed in the HMBC (Figure 8) and HETCOR spectra.

The stereochemistry of the double bonds could not be determined from the coupling constants of the protons resonating at 5.32 ppm since the multiplet was too complex (Figure 7). Both double bonds were assigned as Z-olefins based on the
characteristic upfield shift of C-14 at 25.4 ppm which compared well with the literature value of ~25.7 ppm expected for a methylene bridged Z,Z-diene\textsuperscript{24} and the shift of the protons on C-14 at 2.73 ppm.\textsuperscript{24}

In order to determine the absolute stereochemistry at C-2, the hydroxy dienone (4) was treated with Mosher’s reagent (\(R\)- and \(S\)-\(\alpha\)-methoxy-\(\alpha\)-trifluoromethylphenylacetate (MTPA)) under standard conditions\textsuperscript{25} and only starting material was recovered. This was not unexpected due to a previous observation of a neighbouring effect of the acetate moiety which shields the C-2 hydrogen.\textsuperscript{26} Using more forcing conditions resulted in decomposition of (4). An enantioselective synthesis of both the \(R\)- and \(S\)-isomers of (4) was subsequently carried out in this laboratory by Dr L. Schäffeler, and gave > 90% ee. Measurement of their optical rotations and NMR chiral shift reagent studies showed that the natural compound possessed the \(R\)-configuration.

The active component in \textit{P. americana} leaves that causes selective necrosis of the mammary tissue in lactating mice was therefore the \(R\)-enantiomer of \((12Z,15Z)-2\)-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate (Figure 10). This compound and its synthetic analogues are presently under active investigation for the treatment of breast tumours.\textsuperscript{27}

![Figure 10](image-url)  

\textbf{Figure 10.} The active compound in \textit{P. americana} leaves that causes mammary tissue damage in mice.
EXPERIMENTAL

GENERAL PROCEDURES

See General Procedures in Experimental in Part 1 and Chapter 4.

Optical rotations were measured on a Perkin-Elmer model 241 spectropolarimeter at 22°C. High performance liquid chromatography (HPLC) was performed using a Maxima 820 chromatography workstation with a Waters 510 HPLC pump and either a Waters Lambda-Max 481 AZ LC spectrophotometer or a Waters differential refractometer R401 as detector. Alltech CN-bonded silica column (10µm, 10 x 250 mm) was used for normal phase HPLC and Waters Prep Novapak HR C18 column (60A, 6 µm, 7.8 x 300 mm) was used for reversed phase-HPLC (RP-HPLC).

BIOLOGICALLY ACTIVE EXTRACT OF P. AMERICANA SEEDS

Isolation and Characterisation

The seeds were extracted* with a warm, aqueous methanolic solution. The solvent was removed and the concentrate was taken up in water. It was extracted with ethyl acetate and the solvent was removed. The residue was subjected to size exclusion chromatography (LH-20 gel) and normal phase chromatography (silica gel, mobile

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* Dr P. Oelrichs carried out the extraction and initial purification of the biologically active component from the seeds.
phase: chloroform / water / acetic acid). The fraction toxic to mice afforded a brown solid.

The author received the brown solid as a suspension in methanol. The mixture was centrifuged and a brown pellet was obtained. A variety of solvents (acetone, chloroform, water, dimethyl sulfoxide and warm methanol) were tried and it was found that the pellet was sparingly soluble in warm methanol. The active compound was identified as having a structure the same as or similar to trimeric proanthocyanidin (1) (Found: C, 56.0; H, 5.0; O, 39.0). $^1$H NMR (CD$_3$OD, 300 MHz) $\delta$ 7.37 - 6.69, bm; 6.18 - 5.64, bm; 4.77 - 3.82, bm; 2.82, m. $^{13}$C NMR (CD$_3$OD, 75.4 MHz) $\delta$ 158.2 - 145.4, 24 x $C_{\text{quat}}$; 133.2 - 131.6, 5 x $C_{\text{quat}}$; 124.3 - 115.3, 17 x CH; 109.1 - 100.0, 10 x $C_{\text{quat}}$; 98.5 - 96.1, 9 x CH; 80.3 - 78.7, 5 x CH; 72.6 - 67.0, 10 x CH; 38.3, 38.2, 30.6, 29.9, 28.9, 5 x CH. Positive FAB-MS of major component: $m/z$ 865 [MH$^+$], 543. Negative FAB-MS: $m/z$ 863 [M - 1]$^+$.  

Methylation of the active compound (1)  

The procedure followed was an adaptation of that by Vyas and Shah.$^{10}$

A mixture of the brown solid (25 mg), dry acetone (5 mL) and potassium carbonate (0.54 g) under argon was heated to reflux using a dry-ice condenser. Iodomethane (250 $\mu$L, 3.9 mmol) was added dropwise to the solution. After 2h, another aliquot of iodomethane (1 mL) was added and the mixture was heated for 6h. The mixture was cooled and the pH was adjusted to 4 with dilute hydrochloric acid solution. It was extracted with dichloromethane (3 x 5 mL). The organic fractions were combined, washed with saturated sodium chloride solution (2 x 5 mL) and dried.
(MgSO₄). The solvent and residual iodomethane were removed. The concentrate was chromatographed (silica, ethyl acetate / dichloromethane, 1 : 19) and gave a brown solid (16 mg). Positive FAB-MS: \( m/z \) 1019 [MH⁺], 23%), 1033 (6), 1047 (4).

**BIOLOGICALLY ACTIVE EXTRACT OF *P. AMERICANA* LEAVES**

Milled, freeze-dried *P. americana* leaves (200 g) were extracted* with chloroform for 18 h (soxhlet apparatus) and the extract was evaporated to dryness under reduced pressure. The residue was purified by silica gel and then florosil chromatography (order of solvents: hexane, dichloromethane, 4% ethyl acetate / dichloromethane). The fractions were collected and the activity of each evaluated when administered to lactating mice.

One fraction (fraction 1) reduced the milk flow in the mice and was further purified using alumina chromatography (chloroform / hexane). A yellow, viscous oil was obtained.

Another fraction (fraction 2) damaged the mammary tissue of the mice. It was purified by XAD-2 reversed phase chromatography (chloroform / methanol / acetic acid / water, 5 : 80 : 1 : 14) and preparative HPLC (iso-propanol / hexane) to give a yellow oil (2.0 g).

* Dr P. Oelrichs carried out the extraction and initial purification of the active compounds from the leaves.
Fraction 1 from *P. americana* leaves

The yellow, viscous oil (86 mg) was received by the author and was further purified using normal phase – HPLC with a gradient of ethyl acetate / heptane. A fraction was obtained as a pale yellow, viscous oil (50 mg) identified as a mixture of polyisoprenols (2), where n = 5 – 8 (Figure 2). $v_{\text{max}}$ 3600 – 3200 cm$^{-1}$ (OH), 3040 cm$^{-1}$ (C=CH), 2960, 2920, 2850 (aliphatics), 1660 cm$^{-1}$ (C=C). $^1$H and $^{13}$C NMR spectra see Table 1. EI-MS: $m/z$ 834 (M$^{++}$, <0.1%), 816 (2), 748 (4), 680 (2), 611 (2), 543 (3), 475 (4), 407 (6), 339 (10), 271 (19), 203 (50), 135 (76) (see Figure 4).

The mixture of polyisoprenols was subjected to RP-HPLC (C18, methanol / iso-propanol / water, 60 / 40 / 5, with a gradient of iso-propanol / hexane, 30 / 70, from 0 to 60% over 45 min, flow 1.5 mL / min) and gave seven peaks in a ratio of (2:10:5:6:28:19:2) that were collected individually. Fraction 1 with $t_R = 6.92$ min, EI-MS: $m/z$ 696 (3%), 694 (5), 678 (16), 676 (15). Fraction 2 with $t_R = 7.83$ min, EI-MS: $m/z$ 764 (12%), 762 (16), 746 (25), 744 (13). Fraction 3 with $t_R = 8.78$ min, EI-MS: $m/z$ 832 (4%), 830 (4), 814 (10), 812 (5). Fraction 4 with $t_R = 10.71$ min, EI-MS: $m/z$ 698 (M$^{++}$, 4%), 680 (20). Fraction 5 with $t_R = 11.94$ min, EI-MS: $m/z$ 766 (M$^{++}$, 2%), 748 (8). Fraction 6 with $t_R = 13.18$ min, EI-MS: $m/z$ 834 (M$^{++}$, 1%), 816 (5). Fraction 7 with $t_R = 14.43$ min, EI-MS: $m/z$ 902 (M$^{++}$, 1%), 884 (4).

Fraction 2 from *P. americana* leaves

The yellow oil was received and no further purification was required. The oil was identified as (12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate (4)
(83 mg). (Found [M•• - H2O], 362.2819. C23H3gO3 requires 362.2821). [α]22 0 +11.9 (c = 10 mg / mL, CHCl3). νmax 3460b (OH), 1740s (C=O), 1720s (C=O) cm⁻¹. ¹H NMR (CDCl3, 500 MHz) and ¹³C NMR (CDCl3, 75.4 MHz) see Table 2. EI-MS: m/z 362 ([M•• - H2O], 0.5%), 320 ([M•• - CH₃CO₂H], 0.2), 302 ([M•• - (CH₃CO₂H + H₂O)], 3), 273 (1), 259 (1), 245 (1), 231 (2), 217 (1), 149 (6), 95 (52), 81 (100). Cl-MS: m/z 398 ([M + NH₄]+, 20%), 379 (9), 321 (35), 303 (55), 290 (100), 213 (58), 195 (85). UV (CHCl₃) λmax 296, 332sh, 368sh, 401sh nm.

Ozonolysis and Methylation of (4)

A continuous stream of ozone was bubbled through a mixture of the heneicosadienone (4) (2 mg) and dichloromethane (0.5 mL) for 10 min at -78°C. The reaction mixture was warmed and a slow stream of nitrogen was blown over the top of the mixture to evaporate the solvent. A 30% solution of hydrogen peroxide (200 µL) was added and the mixture was then heated to reflux for 6h. The mixture was cooled and acidified to pH 2 with 2M sulfuric acid. It was extracted with diethyl ether (3 x 2 mL) and dried (MgSO₄).

An ether solution of diazomethane (prepared from an aqueous sodium hydroxide solution and N-methyl-N-nitroso-4-toluenesulfonamide (Diazald)) was added to the mixture until a yellow colour persisted. The methylated material was analysed by GC/MS (40 – 250°C, Δ 10°C / min). The major product was identified as methyl hexanoate when the retention time and mass spectral data were compared with those for an authentic sample recorded under the same conditions: tR = 6.24 min; EI-MS: m/z 130 (M••, 2%), 115 (1), 101 (10), 99 (19), 87 (31), 74 (100).
Attempted Preparation of Mosher's Esters of (4)

The method according to Ward and Rhee\textsuperscript{25} was followed.

Oxalyl chloride (7 µL, 80 µmol) was added to a solution of \( R(+)-\alpha\)-methoxy-\( \alpha\)-
trifluoromethylphenyl acetate (\( R\)-MTPA) (4 mg, 17 µmol), \( N,N\)-dimethyl-
formamide (DMF) (1.5 µL, 17 µmol) and hexane (0.5 mL) at room temperature
under argon. The mixture was stirred for 1h. The solvent and volatile reagents
were removed \textit{in vacuo} to afford a yellow residue. A mixture of compound (4)
(5 mg, 14 µmol), triethylamine (5 µL, 32 µmol), \( N,N\)-dimethylaminopyridine
(DMAP) (1 mg, 8 µmol) and CDCl\textsubscript{3} (300 µL) was added to the residue and stirred
for 24h. The \textsuperscript{1}H and \textsuperscript{19}F NMR spectra of the product contained signals that
corresponded to those in the control solution and those for compound (4). No
other signals were observed.

The above procedure was followed except \( S\)-MTPA (4 mg, 17 µmol) was used.
The \textsuperscript{1}H and \textsuperscript{19}F NMR spectra contained signals that corresponded to those in the
control solution and those for compound (4). No other signals were observed.

The control solution was prepared for both \( R\)-MTPA and \( S\)-MTPA by following the
above procedure except that the substrate (4) was not added.

The above procedure was repeated using the same reagents and amounts with
compound (4) except the solution was stirred for 4h, heated (50°C) for 1h and then
cooled. The \textsuperscript{1}H NMR spectrum showed a complex set of signals which indicated
that decomposition had occurred.
REFERENCES


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