Citation: Prazeres, M. A. (1982). Studies of Some Podocarpus Saligna Constituents. (Unpublished Doctoral thesis, The City University)

STUDIES OF SOME PODOCARPUS SALIGNA CONSTITUENTS

A Thesis presented by

Maria Amélia Prazeres

In partial fulfilment of
the requirements for the
degree of
DOCTOR OF PHILOSOPHY
THE CITY UNIVERSITY

Department of Chemistry,
The City University,
London
March 1982

[redacted]

TABLE OF CONTENTS

Page

SECTION 1. ISOLATION AND STRUCTURAL STUDIES OF NORDITERPENE DILACTONE DERIVATIVES

Chapter 1. Literature survey of norditerpene dilactones

Chapter 2. Chemical studies of Podocarpus saligna constituents: non-lactone components

Chapter 3. HPLC of norditerpene dilactones

Chapter 4. Structure elucidation of norditerpene dilactones

SECTION 2. SYNTHETIC APPROACHES TO NORDITERPENE DILACTONES

Chapter 5. Literature survey of the total synthesis of diterpenes

Chapter 6. Proposal for a novel synthetic approach to norditerpene dilactones

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to my supervisor - Dr. S.A. Matlin for his invaluable help and constant encouragement during the period of this work.

Thanks are also due to my colleagues and friends in the Chemistry Department at The City University, and especially to [redacted], for their help and support.

I am grateful to [redacted] of Cambridge University for providing the 400 MHz n.m.r. spectra, and [redacted] of Concepcion University of Chile, for supplying the Podocarpus saligna extracts and many reference samples.

Finally, I would like to thank the [redacted] and Instituto Nacional de Investigação Cientifica for some financial support in the first year.

ABSTRACT

The literature on the isolation, structure elucidation and biological activities of naturally occurring norditerpene dilactones is reviewed.

The isolation of non-lactone components from Podocarpus saligna is described and discussed in relation to previous literature reports. A new HPLC method has been devised for the analysis and preparative-scale separation of the dilactones in P. Saligna, leading to the isolation of several pure compounds in small quantities. The characterisation of these new compounds has been achieved, mainly by the pioneering use of recently-developed spectroscopic methods, including 400 MHz ^{1}H NMR with nuclear Overhauser enhancement difference spectroscopy and Fast Atom Bombardment Mass Spectrometry.

In the second section of the work, literature methods for the synthesis of diterpenes are reviewed and a proposal is set out for the total synthesis of A-ring oxygenated norditerpene dilactones. This proposal involves a regiocontrolled Diels-Alder reaction, for which substantial literature support is presented. The synthetic approaches to establishing this route are described.

These involve firstly, the efficient synthesis of xyloquinone by catalytic oxidation of 2,6-dimethylphenol; secondly, the synthesis of appropriate dienes including 3-methyl-2-pyrone; and thirdly, the attempted cycloadditions between xyloquinone and the various dienes.

The third section presents some applications of HPLC to the analysis of steroid esters which are of potential interest as long-acting, injectable contraceptive agents in a World Health Organisation development programme.

SECTION 1

ISOLATION AND STRUCTURAL STUDIES OF NORDITERPENE DILACTONE DERIVATIVES

CHAPTER 1. LITERATURE SURVEY OF NORDITERPENE DILACTONES

1.1 Introduction

During the last fifteen years, a number of nor- and bisnorditerpene dilactones have been isolated from the root bark, seeds, leaves and stems of Podocarpus species. The Podocarpaceae are ancient gymnosperms growing in scattered parts of the southern hemisphere and tropical and subtropical areas of eastern Asia[1]. For example, P. sellowii is a conifer growing up to 15 m tall and is distributed throughout southern Brazil[2]. The widespread use of members of the Podocarpaceae as building material has stimulated an interest in their chemical constituents, particularly in Australia, Brazil, Chile, Japan and New Zealand[1-3].

The lactones have been considered of much interest, because of their important and varied types of biological activity:

(i) Many of the lactones show activity as retarders of plant growth, having a potent inhibitory effect on the expansion and mitosis of plant cells.

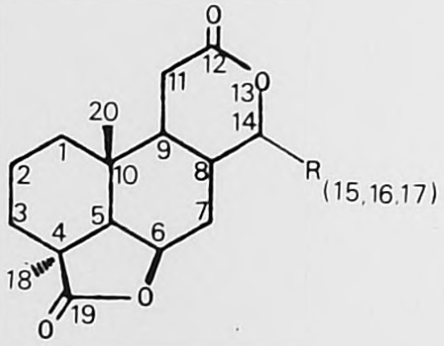
(ii) Some show high toxicity to house fly larvae and production of abberant metamorphosis. Termite resistance of some of the Podocarpaceae is well known, e.g. Podocarpus macrophyllus in Japan, and has been traced to the termiticidal activity of norditerpene lactones such as inumakilactone A.

(iii) Pronounced cytotoxicity and anti-tumor activity has been reported for some of the lactones. Different members of the series show <u>in vivo</u> activity against P-388 leukemia in mice and <u>in vitro</u> activity against cells of human carcinoma of the nasopharynx(9 KB), P-388 murine leukemia and Yoshida carcinoma.

(iv) Norditerpene lactones isolated from an unidentified Acrostalagmus mould and lately from Aspergillus wentii having a close structural similarity to the compounds of plant origin described above, have been shown to possess respectively significant antifungal activity both *in vivo* and *in vitro* and plant growth inhibitory activity.

1.2 Structures of norditerpene dilactones

So far, about 50 lactones have been isolated and their structures determined by spectroscopic techniques. All of the lactones known have the basic ring system shown below. The compounds of plant origin have a side chain (R) with two, three or four carbon atoms (the fourth carbon, when present, is that of a methyl group in an ester), whereas those of fungal origin have a hydroxyl or methoxyl group attached at C-14 or lack of side chain.



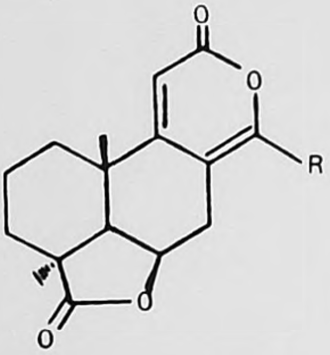
The naming of members of this family of natural products has been essentially arbitrary and based loosely on terminology indicating the first plant origin and chronological sequence of discovery e.g. nagilactones A to G from P. nagi, sellowins A, B, C from P. sellowii, etc. A more systematic classification into structural types has been developed[1], based on the environment of the δ-lactone portion of the B/C ring system:

(i) Sub-group A: α-pyrone ring (8:14, 9:11-dienolide).

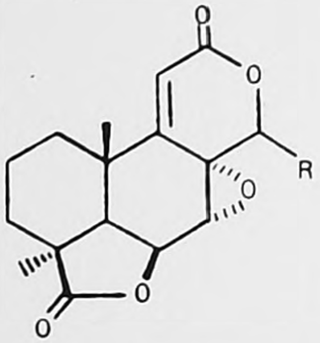
(ii) Sub-group B: dihydro-α-pyrone (9:11-enolide type), often with a 7:8-epoxy group.

(iii) Sub-group C: dihydro-α-pyrone with heteroannular extended unsaturation (7:8, 9:11-dienolide type).

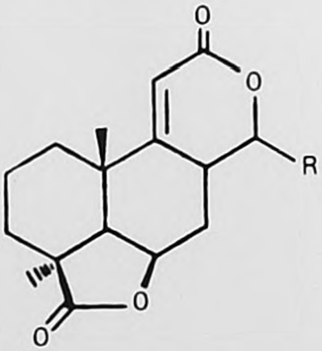
Two compounds, (40) and (41), represent a minor variant to this classification, showing the unsaturation of the dihydro-α-pyrone ring on carbon atoms 8:9 instead of the usual position for sub-group B. These will be classed here as belonging to sub-group B.



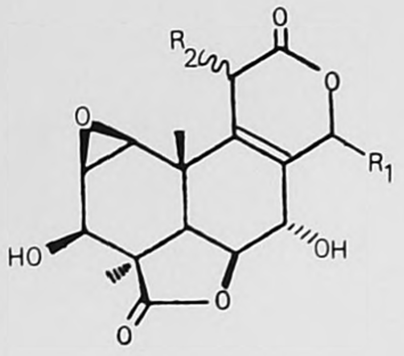
Sub-group A



Sub-group B



Sub-group B

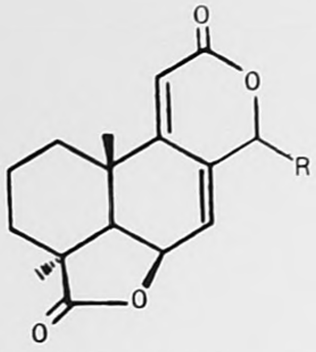


Sub-group B variant

R_1 = $CH(OH)CH_3$

R_2 = H (40)

R_2 = OH (41)



Sub-group C

Names of the dilactones, their structures, melting points, methods for determination of structure, and biological activities are listed in Tables 1-3.

A number of compounds have been included (salignones) which were isolated from P. saligna by our collaborating colleagues in Chile and which were characterised by X-ray crystallography by Prof. William H. Watson at the Texas Christian University in the USA. Most of these structures have not yet been reported in the literature, but Prof. Watson revealed the structures in an unpublished lecture given at an international symposium on natural products[17].

Table 1. Diterpene dilactones of sub-group A.

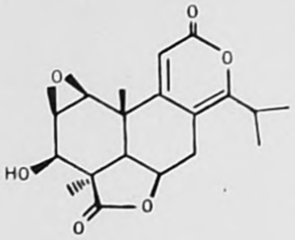
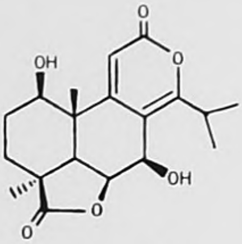
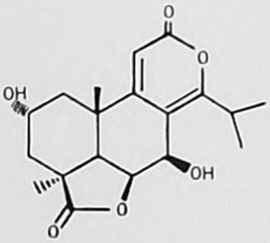
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
1	 Hallactone A $(C_{19}H_{22}O_6)$ mp 266-268° (d) (leaves, stems)	IR, UV, ^1H NMR	Toxic to house fly larvae	4,5
2	 Nagilactone A $(C_{19}H_{24}O_6)$ mp 305° (subl.) (leaves, stems)	IR, UV, ^1H NMR X-ray	Inhibitor of expansion and mitosis of plant cells	4,6
3	 1-Deoxy-2α-hydroxy--nagilactone A $(C_{19}H_{24}O_6)$ mp 243-245° (d) (seeds)	IR, UV, ^1H NMR, MS, CD	-	7

Table 1. Cont.

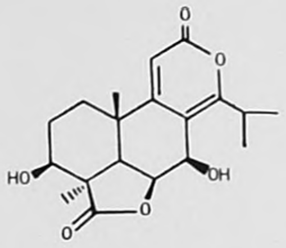
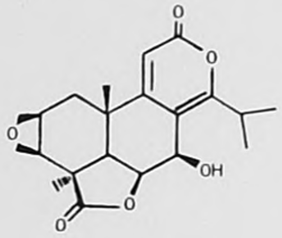
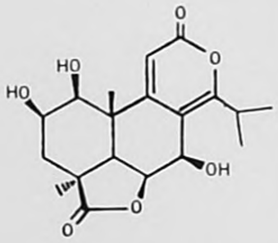
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
4	 Sellowin C ($C_{19}H_{24}O_6$) mp 310° (d)	IR, UV, ^{1}H NMR	-	1,8
5	 1-Deoxy-2β,3β- -epoxy-nagilactone A ($C_{19}H_{22}O_6$) mp 300° (subl.) (root bark)	IR, UV, ^{1}H, ^{13}C NMR, MS	Cytotoxicity	9
6	 Nagilactone B ($C_{19}H_{24}O_7$) mp 258-261° (d) (leaves, seeds, stems)	IR, UV, ^{1}H NMR	(i) Inhibitor of expansion of plant cells (ii) Tumor-inhibitory (P-388) (iii) Cytotoxicity (9KB)	1,6

Table 1. Cont.

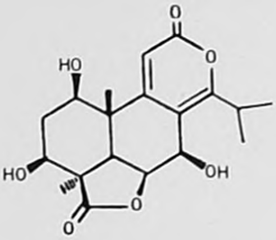
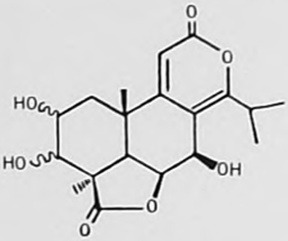
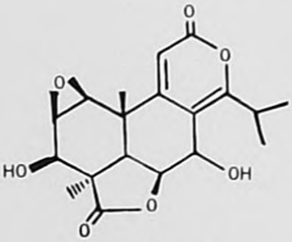
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
7	 3β-Hydroxy- -nagilactone A ($C_{19}H_{24}O_7$) mp 247-249° (d) (seeds)	IR, UV, ^1H NMR, MS, CD	-	10
8	 Urbalactone ($C_{19}H_{24}O_7$) mp 270-272° (d) (leaves, stems)	IR, UV, ^1H NMR	-	11
9	 Nagilactone C ($C_{19}H_{22}O_7$) mp 290° (d) (stems, leaves, bark, seeds)	IR, UV, ^1H NMR, (NOE), CD	(i) Toxic to house fly larvae (ii) Inhibitor of expansion and mitosis of plant cells (iii) Tumor-inhibitory (Yoshida sarcoma cells and P-388)	2,4 5,6 12,13

Table 1. Cont.

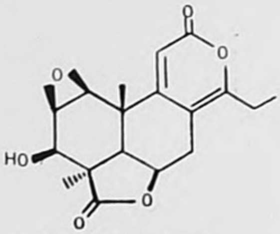
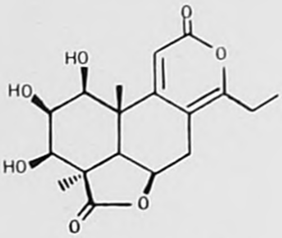
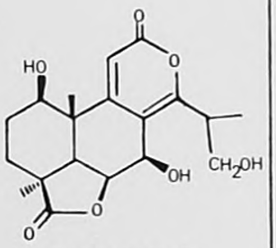
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
10	 Nagilactone D $(C_{18}H_{20}O_6)$ mp 265-266^{o} (leaves, stems, seeds)	IR, UV, ^{1}H NMR (NOE), CD	(i) Inhibitor of expansion of plant cells (ii) Tumor-inhibitory P-388 (iii) Cytotoxicity (9KB)	4,6, 13
11	 1,2-Deepoxy-1,2-dihydroxy-nagilactone D $(C_{18}H_{22}O_7)$ mp 200-210^{o} (seeds)	IR, UV, ^{1}H NMR, MS, CD	-	10
12	 Inumakilactone E $(C_{19}H_{24}O_7)$ mp 220-225^{o} (leaves, stems)	IR, UV, ^{1}H NMR, MS	Inhibitor of expansion and mitosis of plant cells	12

Table 1. Cont.

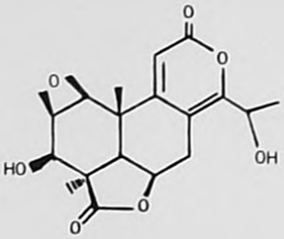
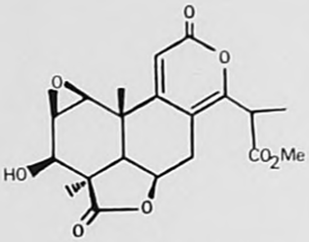
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
13	 15-Hydroxy-nagilactone D ($C_{18}H_{20}O_7$) mp 273° (seeds)	IR, UV, ^{1}H NMR, MS, CD	-	10
14	 15-Methoxycarbonyl-nagilactone D ($C_{20}H_{22}O_8$) mp 238-239° (seeds)	IR, UV, ^{1}H, ^{13}C NMR, MS, CD	-	7

Table 2. Diterpene dilactones of sub-group B.

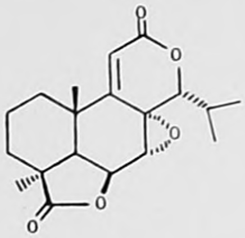
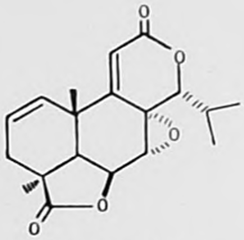
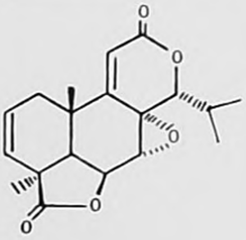
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
15	 Nagilactone G (2,3-dihydro-podolide) $(C_{19}H_{24}O_5)$ mp 296-298^{o} (bark, heartwood)	IR, UV, ^{1}H NMR, MS, CD	(i) Cytotoxicity (9KB) (ii) Tumor-inhibitory activity (P-388)	1,2, 14
16	 Milanjilactone A $(C_{19}H_{22}O_5)$ mp 237-238^{o} (stems)	IR, UV, ^{1}H NMR, MS	Cytotoxicity (9KB)	15
17	 Podolide $(C_{19}H_{22}O_5)$ mp 296-298^{o} (leaves, twigs)	IR, UV, ^{1}H NMR, CD, X-ray	(i) Tumor-inhibitory activity (P-388) (ii) cytotoxicity (9KB)	4,16

Table 2. Cont.

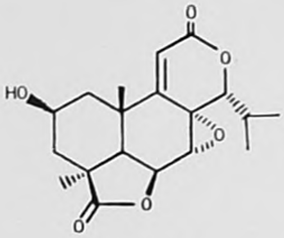
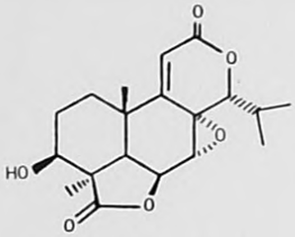
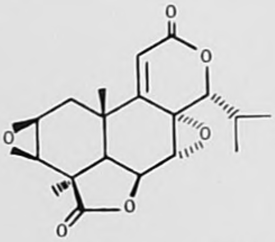
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
18	 Salignone G $(C_{19}H_{24}O_6)$ (root bark)	IR, UV, ^1H NMR, MS, X-ray	-	17
19	 Nagilactone E $(C_{19}H_{24}O_6)$ mp 295° (root bark)	IR, UV, ^1H NMR, CD	(i) Inhibitor of expansion of plant cells (ii) Cytotoxicity (iii) Tumor-inhibitory (P-388)	17,18
20	 2β,3β-epoxy-podolide $(C_{19}H_{22}O_6)$ mp 227° (subl.) (root bark)	IR, UV, ^1H NMR, MS, CD	-	14

Table 2. Cont.

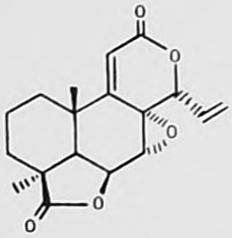
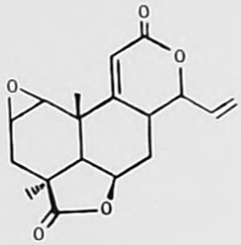
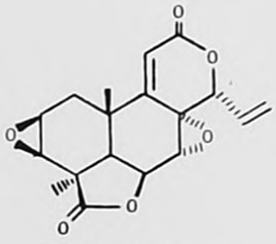
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
21	 Salignone D $(C_{18}H_{20}O_5)$ mp 261-264° (leaves, stems)	IR, UV, ^{1}H NMR, MS, X-ray	-	19
22	 Lactone C $(C_{18}H_{20}O_5)$ (provisional) mp 312-315° (leaves)	IR, UV, MS	-	3
23	 Sellowin B $(C_{18}H_{18}O_6)$ mp 316-317° (d) (leaves, bark, heart-wood)	IR, UV, X-ray	Inhibitor of expansion of plant cells	4,8

Table 2. Cont.

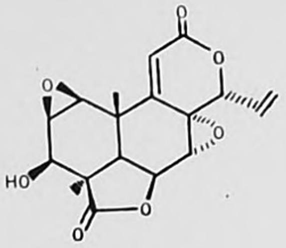
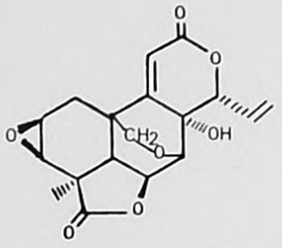
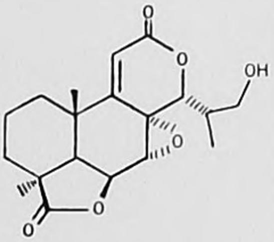
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
24	 Inumakilactone B ($C_{18}H_{18}O_7$) mp 295° (d) (seeds)	IR, UV, ^{1}H NMR, CD	Inhibitor of expansion of plant cells	4,20
25	 Salignone A ($C_{18}H_{18}O_7$) mp 210-225° (leaves, stems)	IR, UV, ^{1}H NMR, MS, X-ray	-	17
26	 Salignone E (lactone D) mp 268° (leaves, root bark)	IR, UV, ^{1}H, ^{13}C NMR, MS, CD, X-ray	-	3,14, 17

Table 2. Cont.

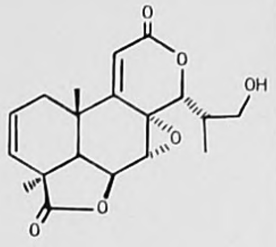
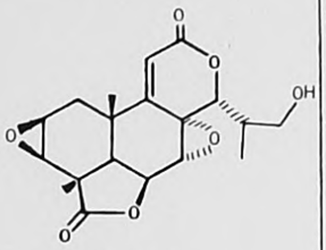
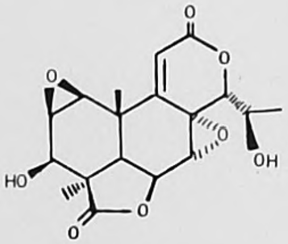
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
27	 Salignone H (16-hydroxy-podolide) $(C_{19}H_{22}O_6)$ mp 225° (root bark)	IR, UV, ^1H, ^{13}C NMR, (NOE), MS, X-ray	-	14,17
28	 Sellowin A $(C_{19}H_{22}O_7)$ mp 298° (d) (bark and heartwood)	IR, UV, ^1H NMR, MS	(i) Inhibitor of expansion of plant cells (ii) Toxic to house fly larvae	4,8,
29	 Inumakilactone A $(C_{18}H_{20}O_8)$ mp 251-253° (d) (bark, heartwood, seeds)	IR, UV, ^1H NMR (NOE), CD, X-ray	(i) Termiticidal (ii) Inhibitor of expansion of plant cells	4,22

Table 2. Cont.

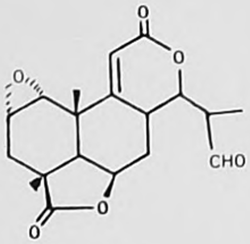
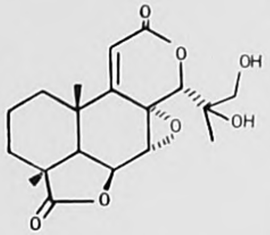
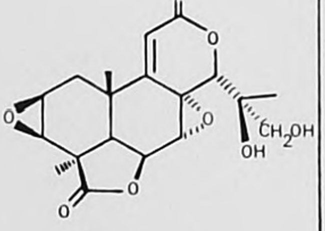
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
30	 Lactone B $(C_{19}H_{22}O_6)$ (provisional) mp 215-220° (d) (leaves)	IR, UV, MS	-	3
31	 Salignone F $(C_{19}H_{24}O_7)$ mp 283-285° (root bark)	IR, UV, ^{1}H NMR, MS, X-ray	-	17
32	 Podolactone A $(C_{19}H_{22}O_8)$ mp 291-293° (d) (bark, heartwood)	IR, UV, ^{1}H NMR, MS, X-ray	(i) Inhibitor of expansion of plant cells (ii) Toxic to house fly larvae	4,23

Table 2. Cont.

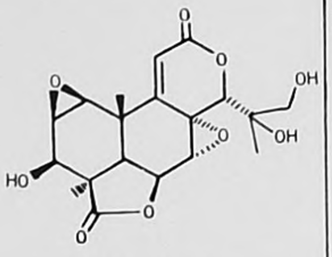
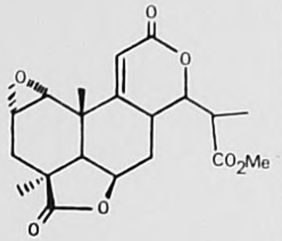
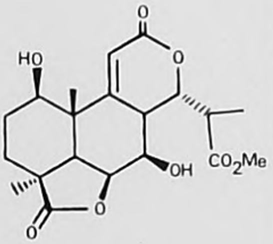
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
33	 Podolactone B $(C_{19}H_{22}O_9)$ mp 272-275^{o} (bark and heartwood)	IR, UV, ^{1}H NMR, MS	Inhibitor of expansion of plant cells	4,23
34	 Lactone A $(C_{20}H_{24}O_7)$ (provisional) mp 258-260^{o} (leaves)	IR, UV, ^{1}H NMR, MS	-	3
35	 Salignone B $(C_{20}H_{26}O_8)$ mp 230-233^{o} (leaves, stems)	IR, UV, ^{1}H NMR, MS, X-ray	(i) Inhibitor of expansion of plant cells (ii) Toxic to house fly larvae	17

Table 2. Cont.

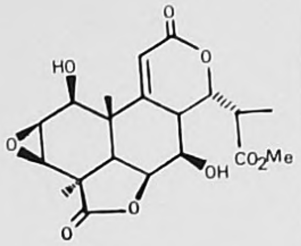
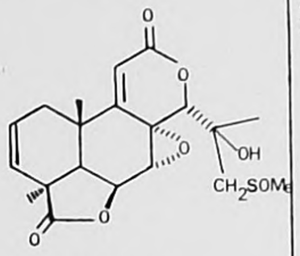
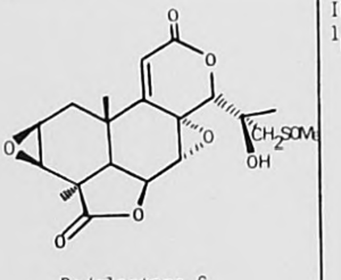
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
36	 Salignone C ($C_{20}H_{24}O_9$) mp 290-292^o (leaves, stems)	IR, UV, ^{1}H NMR, MS, X-ray	-	17
37	 Podolactone D ($C_{20}H_{24}O_7S$) mp 261-266^o (d) (bark, heartwood)	IR, UV, ^{1}H NMR, MS	Inhibitor of expansion of plant cells	4,17, 24
38	 Podolactone C ($C_{20}H_{24}O_8S$) mp 288-290^o (d) (bark, heartwood)	IR, UV, ^{1}H NMR, MS	(i) Inhibitor of expansion of plant cells (ii) Toxic to house fly larvae	4,17, 24

Table 2. Cont.

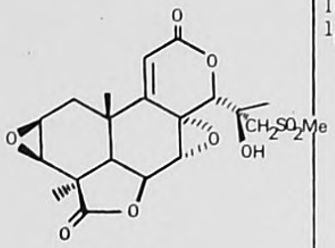
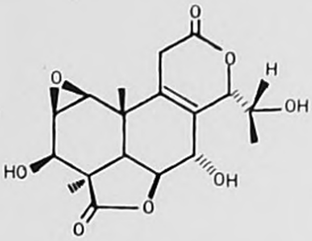
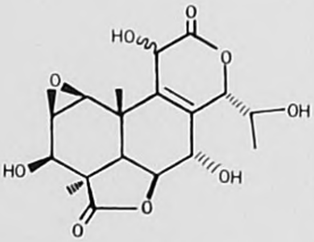
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
39	 Hallactone B ($C_{20}H_{24}O_9S$) mp 325-330° (d) (leaves, stems)	IR, UV, ^{1}H NMR, MS	Toxic to house fly larvae	4,5
40	 Inumakilactone D ($C_{18}H_{22}O_8$) mp 263-265° (d) (seeds)	IR, UV, ^{1}H NMR, X-ray	Inhibitor of plant cells expansion	25
41	 Inumakilactone C ($C_{18}H_{22}O_9$) mp 263-265° (d) (seeds)	IR, UV, ^{1}H NMR, (NOE), CD	-	20

Table 3. Diterpene dilactones of sub-group C.

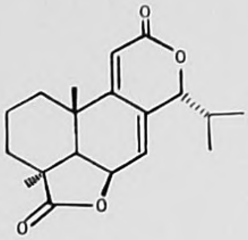
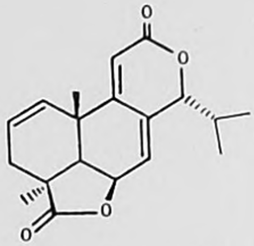
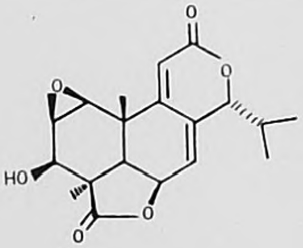
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
42	 Nagilactone F $(C_{19}H_{24}O_4)$ mp 223° (leaves, stems)	IR, UV, ^{1}H NMR, MS, CD	(i) Inhibitor of expansion of plant cells (ii) Cytotoxicity (9KB) (iii) Tumor-inhibitory (P-388)	2,18
43	 Milanjilactone B $(C_{19}H_{22}O_4)$ mp 220-222° (stems, bark)	IR, UV, ^{1}H NMR, MS	Cytotoxicity (9KB)	15
44	 Ponalactone A $(C_{19}H_{22}O_6)$ (root bark)	IR, UV, ^{1}H NMR, (NOE), MS, CD	(i) Inhibition of expansion of plant cells (ii) Toxic to house fly larvae	26

Table 3. Cont.

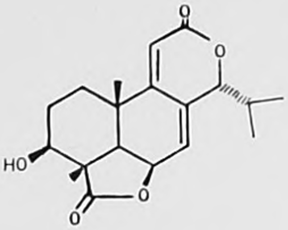
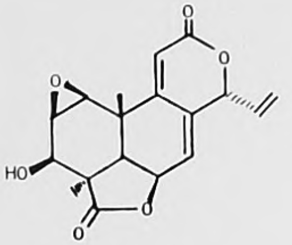
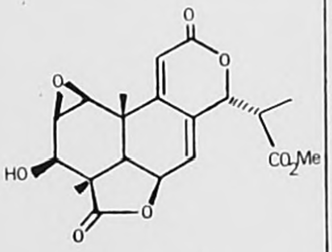
Comp. No.	Structure, name, mp, occurrence	Met. of det.^{tn} of structure	Biol. activity	Refs.
45	 3β-Hydroxy-nagilactone F ($C_{19}H_{24}O_5$) mp 252° (root bark)	IR, UV, ^1H ^{13}C NMR, MS	Cytotoxicity	9
46	 Podolactone E ($C_{18}H_{18}O_6$) mp 261-262° (bark, heartwood)	IR, UV, ^1H NMR	(i) Inhibitor of plant cell expansion (ii) Toxic to house fly expansion	27
47	 Nubilactone A ($C_{20}H_{22}O_8$) mp 288-290° (leaves, stems)	IR, UV, ^1H NMR, MS	Inhibitor of plant cell expansion	28

Table 3. Cont.

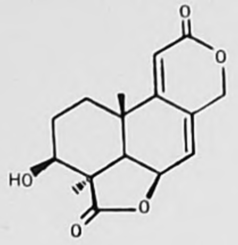
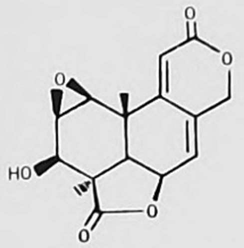
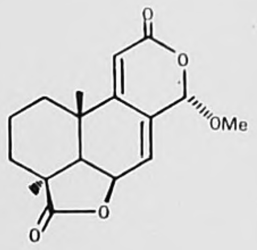
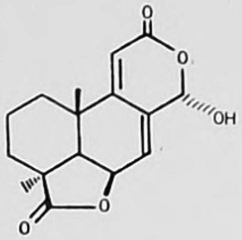
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
48	 Wentilactone B ($C_{16}H_{18}O_5$) mp 260° (mould fermentation)	IR, UV, ^1H, ^{13}C NMR, MS	-	37
49	 Wentilactone A ($C_{16}H_{16}O_6$) mp 258° (mould fermentation)	IR, UV, ^1H, ^{13}C NMR, MS, CD, X-ray	(i) Inhibitor of expansion of plant cells (ii) Toxic to chickens	37
50	 LL-Z1271α ($C_{17}H_{20}O_5$) mp 214-215° (mould fermentation)	IR, UV, ^1H NMR, MS, CD	(i) Antifungal (ii) Inhibitor of expansion of plant cells	29

Table 3. Cont.

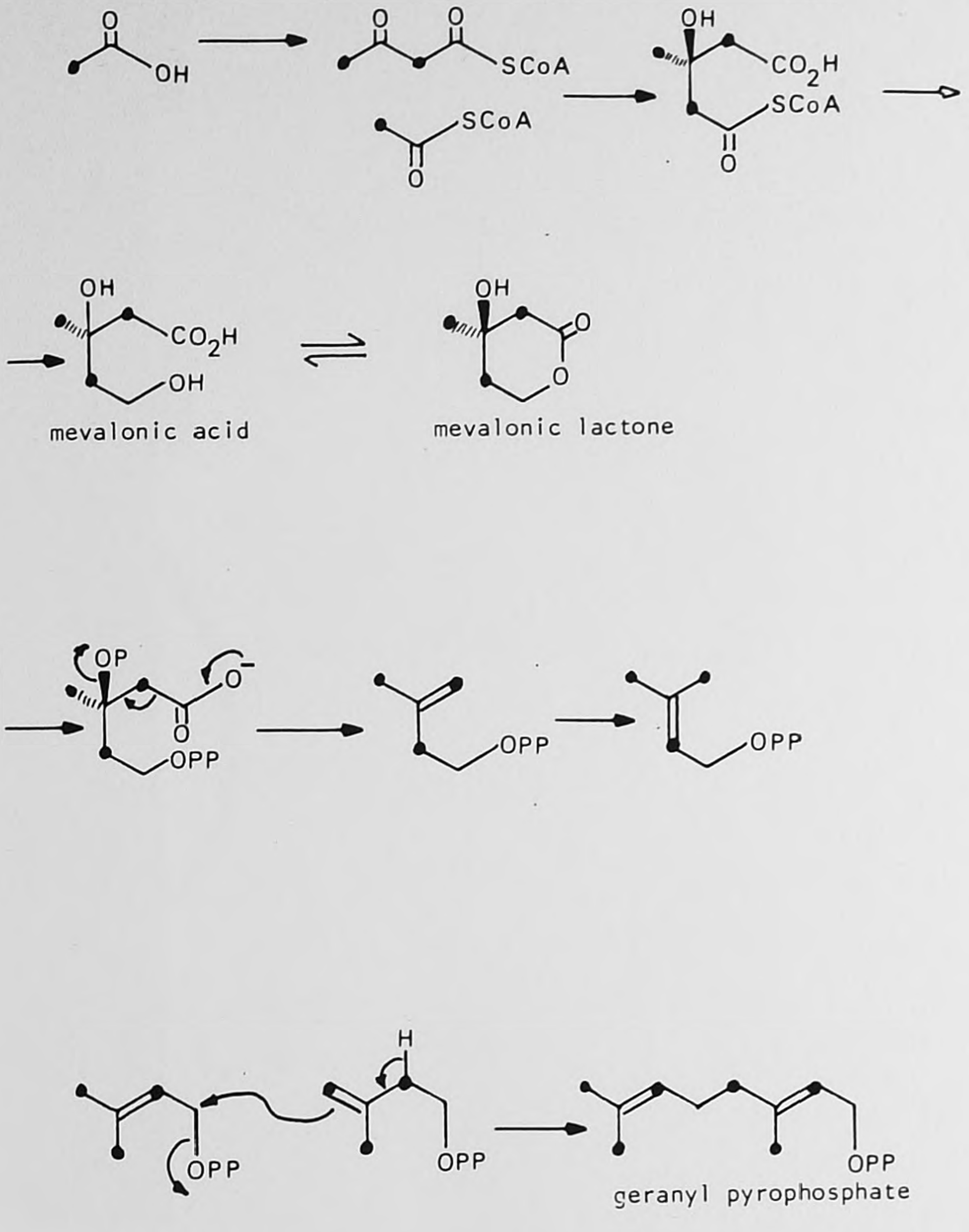
Comp. No.	Structure, name, mp, occurrence	Met. of det^{tn} of structure	Biol. activity	Refs.
51	 LL-Z1271γ $(C_{16}H_{18}O_5)$ mp 241-247° (mould fermentation)	IR, UV, ^1H NMR, MS, CD	Antifungal	29

1.3 Biosynthesis

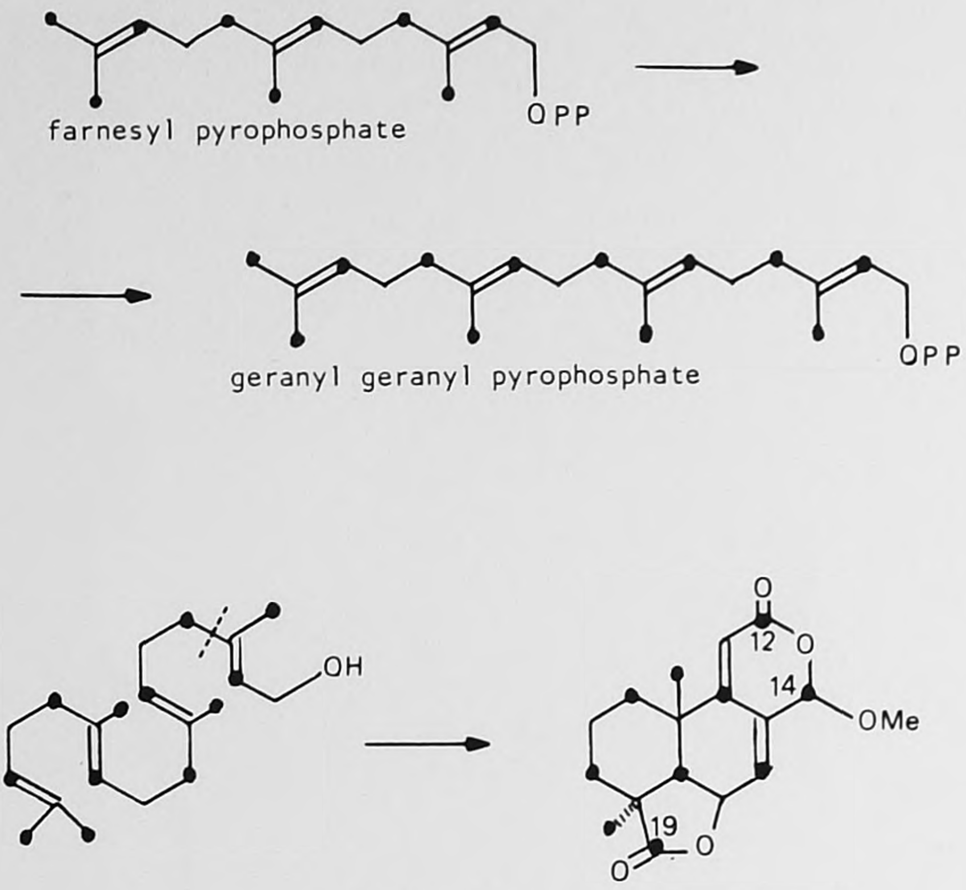
Little direct work on the biosynthetic origin of the norditerpene lactones has been carried out, but their mode of formation is assumed to follow the general pattern of terpenoid biosynthesis in plants. Probably, the difficulty of carrying out biosynthetic labelling experiments with the large, slow-growing gymnosperms has discouraged such studies, besides which the questions of whether the dilactones are products of mature or young plants and whether they show seasonality in their formation remain unanswered.

The only biosynthetic study of direct relevance to the general class of terpene dilactone derivatives under discussion here was that of Kakisawa et al[30], which concerned the Acrostalagmus mould metabolite (50). This compound lacks the carbon side chain at C-14 which is always present in the plant metabolites and whilst the A and B rings are clearly of terpenoid origin, it is not clear whether the C ring is formed by (i) degradation of a diterpene with oxidative loss of four carbon atoms (Scheme 1) or by addition of a C_1 unit (C-12) to a sesquiterpene precursor (Scheme 2). When [2-^{13}C] acetic acid was fed to the Acrostalagmus mould, the lactone isolated had a labelling pattern (determined by ^{13}C NMR) consistent with Scheme 1 (^{13}C = ●), in which the C-12 and C-19 carbonyl carbons showed almost equal, high levels of ^{13}C incorporation.
Furthermore, [2-^{14}C, 5-$^{3}H_2$] mevalonic acid (^{3}H/^{14}C = 5.4) afforded a doubly labelled lactone whose ^{3}H/^{14}C ratio was 2.5 (calculated 2.7). This shows that the lactone is constructed from four molecules of mevalonic acid with loss of four tritium atoms, rather than from three molecules of mevalonic acid (Scheme 3).

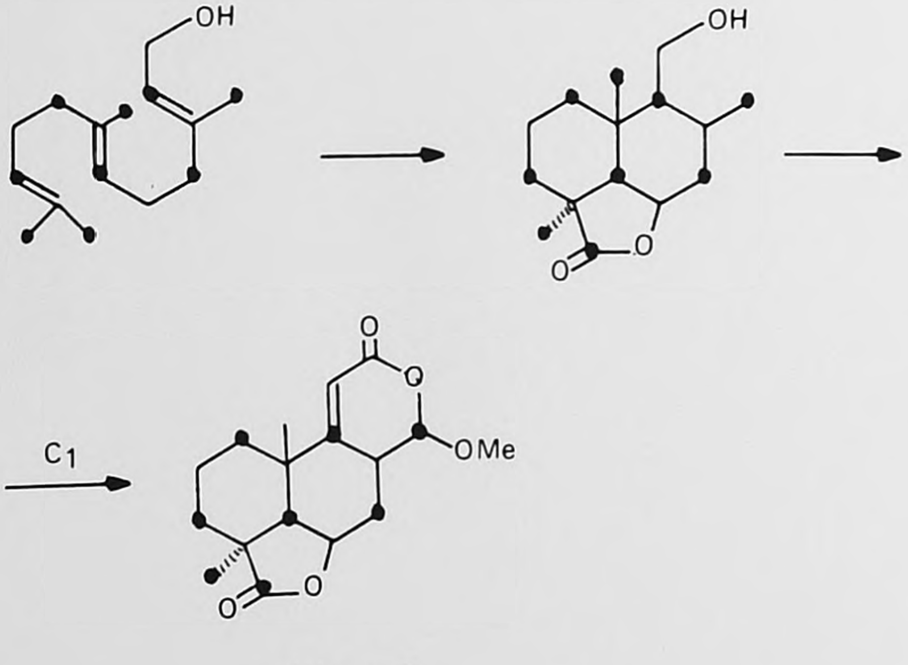
On the basis of these results, Kakisawa et al[30] proposed that the mould metabolite (50) is derived from the degradation of a labdadienol skeleton (52) (i.e. a bicyclic intermediate) by oxidative cleavage between C_{12} and C_{13}.


O
OH
O
O
SCoA
SCoA
O
OH
CO2H
SCoA
O
OH
CO2H
OH
mevalonic acid
OH
O
O
mevalonic lactone
OP
O−
O
OPP
OPP
OPP
H
OPP
OPP
OPP
geranyl pyrophosphate

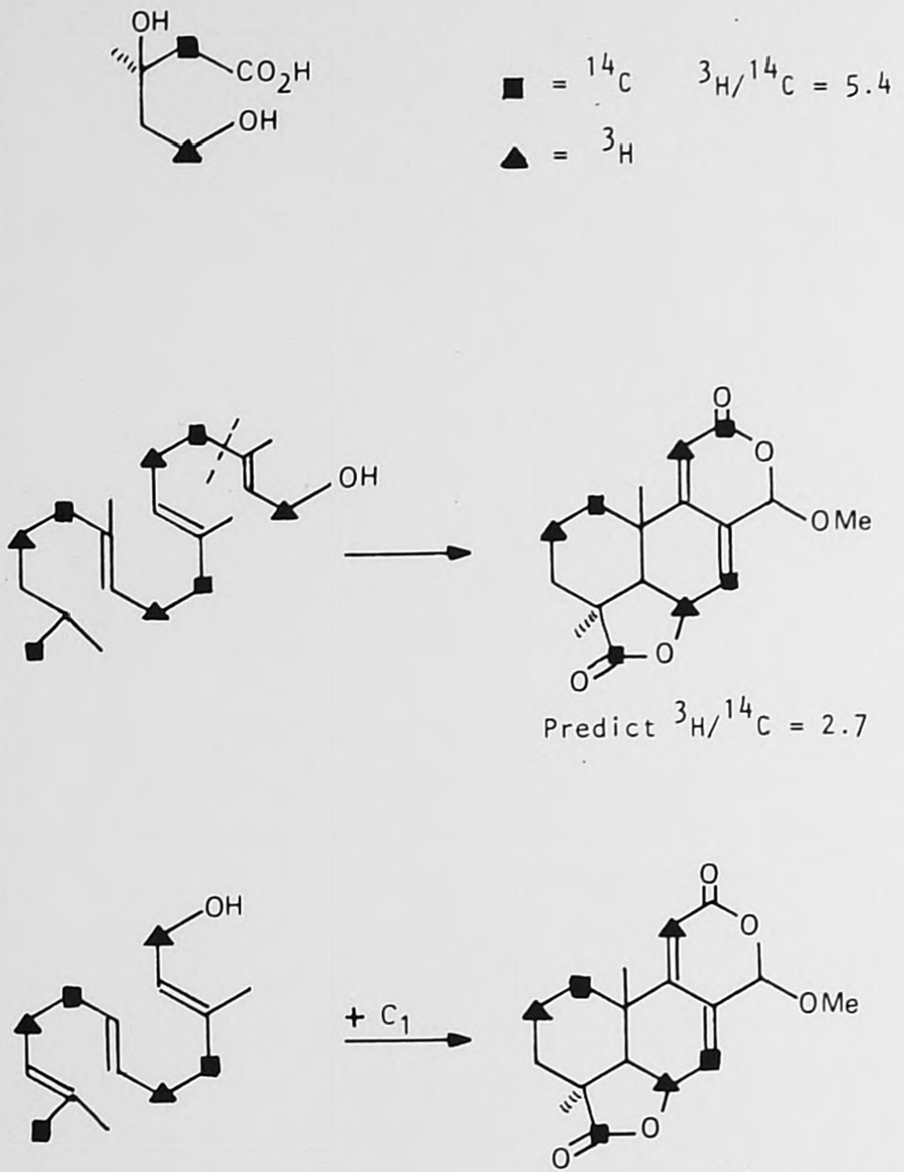
Scheme 1



Scheme 1 (cont.)



Scheme 2



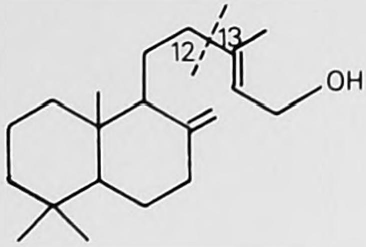
■ = ^{14}C ^{3}H/^{14}C = 5.4

▲ = ^{3}H

Predict ^{3}H/^{14}C = 2.7

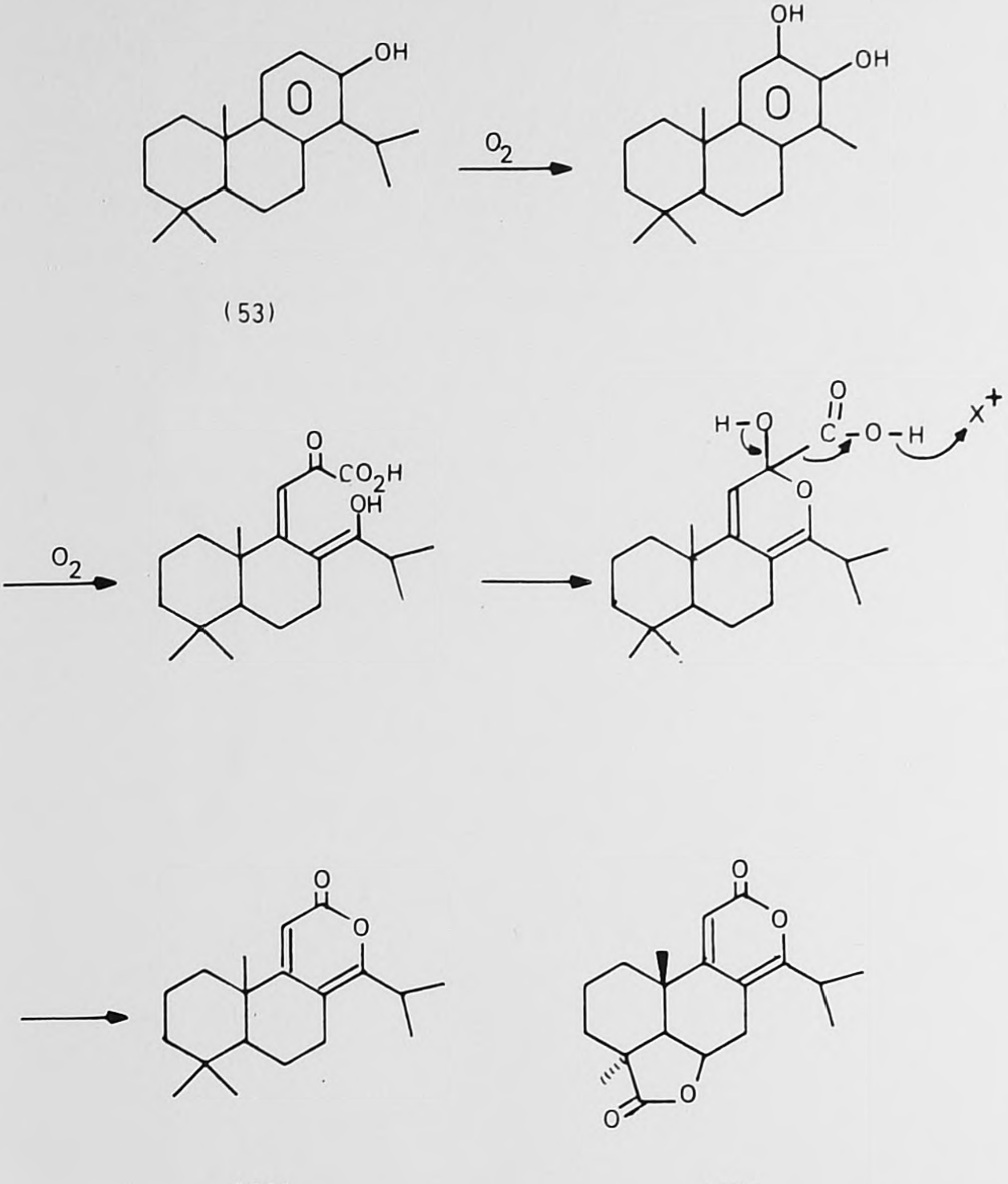
Predict ^{3}H/^{14}C = 3.6

Scheme 3



(52)

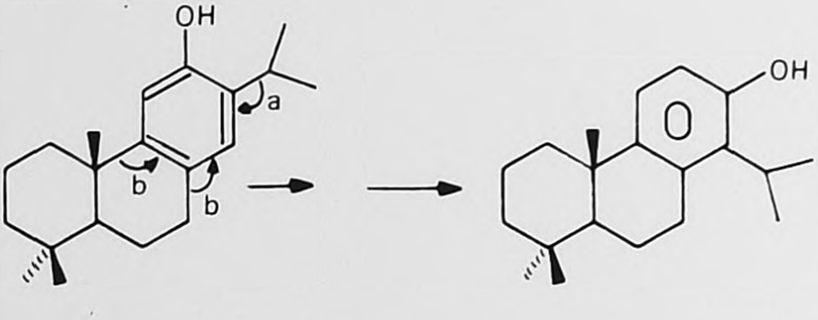
Whilst this remains an acceptable possibility, it is attractive to consider that the mould metabolites may have a biosynthetic origin more closely related to that of the plant dilactones. In the latter, the presence of a carbon side chain attached to C-14 and based on an intact or partly degraded isopropyl group, indicates a biosynthetic precursor in which a carbocyclic C ring is formed, rearranged and oxidatively cleaved. Thus, as long ago as 1968 Hayashi et al[6] proposed that totarol (53), which has also been isolated from some Podocarpaceae, is a key biosynthetic intermediate, being degraded as shown in Scheme 4. This type of oxidative cleavage via a catechol intermediate is a well documented biosynthetic process[31].



Scheme 4

As far as the biosynthesis of totarol is concerned, there do not appear to be any studies reported in the literature to establish its mode of formation.

Oehlschlager and Ourisson[32] suggested that ferruginol (56) may be a biosynthetic precursor of totarol (53) because of their co-occurrence in some Podocarpus species. These authors proposed two possible routes for the rearrangement of ferruginol (56). The first one (a) involves the migration of the isopropyl group from C-13 to C-14 (Scheme 5). In the second route via (b), there is a migration of the appropriate ring B bonds resulting in a "rotation" of ring C. Route (a) suffers from the disadvantage that it fails to explain the position of the OH-group in totarol. Route (b) has the merit of predicting the correct substitution pattern for ring C. However, it is totally inconsistent with the labelling study of the mould metabolite described above and thus cannot be considered as correct.

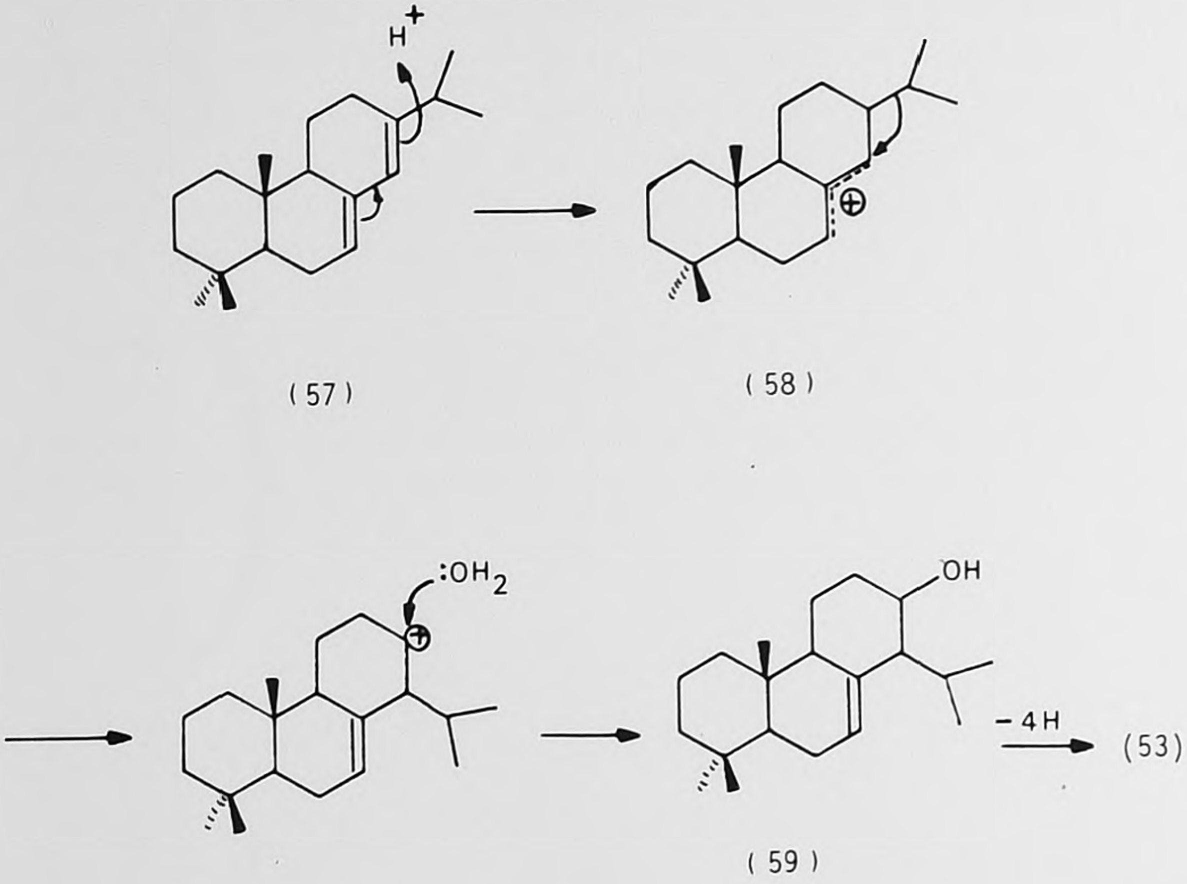


(56) (53)

Scheme 5

In view of the absence of any acceptable mechanism for totarol biosynthesis in the literature, we are left to speculate on this point. A reasonable route is shown in Scheme 6 and involves the protonation of a compound having the abietane skeleton (57), or some equivalent, more oxidized species. A Wagner-Meerwein rearrangement in the re-

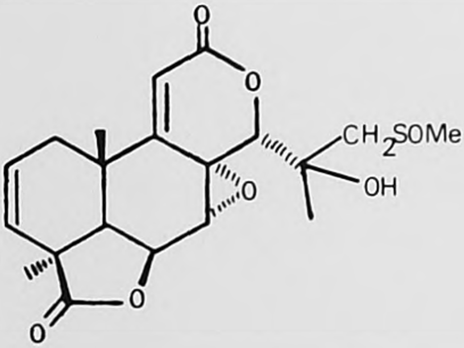
resulting allylic carbonium ion (58) and quenching with water will lead to a structure (59) having the correct substitution pattern and requiring only aromatization to give totarol (53).



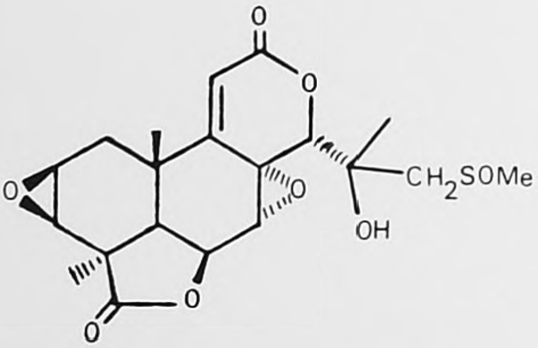
Scheme 6

One of the many outstanding questions about the biosynthesis of the norditerpene lactones concerns the stage at which the γ-lactone ring is formed: either an intermediate having the skeleton (54) undergoes oxidative cyclization, or the γ-lactone ring is formed at an earlier stage, prior to degradation of the totarol C-ring. Similarly, it is not known at what stage the other oxidation processes occur which lead to the introduction of epoxy, hydroxyl and olefinic functions. If these processes take place at a late stage in the biosynthesis, then compound (55) becomes a candidate as a biosynthetic precursor to the entire group. It is interesting that this simple compound has not, so far, been isolated but its double bond isomer nagilactone F (42) is known. However, it is also distinctly possible that the oxidations take place randomly within different plant species, leading to diverse and unrelated sequences for the formation of different members of the series. If compound (55) is an intermediate, it could be transformed into the other known dilactones by sequences of oxidation of the A- and B-rings; migration of the 8:14 double bond followed by its epoxidation or reduction; oxidation and degradation of the side-chain at C-14 up to its total removal to give the Aspergillus mould metabolites.

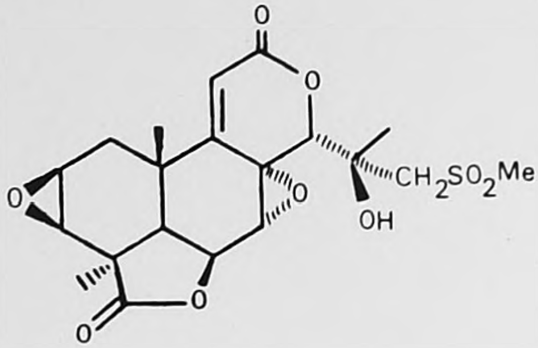
The podolactones (37), (38), and (39) are highly unusual in containing methyl sulfoxide and methyl sulfone groups in the side-chain. Galbraith, Horn and Sasse suggested that their biogenesis involves the methylation and oxidation of the corresponding 16-thiols[24].



(37)



(38)



(39)

An interesting hypothesis for the biosynthesis of this class of compounds was very recently reported by Brown and Sanchez[36] and Dorner, et al[37]. There is a striking structural similarity of diterpene dilactones of sub-group C with the metabolic compounds isolated from fermentation of the fungus Acrostalagmus (genus = Verticillium) and Aspergillus wentii Wehmer (NRRL 6435), which are isolated from soil and are often associated with roots and subterranean stems of plants. It was therefore suggested that norditerpene dilactones may be biosynthesized for Podocarpus plants by mycorrhizal fungi. Furthermore, as wentilactones have no carbon side-chain at C-14, in contrast to the other members of sub-group C isolated from Podocarpus, it seems possible that these diterpene dilactones may be plant-altered fungal metabolites.

1.4 Chemical transformations of norditerpene dilactones

The organic chemistry of these compounds has been relatively little investigated. Two important reasons for this are firstly that the compounds are very poorly soluble and can often be handled only in very polar solvents such as pyridine, DMSO and acetone[1]. Secondly, they have often been isolated only in very small quantities.

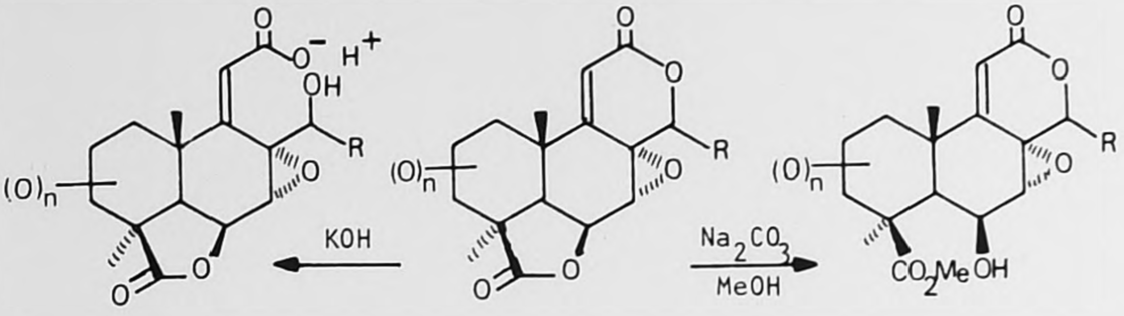
In 1974, when about two dozen of norditerpene lactones had been discovered, Brown and Sanchez[33] published a note on some chemical transformations of norditerpene dilactones from P. sellowii. In summarising what was known at that time, Brown and Sanchez[33] made a number of general assertions:

1. Many standard reactions on these polyfunctional substances give unexpected and frequently uninterpretable results.
2. Starting material is frequently recovered, even under vigorous reaction conditions. The lactone and epoxide groups show an unusual stability to many acids and bases, hydroxyls are often protected from attack by normal reagents, and even a vinyl group resists well-known double bond attacking reagents such as diborane and mercuric acetate.

In the first major review of the Podocarpus dilactones in 1976, Ito and Kodama[1] repeated many of these assertions, although at the same time providing more examples of chemical reactions, many of which contradicted these views. Now, with the hindsight of almost a decade since the work of Brown and Sanchez and with data on double the number of compounds they were considering, a reassessment of these views seems appropriate. The survey of chemistry given below tends to suggest that the majority of transformations which have been attempted have taken a normal course. Relatively few reactions have failed and one or two have taken an "anomalous course" which can be rationalized in terms of the structural features present. Thus, the norditerpene lactones are no more "unusual" than any other complex, polyfunctional class of natural products (e.g. alkaloids, steroids) about which similar comments would be equally true.

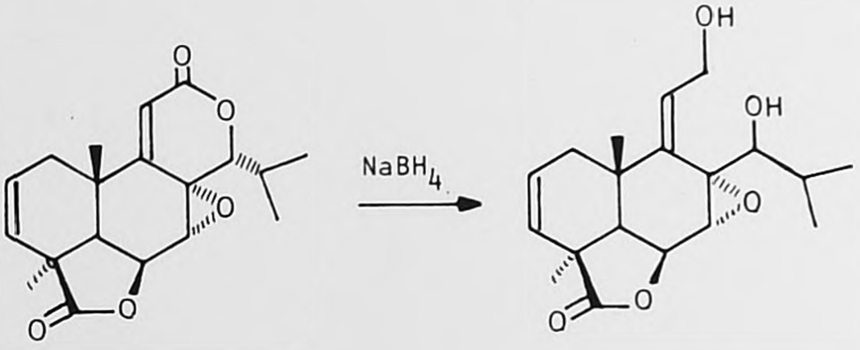
1.4.1 Reactions of the lactone rings

Both the γ- and δ-lactones can be opened by alkali. Within sub-group B, at least, the δ-lactone opens preferentially as shown by infrared, and recloses quantitatively upon acidification[1]. The γ-lactone, on the other hand, is preferentially opened by treatment with sodium carbonate in absolute methanol[1,22], resulting in formation of the corresponding hydroxy methyl ester (Scheme 7).



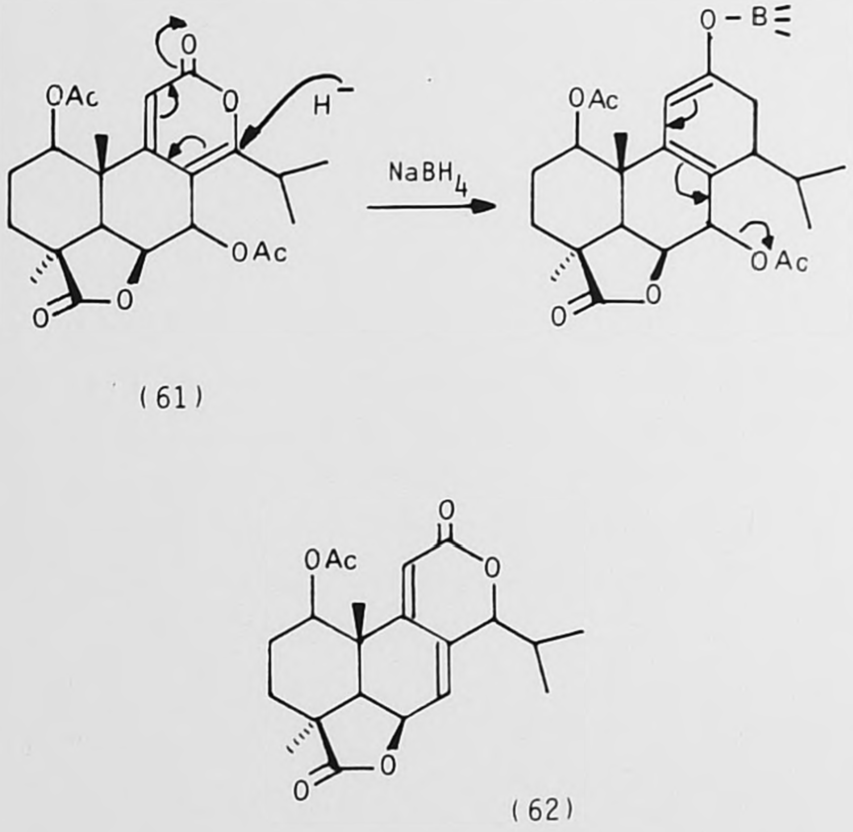
Scheme 7

Hydrogenation[22] of the 9:11 double bond in the diacetate of inumakilactone A gave the expected dihydro derivative. Sodium borohydride reduction of the sub-group B compound podolide (17) afforded the diol (60) resulting from reductive opening of the δ-lactone ring[16]. The diol (60) could be converted back into podolide in excellent yield by oxidation with manganese dioxide.



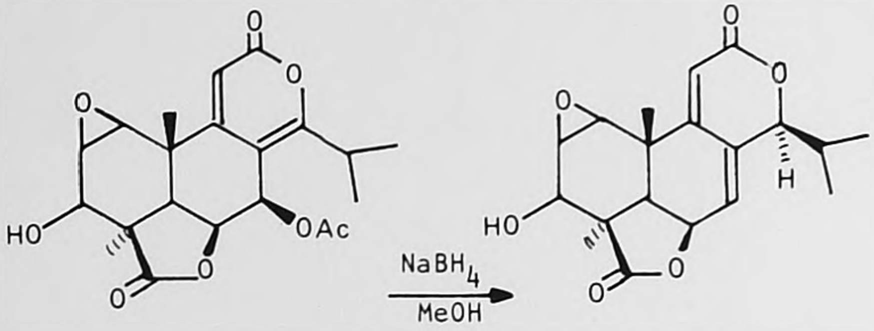
(17) (60)

Several transformations of the δ-lactone in sub-group A type compounds have been reported. Hydrogenation of the δ-lactone to give tetrahydro derivatives[6,13,21] proceeds without difficulty. However, sodium borohydride reduction of nagilactone A diacetate (61) took an anomalous course, leading to the formation of the dienolide (62), the proposed mechanism[6] being shown in Scheme 8. This unusual reaction is significant in two ways. Firstly, it provided the first chemical interconversion between sub-groups A and C. Since sub-group C may be converted to the B-type, in principle, by straightforward methods, this reaction may provide a key to synthetic approaches in the future. Secondly, coming as it did at a very early stage in the exploration of norditerpene lactones, this anomalous reduction is partly responsible for creating the lasting impression that these compounds are "difficult" and "unusual" in their chemistry.



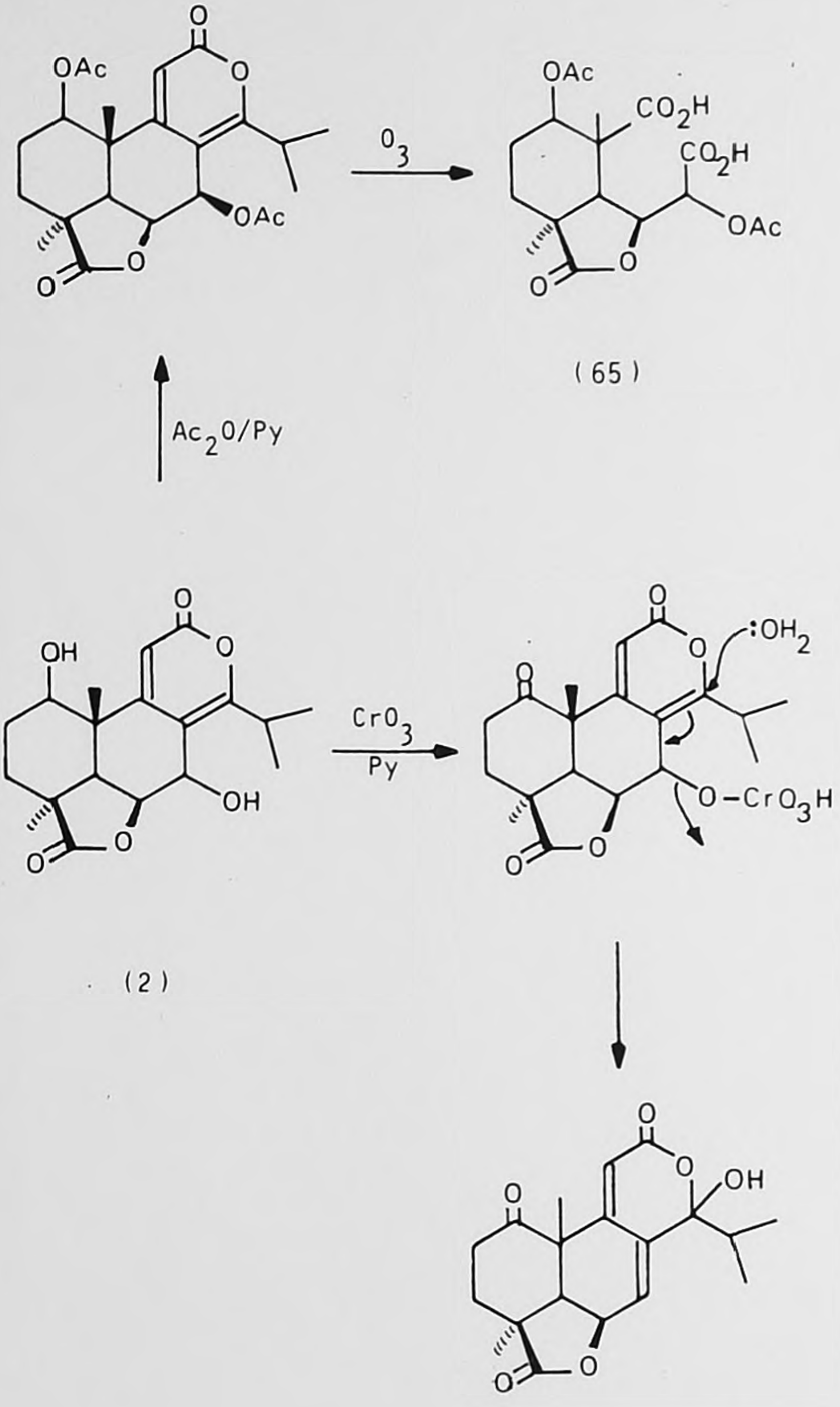
Scheme 8

The reaction may well be a general one for 7-acetoxylated members of sub-group A. One other case of sodium borohydride reduction of such a compound (63) has been reported[26] and this also led to a similar reductive rearrangement (64). In this case, the stereochemistry at C-14 was determined and shown to be β-iPr.



(63) (64)

Two oxidation reactions involving the δ-lactone ring in sub-group A compounds have been described. Ozonolysis of the nagilactone A diacetate (61) gave the expected cleavage to diacid (65) and isobutyric acid. Oxidation of nagilactone A (2) itself with chromic acid in pyridine provided another example of an A- to C-type rearrangement involving loss of the C-7 substituent (Scheme 9).



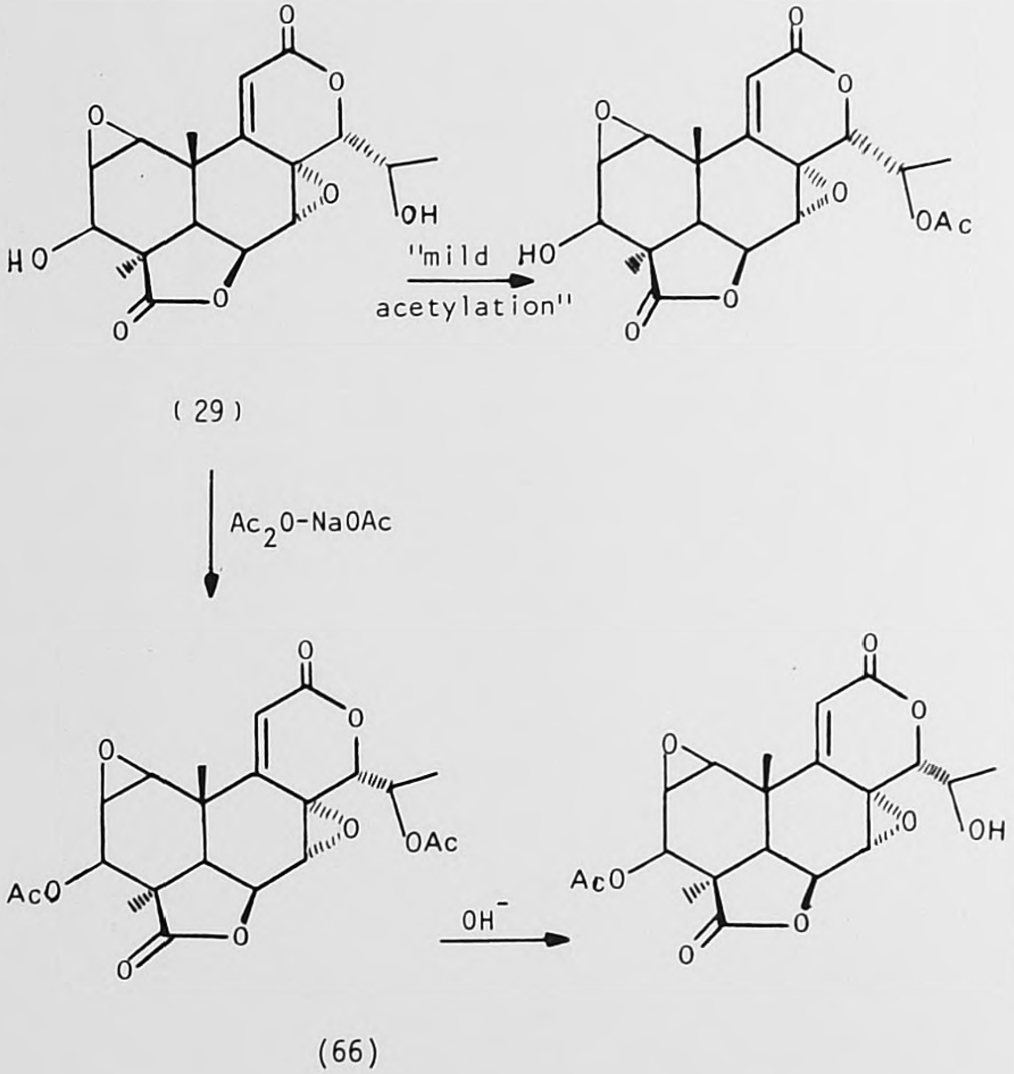
(65)

(2)

Scheme 9

1.4.2 Reactions of hydroxyl groups

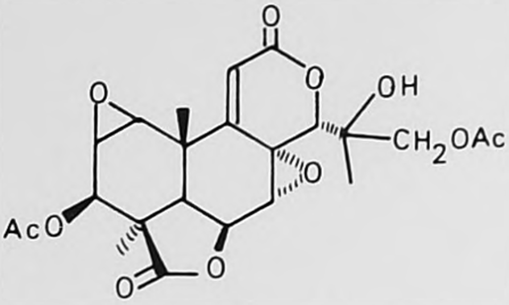
The acetylation of mono, di- and tri-hydroxy derivatives of norditerpene lactones appears to proceed without undue difficulty, in all cases giving the corresponding, fully acetylated products if sufficiently prolonged or vigorous conditions are employed[6,7,9,10,11,12,13,20,22,23,26,28]. In some cases, it has been reported that mild acetylation of some diols gives selective mono acetylation, e.g., a 3,15-diol (29) gave the 15-monoacetate[22]. Conversely, mild hydrolysis of the 3,15-diacetate (66) gave the 3-monoacetate, both reactions showing that the 3-position is rather more hindered than the side-chain 15-position.



(29)

(66)

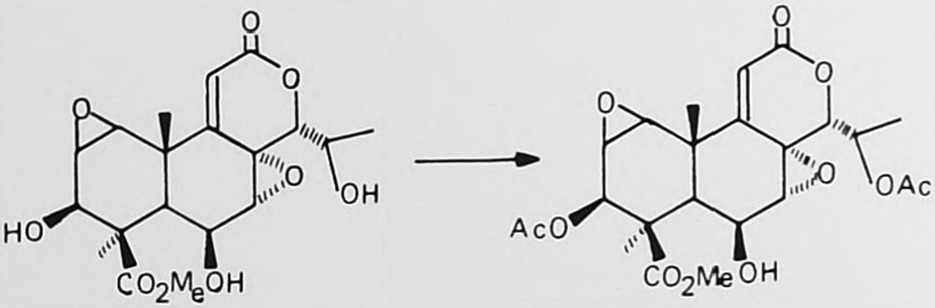
Similarly, mild hydrolysis of the 3,16-diacetate of podolactone B (67) gave the 3-acetate[23].



(67)

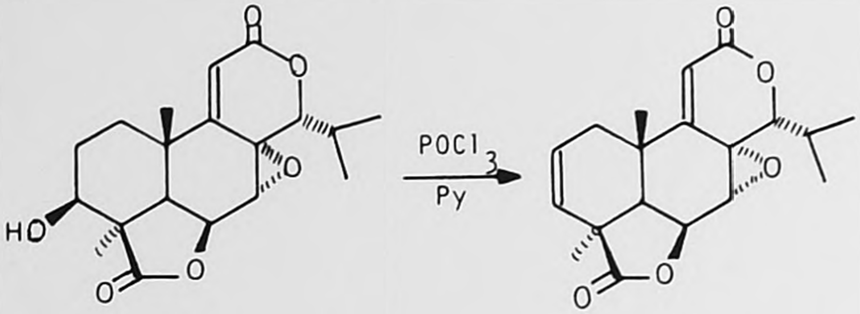
Mild hydrolysis of the 1,7-diacetate (61) of nagilactone A gave the 7-monoacetate, showing the B-ring function to be more hindered than that in the A-ring.

The only reference which could be found to a specific hydroxy-compound failing to undergo acetylation was the case of the degradation product (68). In this, the 6-hydroxy group liberated by methanolysis of the γ-lactone was found to be resistant to acetylation, only the diacetate (69) being obtained. This was attributed[22] to a steric hindrance by the angular methyl group at C-10.



(68) (69)

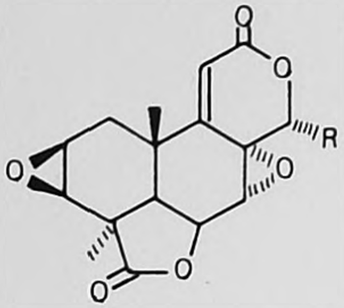
Several examples of the Jones oxidation of ring hydroxyl group to keto groups have been reported[13,22], including one case of the conversion of a 2,3-dihydroxy derivative to an α-diketone[11]. A dehydration reaction has been reported[14] involving conversion of the alcohol (19) to olefin (17).



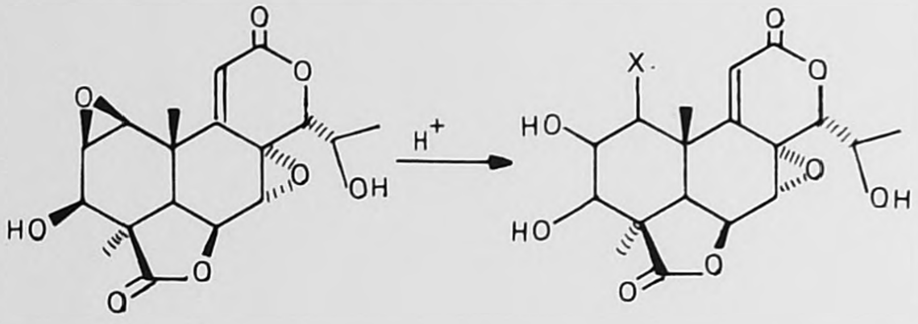
(19) (17)

1.4.3 Reactions of epoxide groups

The A-ring epoxide functions in sellowins A and B, (28) and (23), are attacked by trifluoroacetic acid which causes opening to furnish the monotrifluoroacetates of the corresponding vicinal diols[33]. The A-ring epoxide in inumakilactone A (29) is also attacked by acids (HBr, HCl and H_2SO_4) to give ring opened products (70), (X = Br, Cl and OH respectively)[12].

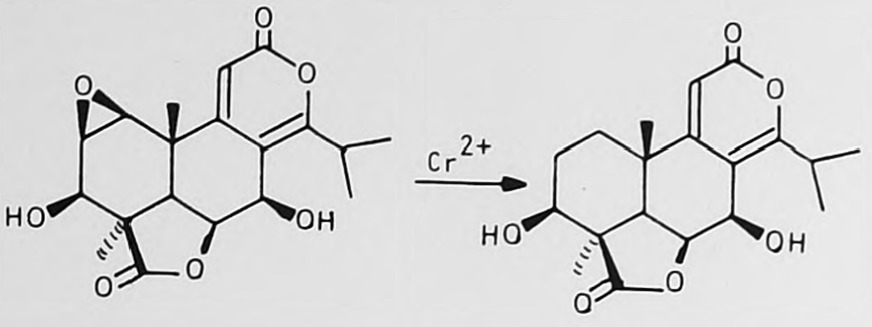


Sel. A (28) R = $CH(CH_3)CH_2OH$
Sel. B (23) R = $CH{=}CH_2$



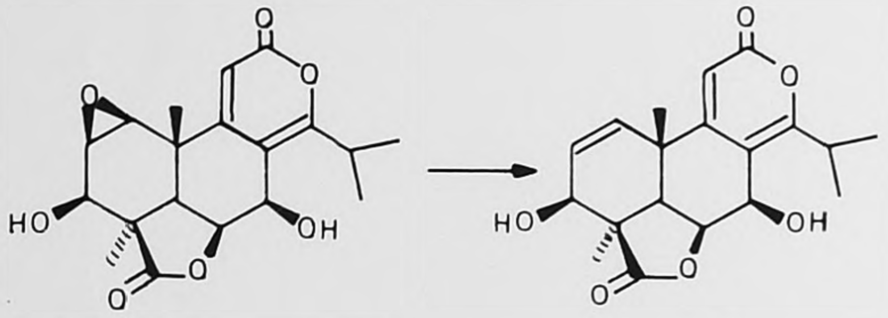
(29) (70)

By contrast, the epoxide ring in nagilactone C (9) was not attacked by trifluoroacetic acid, but was removed in an unusual reduction by chromous ions or Zn-Cu, furnishing the saturated compound, sellowin C[33] (4).



nag. C (9) sel. C (4)

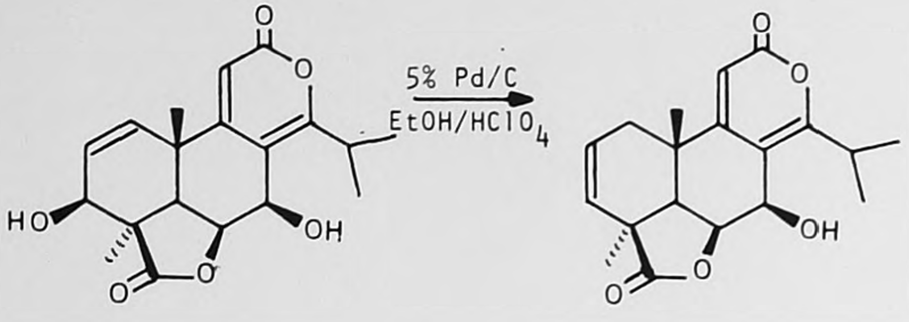
However, the chromous ion (chromous perchlorate-ethylene diamine complex) catalyzed deoxygenation of nagilactone C (9) under N_2 at 30°C for 4 h in DMF afforded the 1,2-unsaturated analogue (71)[38], Scheme 10.



(9) (71)

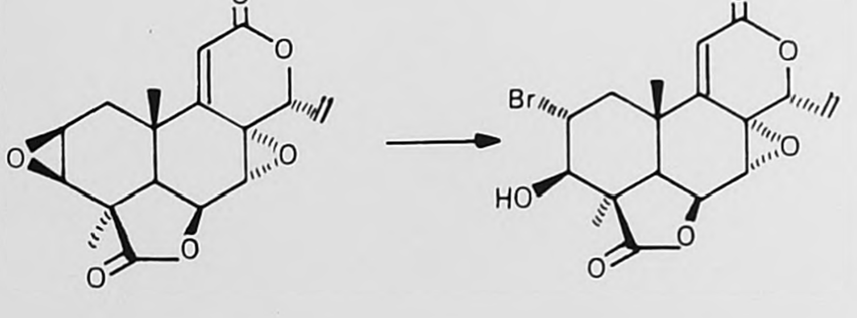
Scheme 10

Hydrogenolysis at C-3 of the olefinic alcohol (71) produced the compound (72) which showed migration of the double bond[38].



(72)

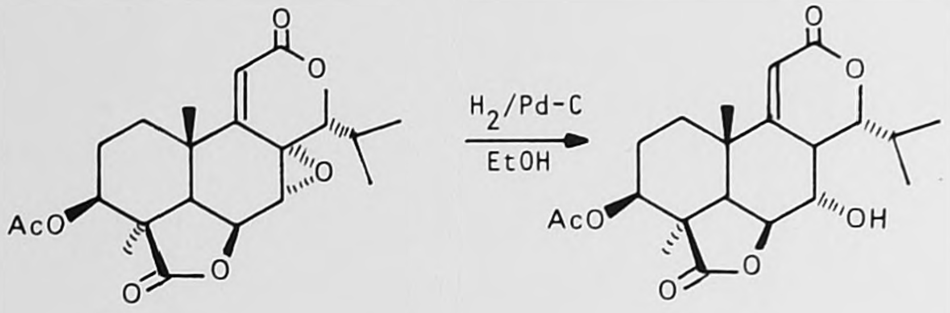
Reaction of sellowin B (23) with N-bromosuccinimide under forcing conditions (in an attempt to functionalize the olefin) gave the bromohydrin (73), the structure of which was determined by X-ray crystallography of the corresponding bromoacetate. The direction of ring opening is unusual in not involving axial attack by Br$^-$, which was attributed[4] to the steric effects of the 18-Me and 20-Me groups.



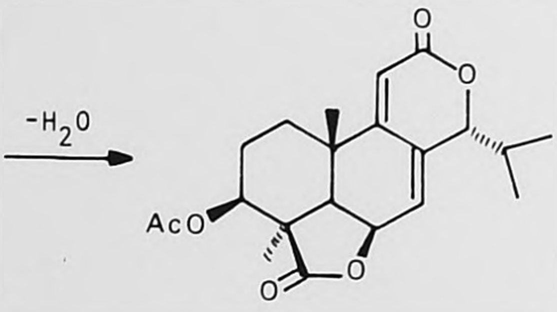
(23) (73)

As implied by many of the reactions above, the reactivity of the 7,8-epoxy group towards acids is considerably less than that of

A-ring epoxides[1,12,33]. Cleavage of the 7,8-epoxide by hydrogenation was accomplished in the nagilactone E acetate (74), the resulting alcohol monoacetate (75) being dehydrated ($POCl_3$, pyridine) to 3β-acetoxynagilactone F (76), thus establishing a link between sub-groups B and C[9]. However, the reduction of a 7,8-epoxide to an alcohol does not appear to be a general reaction[22].



(74) (75)



(76)

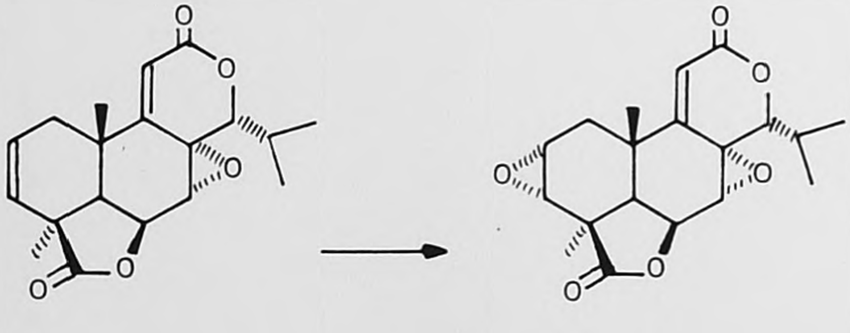
1.4.4 Reactions of the side-chain

Vinyl side-chains have been reported to show resistance to attack by diborane, mercuric acetate and N-bromosuccinimide in aqueous acetone[33]. On the other hand, successful reactions on vinyl side-chains have included hydrogenation[20,27] and bis-hydroxylation with OsO_4 in pyridine[33], so that it is difficult to generalise about the reactivity of this group. The oxidation of side-chain sulfoxide functions to sulfones[5,24], esterifications of hydroxyl groups[22,23,33] and hydrolyses of esters[22,23] have all been carried out, but attempts to reduce a sulfoxide group to a sulphide or to effect its Pummerer rearrangement or desulphurization all failed[24].

It may be concluded that side-chain substituents at C-14 show a great sensitivity to steric and electronic effects in norditerpene lactone derivatives.

1.4.5 Reactions of the 2,3-double bond

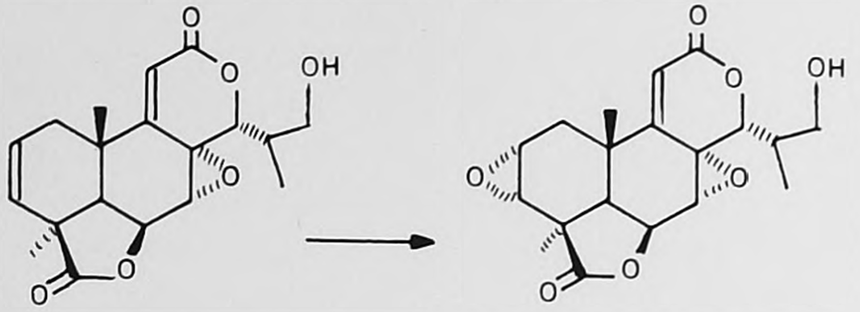
The poor reactivity of some A-ring olefins has been noted in the literature[4,24]. However, the 2,3-double bond of podolide (17) reacted, slowly but definitely, with m-chloroperbenzoic acid in the presence of a radical inhibitor (4,4-thiobis(6-t-butyl-m-cresol)) to give the 2α, 3α-epoxide (77)[38], Scheme 11.



(17) (77)

Scheme 11

A similar result was obtained for 16-hydroxypodolide (27), oxidation giving the epoxide (28)[38], Scheme 12. Thus, the ring A double bond was found to be chemically more reactive at the less hindered α-side, as would be expected. Attempts to prepare the 2β, 3β-epoxide from (17) and (27) were unsuccessful[38].



(27) (Salignone A) (28) (Sellowin A)

Scheme 12

The selective hydrogenation of the double bond at either the 2,3- or 1,2-position was unsuccessful because of the occurrence of undesired transformations, such as saturation of the ring C double bond and the reductive cleavage of the 7α, 8α-epoxide group[38].

1.5 Biological activity

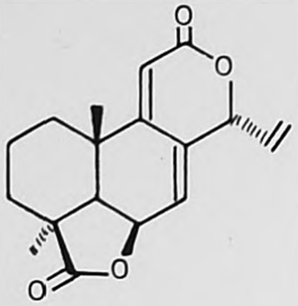
The biological effects of norditerpene lactones are very diverse, as indicated in the Introduction and in Tables 1-3. Unfortunately, testing of biological properties against plant cells, insect pests, tumours and fungi appear to have been largely done at random. Consequently, there is little comparability between different studies and no indication in most cases whether a compound possessing one type of activity also has any of the other types.

By far the most widely reported biological effect of the dilactones is their growth-inhibitory activity on plant cells. Ito and Kodama[1] summarised most of the available data in their review. They concluded that there were widely varying activities within each sub-group, but no significant differences overall between the sub-groups. Less oxidised side-chains and the presence of a 1,2-epoxide function appeared to be generally beneficial for the level of activity.

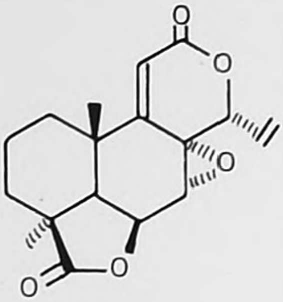
Podolide (17) was the first compound in the norditerpene lactone series reported to show tumour-inhibitory activity, both *in vivo* and *in vitro*[2,16]. Subsequently, there were many sporadic reports of in *vitro* activity of other compounds of this type, culminating in the first serious attempt to develop a systematic structure-activity correlation by Hayashi, Matsumoto and Tashiro[34] in 1979. The cytotoxicity of 32 diterpene dilactone derivatives was reported (Table 4) and the following conclusions drawn:

(i) The most active members have no hydroxyl group in the molecule and generally lack substitution.
(ii) Increasing polarity resulting from hydroxylation and side-chain oxidation appears to have a damaging effect on activity.
(iii) The esterification of the polar groups present in a compound does not help, and frequently actually reduces activity.

Although these and several other minor conclusions were put forward by the authors[34], a close examination of the data (Table 4) suggests that such inferences must be considered tentative, since the lack of complete series of structural relatives makes direct comparisons difficult. Also, if one takes the same liberties in comparing activities and structures which the authors[34] appear to have taken, additional inferences could be drawn which suggest leads to further work. Thus, the data provides support for the idea that replacement of an isopropyl group by a vinyl group enhances activity, indicating that the as yet unknown olefins (78) and (79) would be interesting to test.



(78)



(79)

The deficiencies of this attempted structure-activity correlation point to the disadvantages of working only with natural products and their simple derivatives in trying to establish optimum biological activity. Clearly, in an area like this where the availability of the natural products is severely limited, there is a strong case to be made for developing a rational and flexible total synthesis.

The mould metabolite (50) possesses significant antifungal activity in vitro against a number of fungi and in vivo against some experimental ringworm infections in guinea pigs[35] ("ringworm" is a misnomer since such infections are actually fungal in origin). However, it was noted later that there were also considerable toxic side effects[29]. Although this is the only report of the antifungal activity of a norditerpene dilactone derivative, it does not appear that any other member of the class has ever been tested for such an effect.

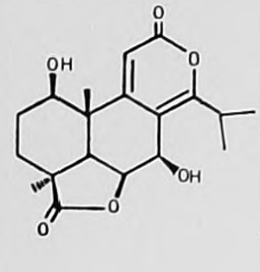
Table 4. Cytotoxicity of the Dilactones against Yoshida Sarcoma.

Lactones	IC_{50} ($\times 10^{-4}$ mM)	Lactones	IC_{50} ($\times 10^{-4}$ mM)
Type A		(19)	10.4
(1)	32.0	(20)	4.11
(2)	17.2	(21)	18.3
(3)	22.5	(22)	87.0
(4)	3.32	(23)	607.0
(5)	16.4	(24)	4.19
(6)	487.0	(25)	20.6
(7)	21.5	(26)	110.0
(8)	305.0		
(9)	1460.0	Type-C	
(11)	1000.0	(27)	1.7
(12)	138.0	(28)	12.2
(13)	119.0	(29)	16.1
		(30)	18.9
Type-B			
(14)	3.36	Others	
(15)	1.48	(10)	inactive
(16)	3.72	(31)	130.0
(17) and (18)*	14.8	(32)	inactive

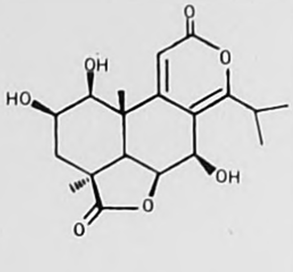
* A 1:1 mixture of the two compounds.

APPENDIX 1. STRUCTURES OF COMPOUNDS LISTED IN TABLE 4.

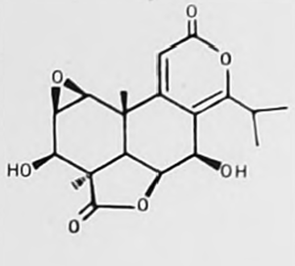
TYPE A



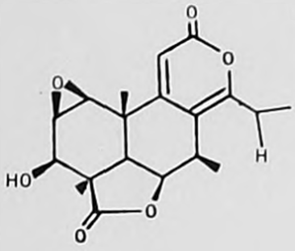
(1)



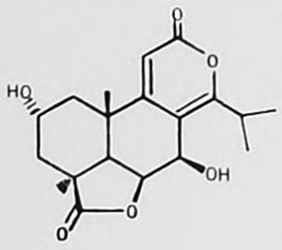
(2)



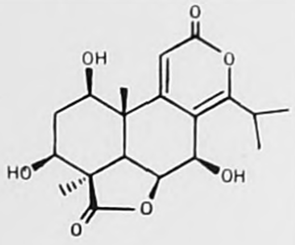
(3)



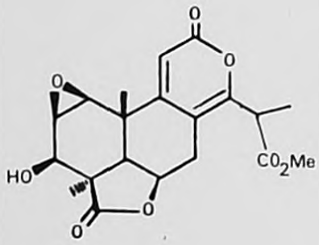
(4)



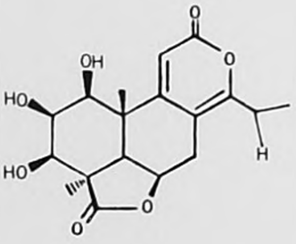
(5)



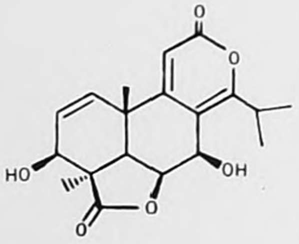
(6)



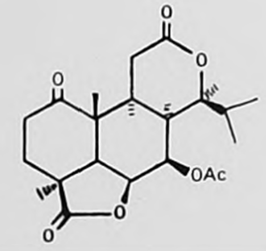
(7)



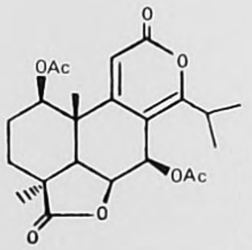
(8)



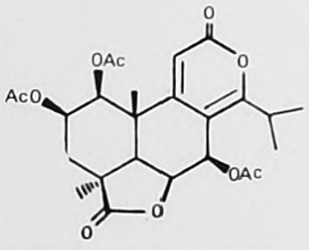
(9)



(10) (inactive)



(11)

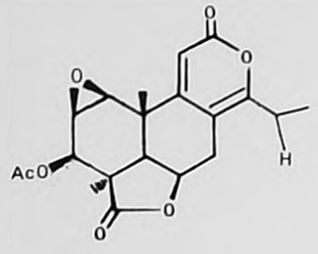


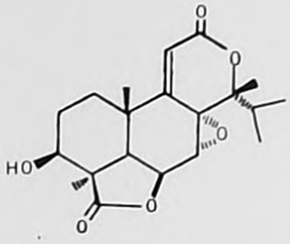
(12)

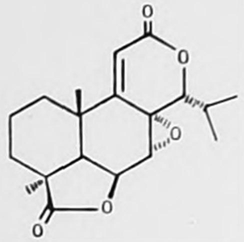
APPENDIX 1. (CONT.)

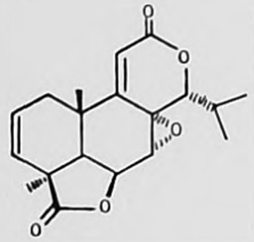
TYPE A (Contd)

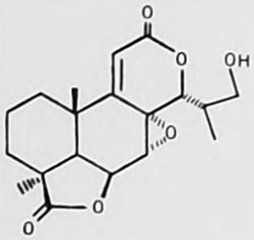
TYPE B

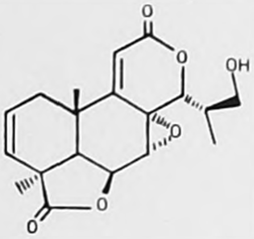

(13)

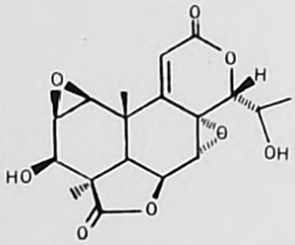

(14)

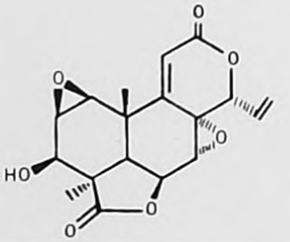

(15)

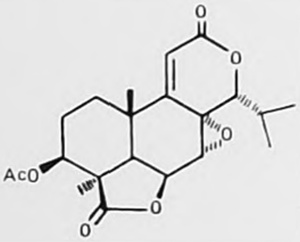

(16)

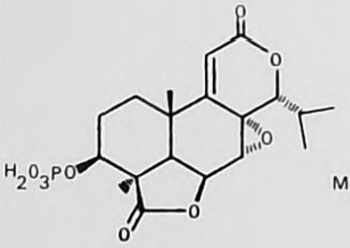

(17)

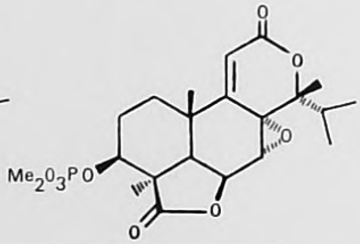

(18)

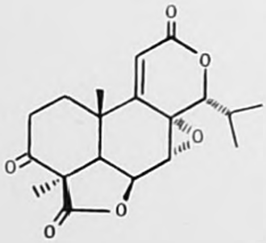

(19)


(20)


(21)


(22)

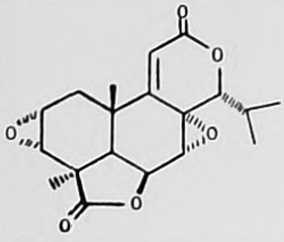

(23)


(24)

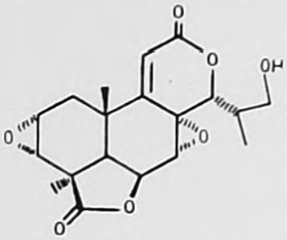
APPENDIX 1. (CONT.)

TYPE B (Contd)

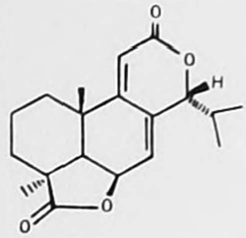
TYPE C



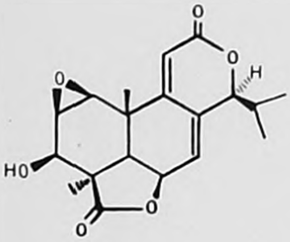
(25)



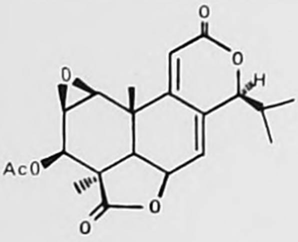
(26)



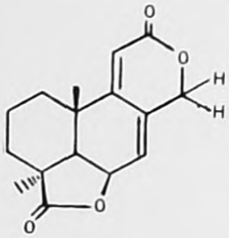
(27)



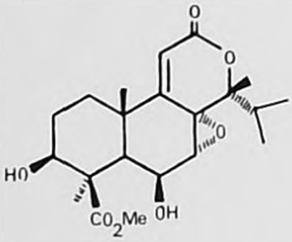
(28)

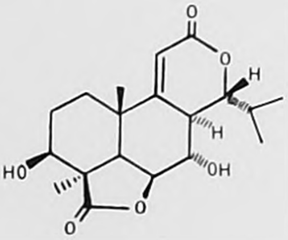


(29)



(30)





(31) (other type)

(32) (other type/inactive)

1.6 References

1. S. Itô, and M. Kodama, Heterocycles, 1976, 4, 595.
2. J.A. Hembree, C. Chang, J.L. McLaughlin, J.M. Cassady, D.J. Watts, E. Wenkert, S.F. Fonseca, and J.P. Campello, Phytochem., 1979, 18, 1691.
3. D.S. Bhakuni, M. Bittner, P.G. Sammes, and M. Silva, Rev. Latinoamer., Quim. 5, 1974, 163.
4. S.K. Arora, R.B. Bates, P.C.C. Chou, W.E. Sanchez L., and K.S. Brown, Jr., J. Org. Chem., 1976, 41, 2458.
5. G.B. Russell, P.G. Fenemore and P. Singh, J.C.S. Chem. Comm., 1973, 166.
6. Y. Hayashi, S. Takahashi, H. Ona and T. Sakan, Tetrahedron Lett., 1968, 2071.
7. Y. Hayashi, Y. Yuki, T. Matsumoto, and T. Sakan, Tetrahedron Lett., 1977, 3637.
8. W.E. Sanchez L., K.S. Brown, Jr., T. Nishida, L.J. Durham, and A.M. Duffield, An. Acad. Brasil Cienc., 1970, 42, Suppl. 77.
9. Y. Hayashi, T. Matsumoto, and T. Sakan, Heterocycles, 1978, 10, 123.
10. H. Hayashi, Y. Yuki, T. Matsumoto, and T. Sakan, Tetrahedron Lett., 1977, 2953.
11. B. Dasgupta, B.A. Burke and K.L. Stuart, Phytochem., 1981, 20, 153.
12. T. Hayashi, H. Kakisawa, S. Itô, Y.P. Chen, and H. Hsû, Tetrahedron Lett., 1972, 3385.
13. S. Itô, M. Kodama, M. Sunagawa, H. Honma, Y. Hayashi, S. Takahashi, H. Ona, T. Sakan, and T. Takahashi, Tetrahedron Lett., 1969, 2951.
14. H. Hayashi, T. Matsumoto, Y Yuki, Sugimotocho, Sumiyoshiku, and T. Sakan, Tetrahedron Lett., 1977, 4215.
15. J.A. Hembree, C. Chang, J.L. McLaughlin, and J.M. Cassady, Experientia, 1980, 36, 28.
16. S.M. Kupchan, R.L. Baxter, M.F. Ziegler, P.M. Smith, and R.F. Bryan, Experientia, 1975, 31, 137.

17. W.H. Watson, paper presented at 12th International Symposium on the Chemistry of Natural Products, Tenerife, Canary Islands, Spain, 21-27th September, 1980.

18. Y. Hayashi, J. Yokoi, Y. Watanabe, T. Sakan, Y. Masuda, and R. Yamamoto, Chemistry Lett., 1972, 759.

19. V. Zabel, W.H. Watson, M. Silva, M. Bittner, and D.A. Langs, Acta Cryst., 1980, B36, 2660.

20. S. Itô, M. Sunagawa, M. Kodama, H. Honma, and T. Takahashi, Chem. Comm., 1971, 91.

21. R.C. Cambie and G.B. Russell, Phytochem., 1973, 12, 2057.

22. S. Itô, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, and O. Honda, Tetrahedron Lett., 1968, 2065.

23. M.N. Galbraith, D.H.S. Horn, J.M. Sasse, and D. Adamson, Chem. Comm., 1970, 170.

24. M.N. Galbraith, D.H.S. Horn, and J.M. Sasse, Chem. Comm., 1971, 1362.

25. M. Kodama, C. Kabuto, M. Sunagawa, and S. Itô, Tetrahedron Lett., 1977, 2909.

26. S. Itô, M. Kodama, M. Sunagawa, M. Koreeda, and K. Nakanishi, Chem. Comm., 1971, 855.

27. M.N. Galbraith, D.H.S. Horn, and J.M. Sasse, Experientia, 1972, 28, 253.

28. M. Silva, M. Bittner, and P.G. Sammes, Phytochem., 1973, 12, 883.

29. G.A. Ellestad, R.H. Evans, Jr., M.P. Kunstmann, J.E. Lancaster, and G.O. Morton, J. Amer. Chem. Soc., 1970, 92:18, 5483.

30. H. Kakisawa, M. Sato, T. Ruo, and T. Hayashi, J.C.S. Chem. Comm., 1973, 802.

31. S. Senoh, S. Imamoto, Y. Maeno, T. Tokuyama, T. Sakan, A. Komamine, and S. Hattori, Tetrahedron Lett., 1964, 3431.

32. A.C. Oehlschlager and G. Ourisson, "Terpenoids in Plants", Ed. G.B. Pridham, Acad. Press. London, New York.

33. K.S. Brown, Jr., and W.E. Sanches L., Tetrahedron Lett., 1974, 675.

34. Y. Hayashi, T. Matsumoto, and T. Tashiro, Gann, 1979, 70, 365.

35. G.A. Ellestad, R.H. Evans, Jr., M.P. Kunstmann, J. Amer. Chem. Soc., 1969, 91:8, 2134.

36. K.S. Brown, Jr., and W.E. Sanchez, Biochem. Syst. Ecol., 1974, 2,11.

37. J.W. Dorner, R.J. Cole, J.P. Springer, R.H. Cos, H. Cutler and D.T. Wicklow, Phytochem., 1980, 19, 1157.

38. Y. Hayashi, T. Matsumoto, T. Hyono, and T. Sakan, Chemistry Lett., 1977, 1461.

CHAPTER 2. CHEMICAL STUDIES OF PODOCARPUS saligna CONSTITUENTS: NON-LACTONE COMPONENTS

2.1 Introduction

Podocarpus saligna D. Don is one of the three species of the genus Podocarpus growing in Chile. The other two are Podocarpus andina and Podocarpus nubigena.

During the last decade these members of Podocarpaceae have been the subject of rather extensive chemical investigation because of their biological activity and also for current examination of Chilean flora.

As a consequence of these studies, various types of compounds such as hydrocarbons, diterpene hydrocarbons, diterpene dilactones, steroids and phenolic compounds such as flavonoids and bisflavonoids have been isolated and their structures determined. In particular, with Podocarpus saligna the chemical studies have mainly been concentrated on the chemistry of the norditerpene dilactones.
Very little work has been done on the other components and only a small number are described in the literature.

2.2 Survey of compounds previously isolated from Podocarpus saligna extracts

All the compounds extracted and identified from leaves and stems of P. saligna mentioned in literature are listed in Table 1, not including the norditerpene dilactones already described in detail in Chapter 1.

Table 1. Compounds previously isolated from Podocarpus saligna D. Don Chile

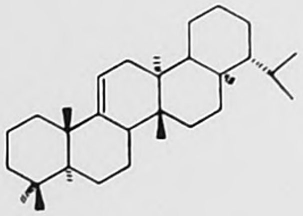
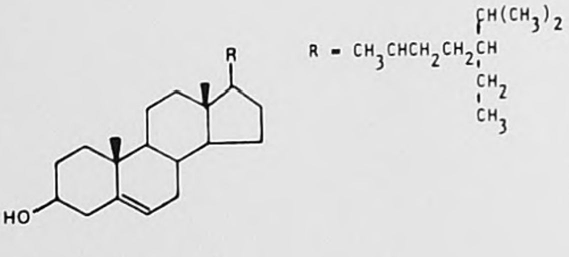
Comp. No.	Structure, name, mp	Extract; plant material	Refs.
1	n-Nonacosane ($C_{29}H_{60}$) mp 56-80°	benzene; leaves	1,2
2	 Fer-9,11-ene ($C_{30}H_{50}$) mp 170-171°	benzene and ethyl acetate; leaves	1,2
3	Isofernene	benzene and ethyl acetate; leaves	1,2
4	 β-Sitosterol ($C_{29}H_{50}O$) mp 132-135°	benzene; leaves	1,2

Table 1. Cont.

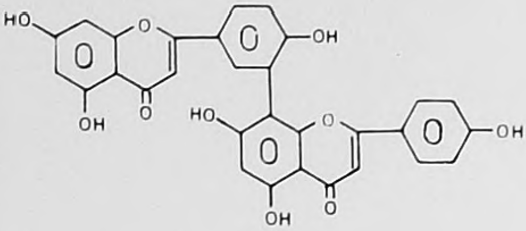
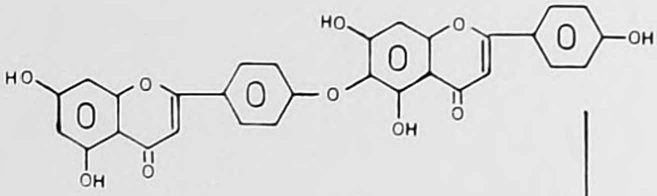
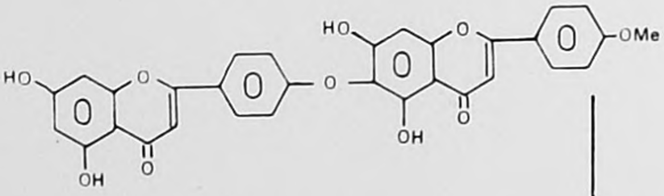
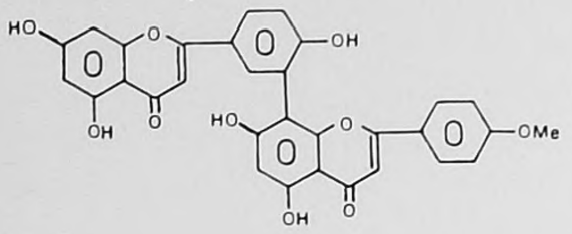
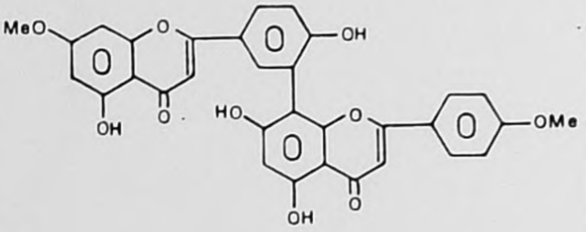
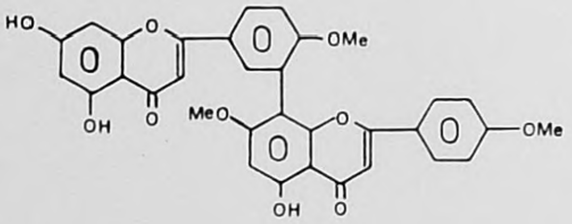
Comp. No.	Structure, name, mp	Extract; plant material	Refs.
5	 Amentoflavone ($C_{30}H_{18}O_{10}$)	ethyl acetate; leaves	2,3
6	 Hinokiflavone ($C_{30}H_{18}O_{19}$) mp 351-354°	ethyl acetate; leaves	2,3
7	 Cryptomerin ($C_{31}H_{20}O_{10}$)	ethyl acetate; leaves	2,3

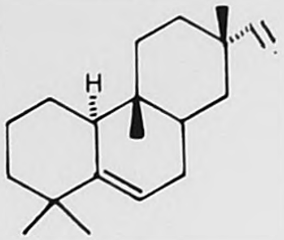
Table 1. Cont.

Comp. No.	Structure, name, mp	Extract; plant material	Refs.
8	 Podocarpusflavone A ($C_{31}H_{20}O_{10}$) mp 231-233°	ethyl acetate; leaves	2,3
9	 Podocarpusflavone B ($C_{32}H_{22}O_{10}$)	ethyl acetate; leaves	2,3
10	 Kayaflavone ($C_{33}H_{24}O_{10}$) mp 310-312°	ethyl acetate; leaves	2,3

2.3 Results and discussion

The petroleum ether extract of Podocarpus saligna was chromatographed on a silica column and furnished three principal components. The least polar of these was the hydrocarbon n-nonacosane, $C_{29}H_{60}$. Its mass spectrum showed a principal $M^{+\cdot}$ at m/e 408 and co-occurring small peaks at m/e 422 and 436, suggesting the presence of small amounts of higher homologues. The fragmentation pattern was entirely in accord with the assignment as a saturated, linear hydrocarbon, being characterised by clusters of peaks at intervals of 14 mass units, the principal peak of each cluster corresponding to a $(C_nH_{2n+1})^+$ ion (393, 379, etc.). The most intense fragments were at m/e 57 and 43. As indicated in Table 1, n-nonacosane has previously been observed in a benzene extract of P. saligna.

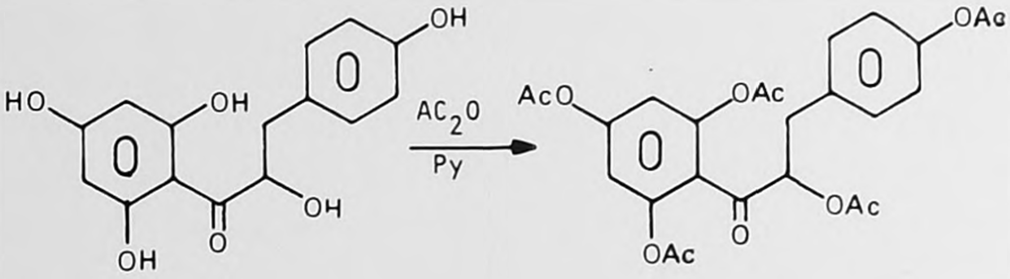
The second compound isolated from the petroleum ether extract was identified as the diterpene rimuene (11). The presence of four methyl singlets and a vinyl group were indicated by the nmr spectrum, which also showed one other olefinic signal. Taken together with the mass spectrum ($M^{+\cdot}$ 272, $C_{20}H_{32}$), this data suggested a tricyclic diterpene. Rimuene has previously been observed in P. andina and P. nubigena[2], as well as other Podocarpus species[9,10]. The identity of the diterpene in the petroleum ether extract of P. saligna was confirmed by matching the mass spectral fragmentation pattern with the published spectrum for rimuene[4,5,6,7] and, finally, by TLC and GLC comparison with an authentic sample of rimuene provided by Prof. Silva in Chile.



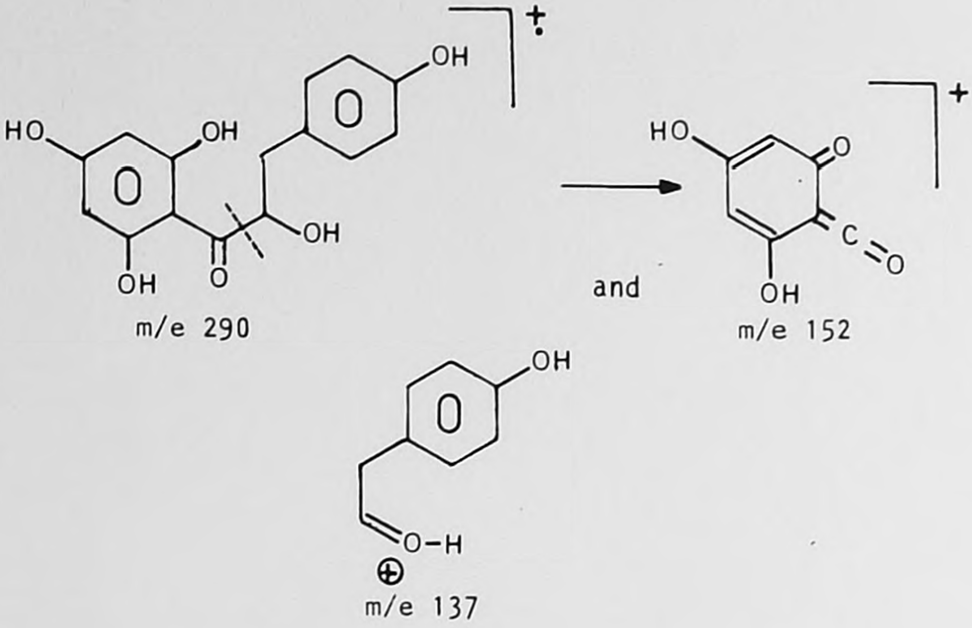
(11)

The third component isolated from the petroleum ether extract was readily identified as hydroquinone, $C_6H_6O_2$. Its mass spectrum showed a molecular ion at m/e 110 and peaks at m/e 82 (-CO) and 81 (-CHO). Its nmr spectrum showed singlets at δ 6.52 (2H) and 8.65 (1H). Finally, a mixed melting point with an authentic sample of hydroquinone showed no depression. Hydroquinone was by far the most abundant component present in the petroleum ether extract and it is therefore surprising that its presence in P. saligna has not previously been reported.

An ethyl acetate extract of P. saligna was subjected to precipitation followed by preparative TLC, yielding a phenolic compound as the major component. This was identified as the α-hydroxydihydrochalcone, nubigenol (12) which has previously been isolated[8] only from another species, P. nubigena, which also grows in Chile. The compound is only very sparingly soluble in organic solvents and its nmr spectrum (d_5-pyridine) was of poor quality and gave little clue, initially, to the structure. The mass spectrum was more informative, showing m/e 290 ($M^{+\cdot}$), a strong peak at 272 ($M-H_2O$) and fragment ions at 152 and 137 due to α-cleavage, consistent with the formulation as nubigenol (Scheme 1). This assignment was further supported by the infrared spectrum, νmax 1630 cm^{-1} in agreement with the presence of an aryl α-hydroxyalkyl ketone. It was confirmed by mp, mixed mp and chromatographic comparison with an authentic sample of nubigenol from Chile. As a further structural proof, the sample was also converted to its pentaacetate by prolonged treatment with acetic anhydride and pyridine. The product (13) had a mp and spectroscopic properties, including nmr, entirely in agreement with the literature values.



(12) (13)



Scheme 1

The chloroform extract contained the most polar components from the P. saligna. Chromatography on a silica column, followed by preparative TLC, yielded several fractions of materials which could be recognised as norditerpene lactones from their general spectroscopic properties. However, no completely pure materials could be obtained by the use of TLC alone and attention was therefore turned to the application of the higher resolution method of HPLC in order to further refine these sub-fractions of the chloroform extract. The results of the analytical and preparative HPLC work will be presented in the next chapter.

2.4 Experimental

Petroleum ether extract. This extract (800 mg) was chromatographed on a silica column (70 g of silica GF_{254} Typ 60) with gradient elution with cyclohexane/THF to yield the following components: n-nonacosane (18 mg) eluted with cyclohexane, mp 57-59^0 (methanol), ν_{max}^{film} 2920, 2874, 1450, 737, 720 cm^{-1}, MS peak at m/e 408 corresponding to $C_{29}H_{60}$. Small peaks also occurred at 422, 436, as well as peaks characteristic of fragmentation of a saturated hydrocarbon, 393, 379, 365, ..., 57, 43.

Rimuene. (10 mg), $C_{20}H_{32}$, eluted with cyclohexane/THF (95:5), mp 55-56°, $\nu_{max}^{CHCl_3}$ 2920, 2850, 1620, 1450, 1375 cm^{-1}. MS showed $M^{+\cdot}$ at 272 and one strong peak at 257 very characteristic of rimuene, other MS peaks at 229, 211, 204, 197, 161, 109, 105, 85, 57, 55. NMR $\delta(CDCl_3)$ 0.67 (S, 3H), 0.95 (S, 3H), 1.02 (S, 3H), 1.08 (S, 3H), 1.38 ($-CH_2$-groups), 4.64 (1H, dd, 1.4, 15.5), 4.96 (1H, dd, 1.4, 9.0), 5.36 (1H broad multiplet unresolved), 5.72 (1H, dd, 9.0, 15.5) ppm. Retention time on TLC (SiO_2 plates, EtOAc/C_6H_{12} eluent) and gas chromatography (OV 101 5% on Porapak) identical to an authentic sample.

Hydroquinone. (87 mg), $C_6H_6O_2$, white needles, mp 173° eluted with cyclohexane/THF (80:20), $\lambda_{max}^{H_2O}$ 288 (3.36), ν_{max}^{nujol} 3140, 2910, 1360, 1195, 835, 760 cm^{-1}. MS $M^{+\cdot}$ 110, 82, 81, NMR δ(DMSO) 6.52 (S, 2H), 8.56 (S, 1H) ppm.

Ethyl acetate extract. 500 mg mixed with ethyl acetate/CH_2Cl_2 afforded 100 mg of white precipitate. This precipitate was further fractionated by preparative TLC (SiO_2 plates), eluent C_6H_6/EtOAC. One of the fractions obtained from TLC (40 mg) presented spectral data and mp very similar to nubigenol $C_{15}H_{14}O_6$. Co-chromatography with authentic nubigenol from Chile showed the same retention time.
mp 168-173° (d)(Lit.[8] mp 168-170°), λ_{max}^{MeOH} 212, 230, 282^{nm} + $AlCl_3$ 288 nm + MeONa 298, 424, + NaOAc + H_3BO_3 283, 287 nm.
ν_{max}^{nujol} 3360, 3260, 1630, 1605, 1510, 1460, 1373, 1285, 1145, 1030, 830, 720 cm^{-1}.
NMR δ(py d5) 3.15 (1H), 3.60 (1H), 4.40 (1H, m), 4.84 (1H) ppm. (very poor spectrum).
MS $M^{+\cdot}$ 290, 274, 272, 168, 164, 152, 149, 137, 121, 107.

Nubigenol pentaacetate. A sample of the free alcohol (30 mg) was acetylated with acetic anhydride and pyridine to give the pentaacetate (48 mg) as an amorphous solid. mp 68-70° (lit.[8] mp 68-70°), λ_{max}^{MeOH} 213, 237, 278 nm.
$\nu_{max}^{CHCl_3}$ 1780, 1730, 1620, 1595, 1485, 1425, 1370, 1235, 1175, 1130 cm^{-1}.

δ($CDCl_3$) 1.95 (1xAcO), 2.32 (4xAcO), 2.90 (2H, m), 5.10 (1H, m) 6.60 (1H), 6.72 (1H), 7.30-7.92 (4H) ppm.
MS small peak at 500, 498, 474, 456, 440, 414, 398, 356, 314, 287, 279, 194, 163, 139, 123,

2.5 References

1. M. Silva, M. Hoeneisen and P.G. Sammes, Phytochem., 1972, 11, 433.
2. D.S. Bhakuni, M. Bittner, P.G. Sammes and M. Silva, Rev. Latinoamer., 1974, Quim.5, 163.
3. H. Miura, T. Kihara and N. Kawano, Tetrahedron Lett., 1968, 2339.
4. R.E. Corbett and S.G. Wyllie, Tetrahedron Lett., 1964, 1903.
5. J.D. Connolly, R. McCrindle, R.D.H. Murray and K.H. Overton, Tetrahedron Lett., 1964, 1983.
6. E. Wenkert, A. Afonso, P. Beak, R.W.J. Carney, P.W. Jeffs and D. McChesney, J.Org. Chem., 1965, 30, 713.
7. R.M. Carman, Aust. J. Chem., 1963, 16, 1104.
8. D. Bhakuni, M. Bittner, M. Silva and P.G. Sammes, Phytochem., 1973, 12, 2777.
9. R.T. Aplin, R.C. Cambie and P.S. Rutledge, Phytochem., 1963, 2, 205.
10. L.H. Briggs and J.A. Loe, J. Chem. Soc., 1950, 958.

CHAPTER 3. HPLC OF NORDITERPENE DILACTONES

3.1 Introduction

High performance liquid chromatography (HPLC) has emerged during the last decade as the foremost technique for the separation of complex mixtures of involatile components[1-3]. It is based upon the principle of using a densely packed column containing a packing material which is closely sized and of small diameter, in order to provide minimum peak broadening. Because such columns by their nature, generate high resistance to solvent flow, the mobile phase is delivered by a high pressure liquid pump, the sample being introduced either through a high-pressure septum injector or by means of a valve-loop system. The eluent stream from the column is passed to a detector which provides a continuous output to a recorder. All unions and connection tubing in the system are designed to have minimum dead-volume to avoid peak broadening. Although all of the modes of liquid chromatography, including ion exchange and molecular exclusion, are currently used in HPLC, by far the most important ones are adsorption (SiO_2) and partition (principally with non-polar bonded phases such as "ODS" - i.e. octadecylsilylated silica). During the last decade there has been a gradual improvement in the quality of packing materials for HPLC columns, which have moved from the pellicular type (e.g. Corasil) through the irregular microparticulates (e.g. 10 and 5 μm Lichrosorb and Partisil) to the spherical microparticulates (e.g. 5 μm and 3 μm Hypersil, Spherisorb, Apex, etc.). The quality and range of detectors has also steadily improved. Initially, refractive index detectors (of limited sensitivity) and fixed-wavelength UV detectors (254 nm) were available, but now the principal types of monitors being used are high-sensitivity electrochemical detectors, variable wavelength UV detectors and fluorescence detectors. Of these, the variable wavelength UV monitors generally provide the widest scope for diverse applications requiring high sensitivity. Considerable progress has also been made in improving the techniques of uniting HPLC directly with mass spectrometry and commercial equipment for doing this is now available.

Whereas analytical HPLC, as described above, has now attained very high levels of performance, both in terms of resolution (50,000 - 100,000 plates per metre being common) and sensitivity (nanomole-picomole being typical for reasonable UV chromophores), preparative HPLC has lagged far behind. During the 1970's, only two commercial manufacturers offered equipment designed for large-scale (multi-gram) operation. Both the Waters Prep 500 and Jobin-Yvon Prep 100 systems were very costly in initial outlay. They differed considerably in the sophistication of mechanical equipment and correspondingly also in the cost of running (the Waters providing faster and better-controlled separations and greater cost). However, both systems were designed for use with relatively coarse grades of silica at rather low operating pressures and compromised heavily on resolution in favour of scale and speed. Experience with these two types of equipment in our own laboratories indicated that operations could only be achieved at a few hundred plates per metre and such resolution is quite inadequate for tackling complex mixtures of natural products.

Numerous applications of HPLC to natural products of many classes have been reported in recent years for example, by Nakanishi[4,5]. Most of these have been concerned either with analysis or with small-scale preparative work and there are very few examples in the literature of the careful fractionation of large quantities of crude extracts by true preparative HPLC. To date, no HPLC work with nor-diterpene dilactones has been published to our knowledge.

3.2 Experimental techniques

The analytical HPLC equipment used in this work included a Waters 6000M pump, a Rheodyne 7125 valve-loop injector and a Cecil 2012 variable wavelength UV detector fitted with an 8 μl flow cell. Columns of silica gel were packed by high-pressure slurry techniques, as described in the literature[6].

The selection of the adsorption chromatography mode was based upon the consideration that it would be necessary to scale-up to multigram quantities on large columns for preparative work. High-efficiency grades of silica are much more readily available, and considerably cheaper, than the corresponding grades of bonded phases.

The choice of solvents for this work was dictated first of all by the requirement of detection. In order to be certain that no nor-diterpene lactones would be missed, it was necessary to select a common wavelength for all three subgroups, namely the λ_{max} of the subgroup B at around 220 nm. This rather short wavelength places severe restrictions on the choice of solvents, limiting it to those which are transparent in this region - principally hydrocarbons (e.g. hexane), saturated alcohols (e.g. isopropanol), ethers (e.g. Et_2O) and acetonitrile. Within these groups, it was necessary to find solvent combinations which would:

(i) provide an appropriate polarity to give reasonable retention times and sharp peaks and

(ii) provide some flexibility so that small adjustments could be made in a reasonably predictable way.

After numerous trials, it was concluded that binary solvent systems (e.g. hexane-acetonitrile) were inadequate and failed to give sufficient resolution or sharpness of peaks. Further trials with ternary solvent combinations eventually led to the adoption of a mixture of hexane, acetonitrile and isopropanol. This combination provides a chromatogram of good resolution for complex mixtures of the dilactones, with the isopropanol being important to reduce tailing, and its composition can easily be varied to provide subtle adjustments in resolution as the process of refining the crude mixture progresses.

The problem of preparative HPLC was tackled by assembling our own equipment to try to achieve high resolution. A preparative column was made by packing a 1" i.d. x 45 cm long steel tube with Lichroprep silica (15-25 μm), which was fitted with commercially available end unions. A Metering Pumps Ltd E1 Metripump was used for solvent delivery, giving a typical flow rate of ca. 40-45 ml/min at 1000 psi. Detection was accomplished by using the Cecil 2012 monitor, fitted with a 1 mm preparative flow cell. This allowed the high flow rates to be used without difficulty and also provided a reduction in signal intensity by a factor of 10 compared with the analytical flow cell (10 mm). Further "backing off" to overcome the problem of detector overload was readily achieved by using the variable wavelength control to "tune out" any peaks which became too intense. General experience with this type of preparative system, both in this natural product area and in other fields such as steroids (see Chapter 9) indicated that the system could provide separations at efficiencies of several thousand plates per metre and with a loading capacity in the 0.5 g - 1 g range.

3.3 Results and discussion

We were originally provided, in 1978, with a series of extracts of the leaves and stems of P. Saligna, prepared in Professor Mario Silva's laboratory in Concepcion, Chile. As described in Chapter 2, the chloroform extract (1 g) was rich in norditerpene lactones but only partial separations of these could be obtained by classical chromatographic techniques.

Subsequently, in 1980, we were provided with a fresh ethanol extract of P. saligna leaves and stems. Preliminary HPLC analysis of this total, crude extract showed only small amounts of norditerpene lactones to be present. This extract was then distributed between water and chloroform and after concentration, the new chloroform extract was again examined by HPLC. This revealed that the norditerpene lactones had been concentrated in this phase.

Figure 1 shows the HPLC analyses of the 1978 chloroform extract and the 1980 chloroform extract. The earlier extract was analysed on Lichrosorb SI60 10 μm irregular SiO_2, whereas the 1980 extract was

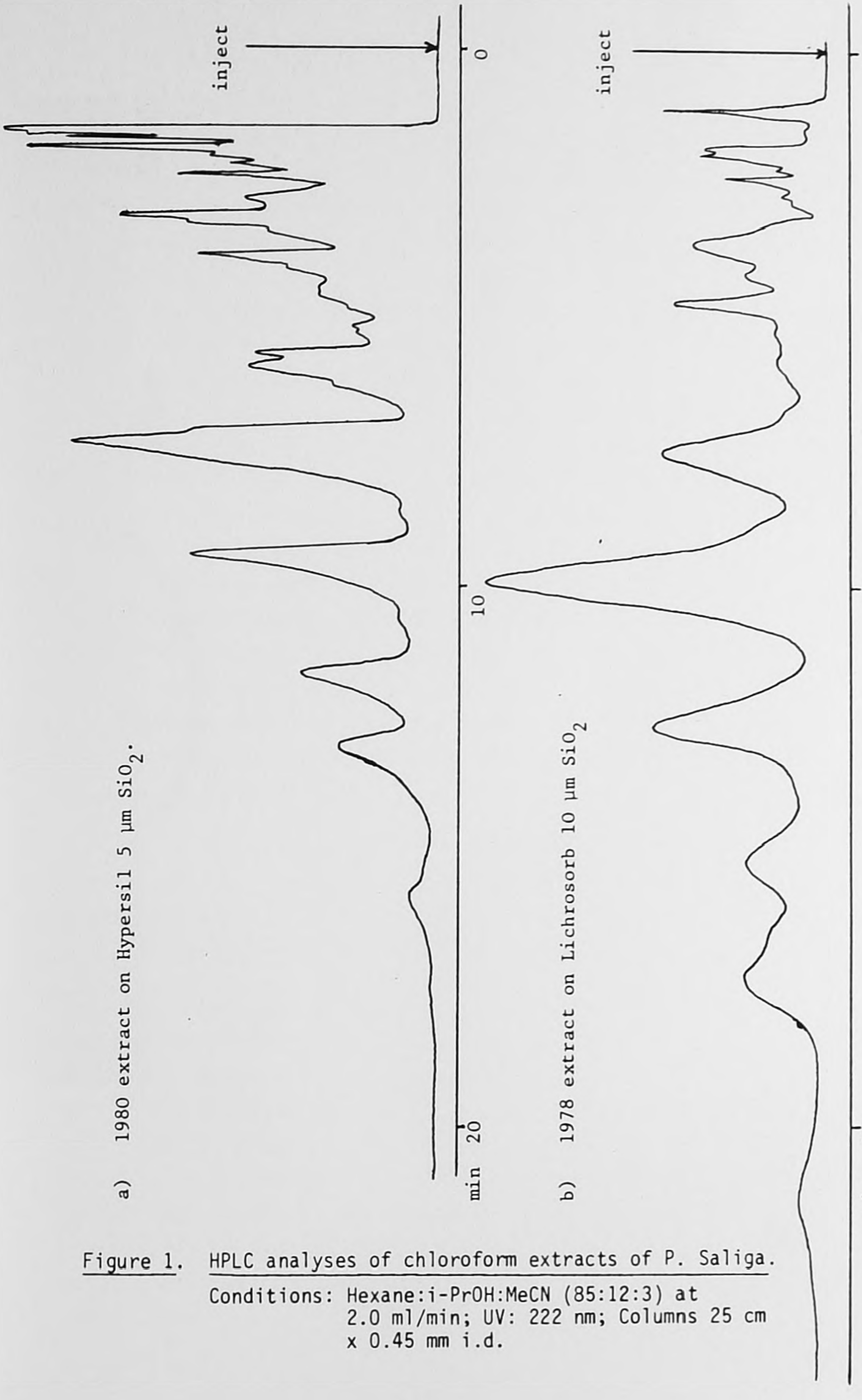


Figure 1. HPLC analyses of chloroform extracts of P. Saliga.

Conditions: Hexane:i-PrOH:MeCN (85:12:3) at 2.0 ml/min; UV: 222 nm; Columns 25 cm x 0.45 mm i.d.

analyzed on Hypersil 5 µm spherical SiO_2. Both analyses were performed using the same mobile phase of hexane : isopropanol : acetonitrile, 85:12:3, which had been developed during the preliminary optimization investigations. The two results are interesting to compare in detail:

(i) Despite the slightly greater polarity of the Lichrosorb column, leading to greater retention times, the Hypersil column shows, as would be expected, better resolution.

(ii) Apart from the differences related to performance of the analytical columns, the two traces are remarkably similar, considering that they represent samples prepared separately more than two years apart and by somewhat different procedures.

(iii) Assuming that all the peaks are due to norditerpene lactones (which may well be untrue), there are evidently more than twenty of these compounds present.

(iv) With the exception only of two or three of the most polar components, resolution in this chromatographic system is inadequate for a single-stage separation. It is not at all surprising that preparative TLC failed to provide complete separations.

Preparative HPLC work was then commenced, both to further refine the partially purified sub-fractions obtained from the original (1978) chloroform extract and also to effect a step-wise HPLC fractionation of the later (1980) chloroform extract.

In carrying out these preparative HPLC purifications, it was necessary to adapt and modify the standard mobile phase conditions in order to improve separations. Analytical HPLC columns typically operate at a plate count very considerably greater than preparative columns (100 times for commercial systems such as Waters and Jobin-Yvon, 10 times for our own system) because of factors related to particle diameter and phase overload. Consequently, the best possible baseline separations must be obtained on the analytical column if adequate resolution is to be maintained on transferring to the preparative one.

One example illustrating this process will be given. A portion of the 1978 chloroform extract was treated with a mixture of chloroform and methanol (1:1), which caused precipitation of a white solid. HPLC analysis of this solid (Figure 2a) in the standard solvent system revealed it to be a somewhat simplified mixture of several norditerpene lactones. The separation was then "stretched out" very considerably by lowering the content of polar components in the mobile phase to give a chromatogram (Figure 2b) much more suitable for transfer to the preparative column.

After each preparative run, the fractions collected were immediately re-analyzed and those which were impure were re-submitted to further preparative HPLC. Typically, 4-6 repeated runs were sufficient to break down the original complex mixture into individual components having purities greater than 95%, as established by analytical HPLC.

Eventually, roughly one dozen of individual compounds were isolated from the preparative HPLC process. In some cases, a few milligrams of material were obtained; in many other cases, only sub-milligram amounts emerged from the laborious process of repeated fractionation. Each compound was then submitted to as many spectroscopic techniques as possible: generally, IR, UV and mass spectrometry. In most cases, the amount of material was too small for ^{1}H NMR on the machine available in our Department (a Jeol MH100 continuous wave NMR). This problem was eventually solved by submitting the samples to 400 MHz ^{1}H NMR on the much more sensitive Fourier Transform machines with superconducting magnets, first in Cambridge and later in Sheffield.

The results of the spectroscopic investigations of the isolated compounds are reported in the next Chapter.

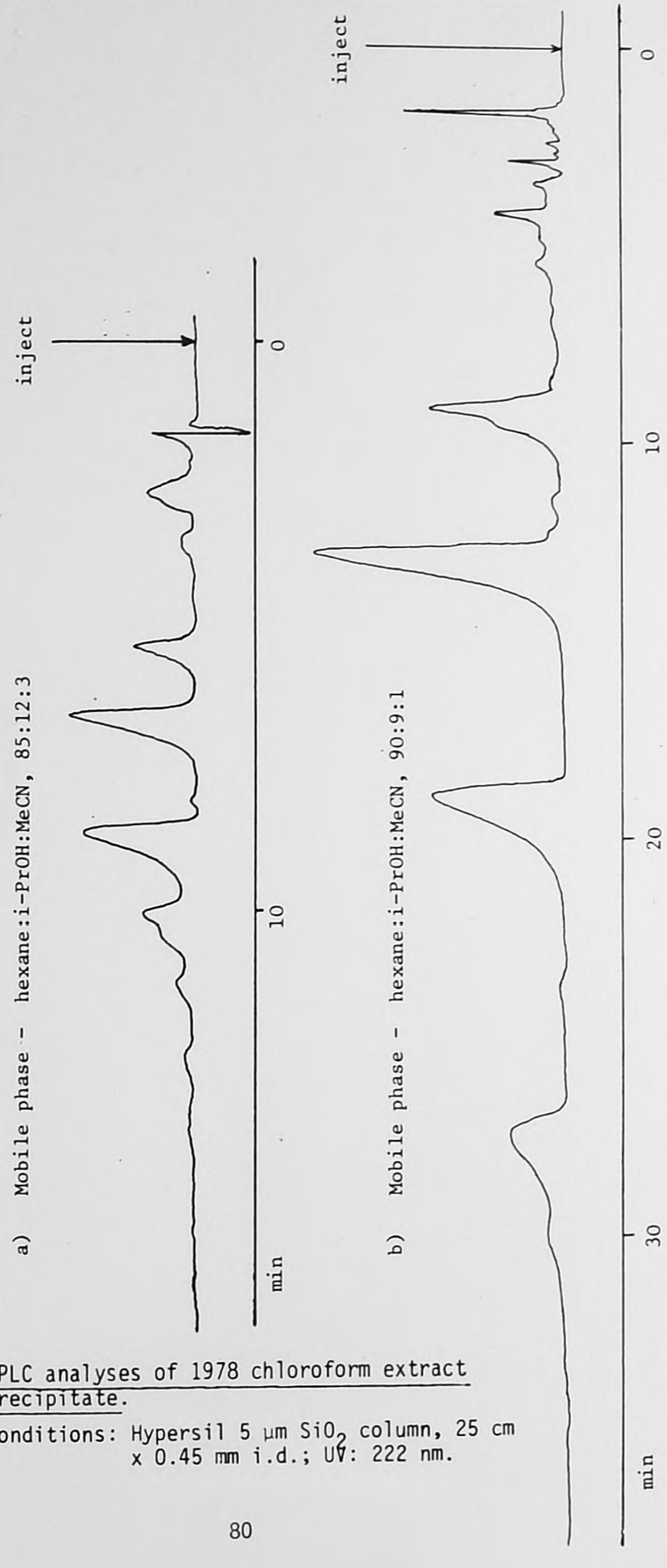


Figure 2. HPLC analyses of 1978 chloroform extract precipitate.

Conditions: Hypersil 5 μm SiO_2 column, 25 cm x 0.45 mm i.d.; UV: 222 nm.

3.4 References

1. W.A. Court, J. Chromatog., 1977, 130, 287.
2. R.L. Rouseff and S.V. Ting, J. Chromatog., 1979, 176, 75.
3. D. Strack, K. Fuisting and G. Popovici, J. Chromatog., 1979, 176, 270.
4. M.A. Adams and K. Nakanishi, J. Liq. Chromatog., 1979, 2, 1097.
5. M.J. Pettei, F.G. Pilkiewicz, and K. Nakanishi, Tetrahedron Lett., 1977, 2083.
6. P.A. Bristow, J. Chromatog., 1978, 149, 13.

CHAPTER 4. STRUCTURE ELUCIDATION OF NORDITERPENE DILACTONES

4.1 Literature methods for structure elucidation

As indicated in Tables 1-3 in Chapter 1, fifteen of the norditerpene dilactones have had their structures confirmed by X-ray chrystallography. This is less than one third of the fifty or so compounds currently known and moreover eight of these X-ray determinations (salignones A-H) have only been accomplished in the last two years by Prof. William Watson[1] in Texas, in collaboration with our co-workers in Chile, and the majority of these eight structures remain so far unpublished.

The first X-ray structures, which emerged[2] in the period 1972-1976, caused several revisions to be made to previous structures, and by implication several other structures in the literature were also affected. Consequently, much of the early chemistry and many of the original spectroscopic assignments of norditerpene lactones are erroneous and great care must be exercised in interpreting the early literature. The structures shown in Tables 1-3 of Chapter 1 represent the most recent, revised versions as far as possible, and may differ significantly (especially in sterochemistry and in the location of ring A epoxide and hydroxyl groups) from the structures quoted in some papers.

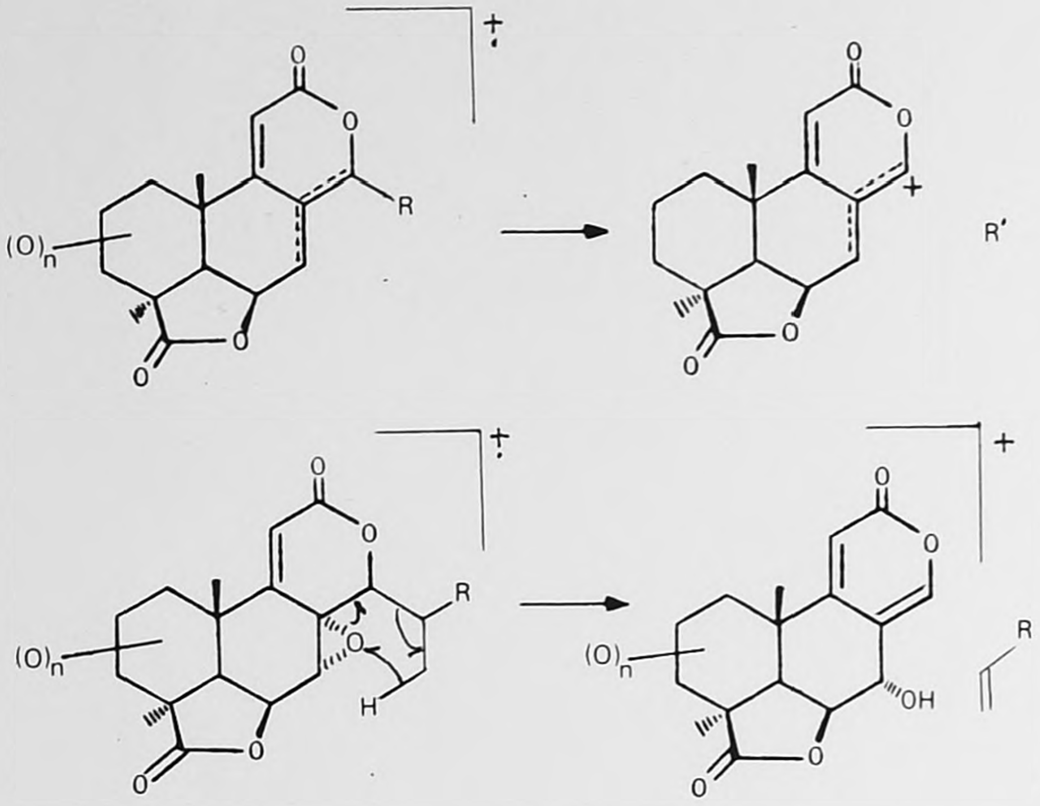
Much of the structural confusion has arisen from the fact that the ^{1}H NMR spectra are very complex and, with the older, low-resolution machines, difficult to interpret. The emergence of high-field NMR and of sophisticated NMR methods of spectral simplification (n.O.e, difference techniques, 2-dimensional J spectroscopy, etc.) has made the assignment of spectra much more reliable in the last couple of years.

The general class of diterpene dilactone derivatives is readily recognized by IR spectroscopy, which shows bands at ν_{max} 1780-1760 cm^{-1} (γ-lactone) and 1730-1700 cm^{-1} (δ-lactone). A compound can be placed into one of the three sub-groups on the basis of its characteristic UV spectrum, which may show λ_{max} (EtOH) 300 nm (ε 5,000-10,000) for sub-group A; 220 nm (7,000-13,000) for sub-group B; or 260 nm (10,000-25,000) for sub-group C.

^{1}H NMR spectroscopy provides the major source of detailed structural information. The nature of the side-chain attached to C-14 is easily recognised by characteristic signals, e.g. a large doublet at δ1.1 for the $-CHMe_2$ group; a doublet at δ1.6 and singlet at δ3.6 for the $-CH(Me)CO_2Me$ group; multiplets at δ5.5 and δ6.1 for the $-CH=CH_2$ group. The presence of hydroxyl and epoxy groups can be inferred from characteristic signals at around δ4.0 ($-C\underline{H}(OH)$) and δ3.3-3.7 (-CH in epoxide rings).

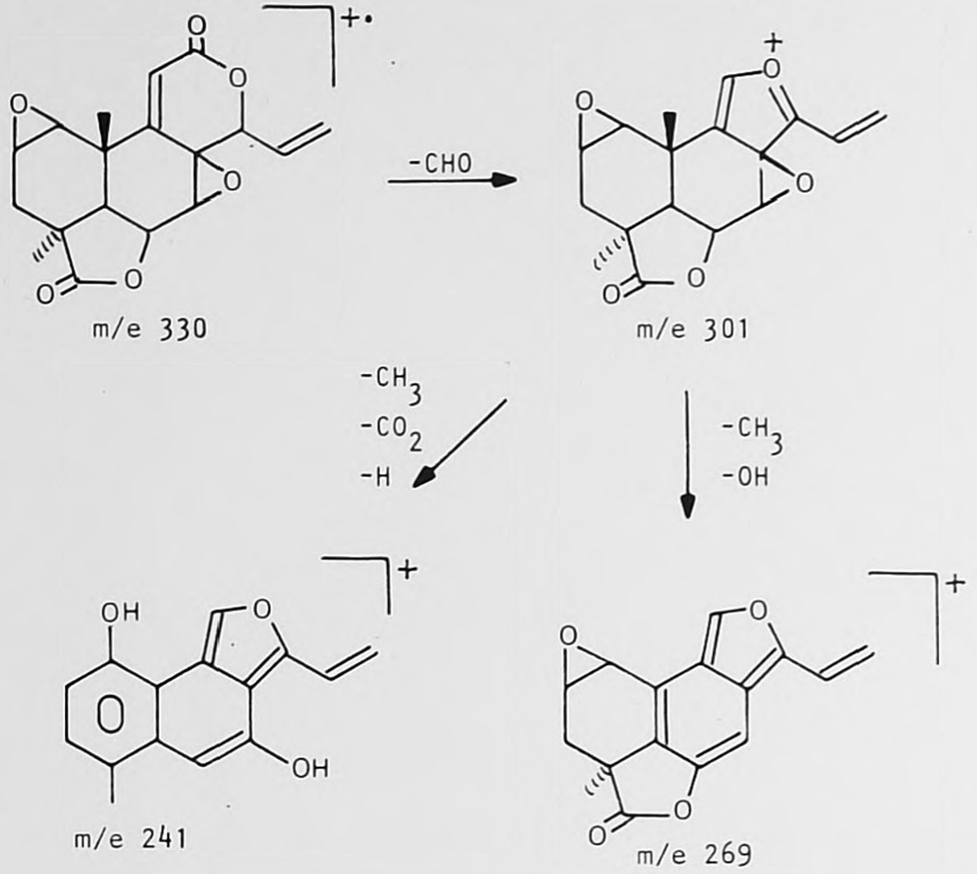
Mass spectrometry has been an important technique for establishing the molecular weight and formula of diterpene dilactones. Unfortunately, this class of compounds frequently fails to produce molecular ions with the standard electron impact (EI) method (70 eV). The use of lower energy EI (20 eV) gives only limited improvement. Surprisingly, there has been no attempt reported in the literature so far to apply the new "soft" ionization techniques such as chemical ionization (CI) or field desorption (FD), which would be expected to solve these problems.

No systematic correlation of the mass spectral fragmentation of the dilactones has even been published. The principal use made of mass spectra in the literature has been to confirm the nature of the C-14 side-chain, which normally cleaves off readily to form a first fragment from the molecular ion, of odd mass value. In cases where there is a 7,8-epoxy group the fragmentation takes place with a H-transfer[3,4] from the side-chain (McLafferty type process), leading to an ion of even mass value (Scheme 1).



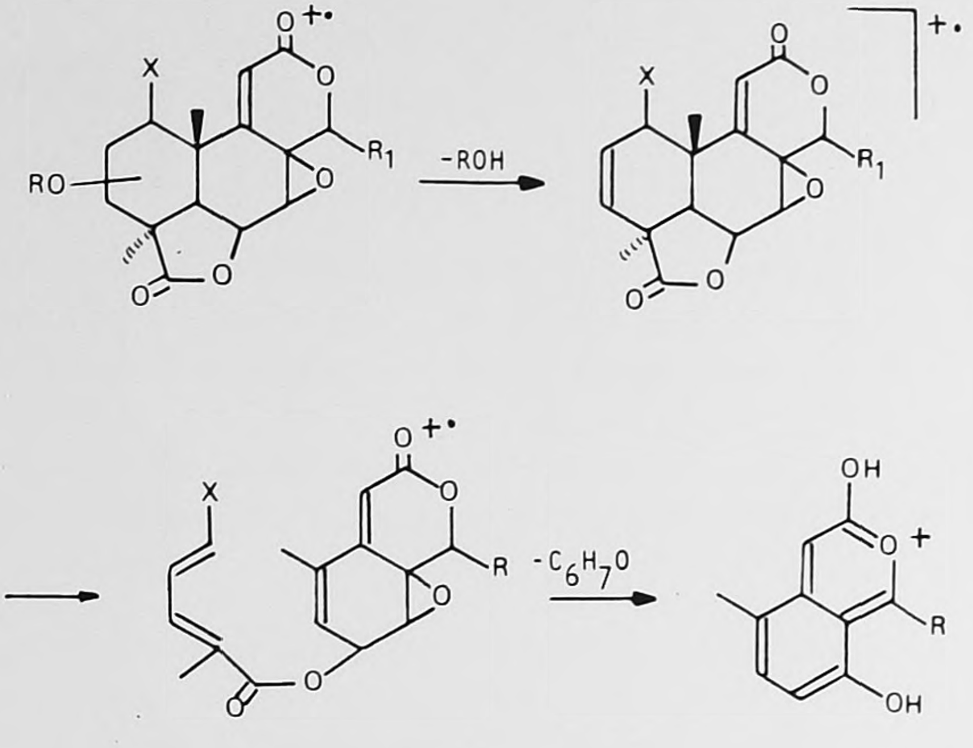
Scheme 1

Losses of 29 mass units from molecular ions frequently occur, and have been attributed to the elimination of CHO from ring C, leading to an ion with a 5-membered ring. This may be followed by further losses of the C-20 methyl group and other substituents to give ions of increasing aromaticity[4] (e.g. Scheme 2).



Scheme 2

Brown and Sanchez[5] suggested a fragmentation process involving a retro Diels-Alder cleavage (Scheme 3) for compounds carrying a hydroxy or acyloxy group at C-2 or C-3 in the A-ring.



Scheme 3

Only recently has an extensive study of the ^{13}C NMR diterpene dilactones been published[6], although sporadic reports of data on a few compounds had appeared earlier[7,8,9,10,11]. Although it is clear from these reports that ^{13}C spectra can, in principle, play a useful role in confirming the presence of structural elements (C=O, epoxide, vinyl, etc.), in practice the real applications of this technique have been very limited so far. This is because of the need for a greater sample size in ^{13}C than in 'H NMR (a factor which prevented our own use of the ^{13}C technique).

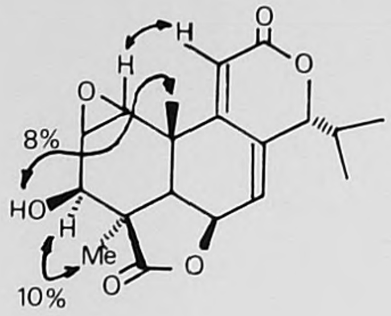
Chiroptical properties of norditerpene lactones have occasionally been reported and have been used in particular to establish the absolute configurations of some members of the series[4]. Since we have not had the opportunity to use this technique in our own work, because of the low quantities of materials isolated, this area will not be reviewed in any detail. However, it is worth summarizing the literature results by pointing out that all of the published work shows the same absolute configuration for all the compounds studied.

4.2 Nuclear Overhauser enhancement in ^1H NMR

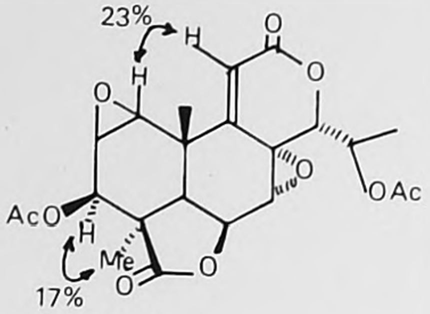
The observation of nuclear Overhauser enhancement (n.O.e) effects has played a useful role in confirming details of the structures of norditerpene lactones. However, some erroneous stereochemical inferences have been made about the orientation of 1,2-epoxy groups, based on the observation of large n.O.e.'s between H-1 and H-11. Those instances of n.O.e.'s reported in the literature are shown in Figure 1.

In the classical n.O.e. experiment, irradiation of one proton causes an increase in the signal intensity for another proton which is close to it in space. When conventional techniques of signal integration are used, it is normally not possible to detect n.O.e. effects below the level of about 5-10%, because of the limited accuracy of NMR integrators. This has, until recently, been the major factor limiting the more widespread application of n.O.e. effects in structure elucidation. It is interesting to note that all of the effects illustrated in Figure 1 are above 8%.

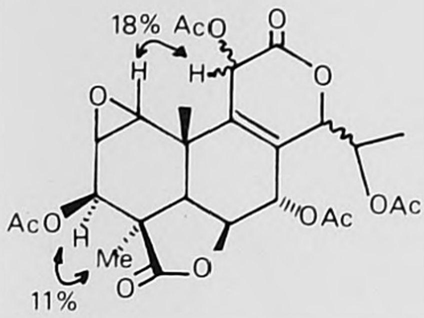
Recently, Dr. Jeremy Sanders of Cambridge University, in collaboration with L.D. Hall of the University of British Columbia, has developed[16,17] a very refined and sensitive technique for measuring n.O.e. effects, which makes use of difference spectroscopy (Figure 2). A spectrum is recorded (pulsed FT ^1H NMR) with a second irradiating frequency in a "blank" portion of the spectrum (Figure 2a). This irradiating frequency is then moved under the first proton and the spectrum re-recorded (Figure 2b). The two spectra are then subtracted by computer giving the difference spectrum (Figure 2c), which may initially be very weak (i.e. poor signal/noise ratio). However, by repeating this cycle of events many times, the difference spectrum can be accumulated so as to give a good signal/noise ratio (Figure 2d).



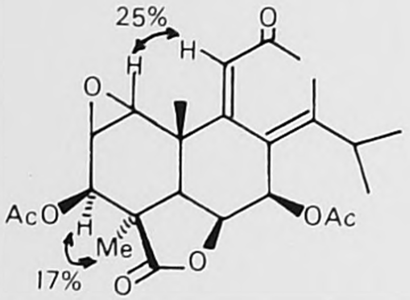
Ref. 13.



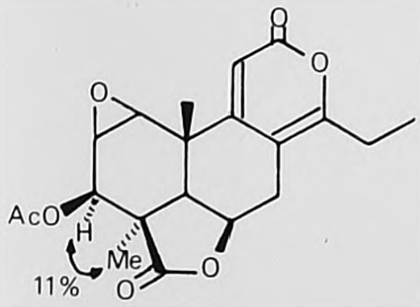
Refs. 12, 4.



Ref. 14.



Refs. 15, 4.



Ref. 15.

Figure 1

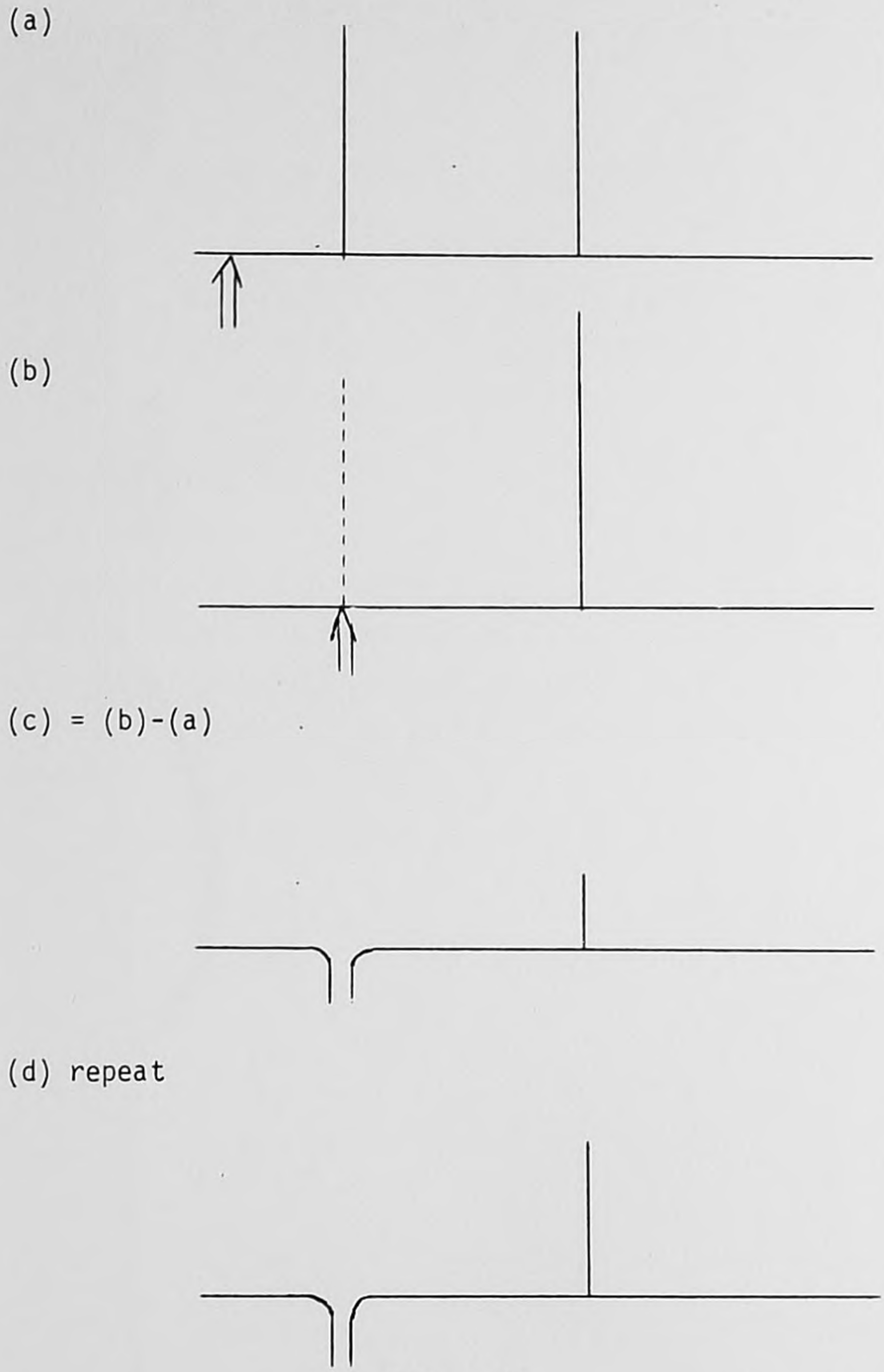


Figure 2

Using this technique, extremely small n.O.e. effects can be detected easily, down to levels well below 1% enhancement[18,19,20].

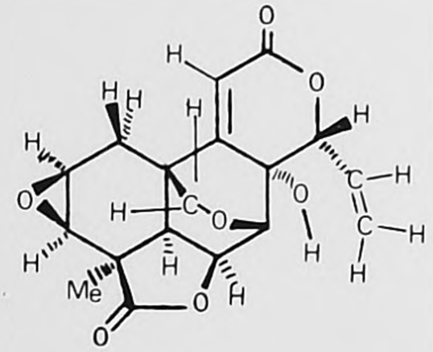
We have made extensive use of this new technique of nuclear Overhauser enhancement difference spectroscopy (n.O.e.d.s.) in our own structure elucidation studies. These are described in the following section.

4.3 Structure determination of compounds isolated

Each of the compounds isolated by preparative HPLC of the P. saligna extracts (Chapter 3) was submitted to as many as possible of the available spectroscopic techniques. The assignments of structures on the basis of the data obtained are presented below.

Compound A, mp 280-289°C

This compound (M^+ 346, $C_{18}H_{18}O_7$) was classified as belonging to sub-group B by its UV spectrum, λ_{max} (EtOH) 218 nm (ε 15,000). Its 400 MHz ^1H NMR spectrum was recorded in d_5-pyridine (Figure 3) and extensive decoupling and n.O.e. difference measurements were performed (Table 1), allowing the structure (1) to be assigned to compound A.



(1)

This structure notably contains an oxymethylene bridge on the β-face between C-10 and C-7. The two prochiral hydrogens of the bridging methylene group provide useful structural probes. The pro-S hydrogen (H-20S, 2.98δ) shows a long-range (W) coupling to H_5. It also has an n.O.e. interaction with H-14, thus defining the α-orientation of the vinyl group attached to C-14. The α-orientation of the C-8-OH is revealed by its n.O.e.'s with H-6 and H-7.

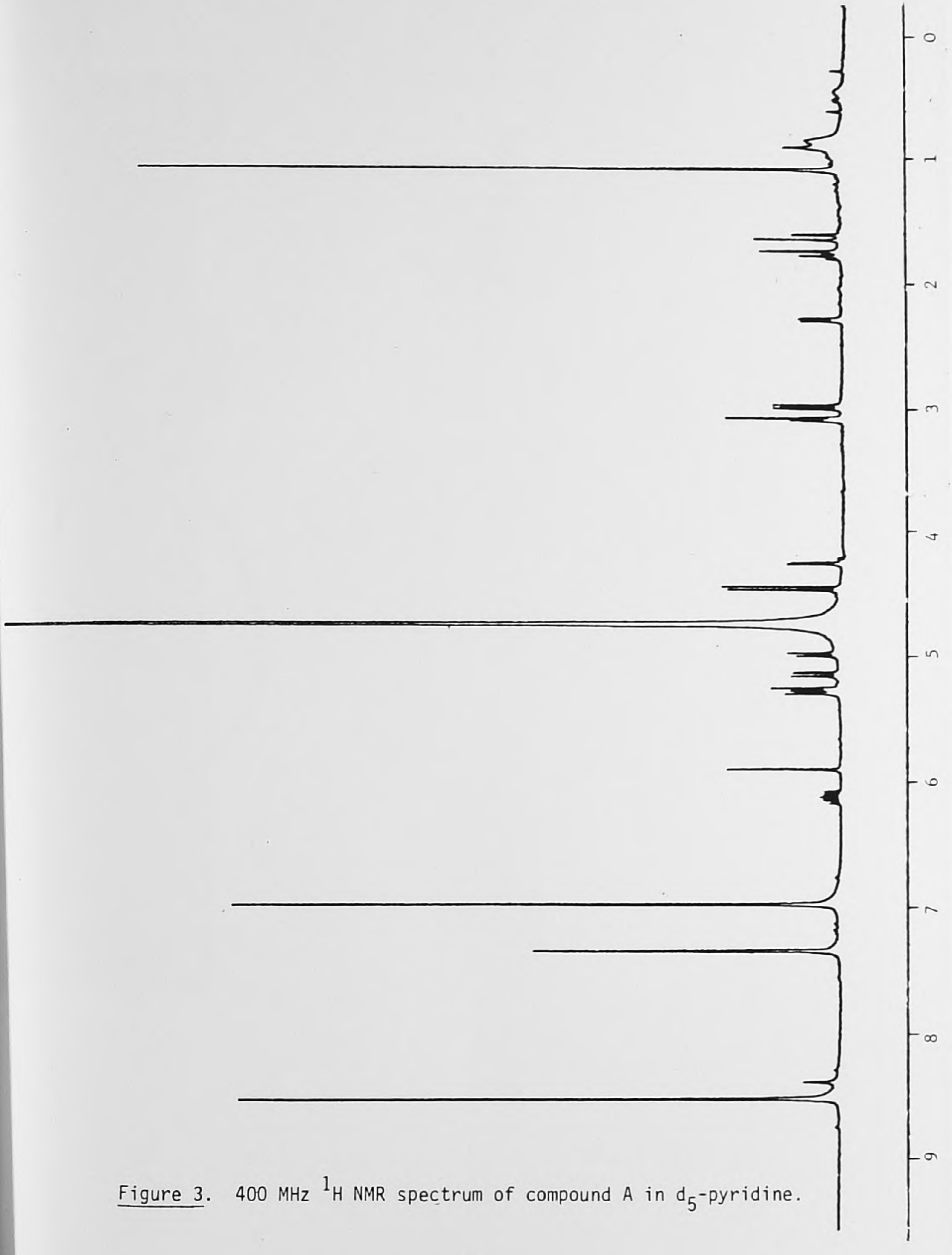
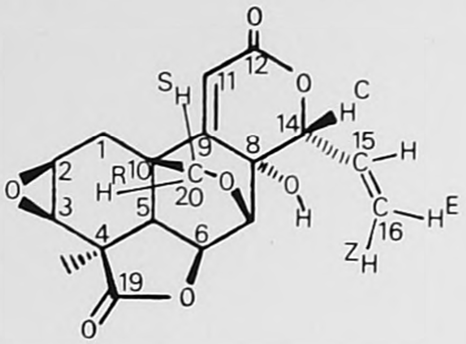


Figure 3. 400 MHz ^{1}H NMR spectrum of compound A in d_5-pyridine.

Table 1. 400 MHz ^1H NMR spectrum of compound A in d_5-pyridine.



Proton	δ/ppm	multi-plicity	J/Hz	n.O.e
1α	1.64	dd	$J_{1\alpha,1\beta}$ =15.5 $J_{1\alpha,2\alpha}$ = 1.5	1β,2,5,11
1β	1.75	dd	$J_{1\beta,1\alpha}$ =15.5 $J_{1\beta,2\alpha}$ = 3.0	1α,2,11,20S
2α	3.10[a]	m	$J_{2\alpha,1\alpha}$ = 1.5 $J_{2\alpha,1\beta}$ = 3.0 $J_{2\alpha,3\alpha}$ = 3.9	} 1α,1β,18,20R
3α	3.10[a]	m	$J_{3\alpha,2\alpha}$ = 3.9	
5α	2.30	dd	$J_{5,6}$ = 7.3 $J_{5,20S}$ = 2.2	1α,6,18
6α	5.29[b]	m	$J_{6,5}$ = 7.3 $J_{6,7}$ = 3.7	(5,7,18)
7α	4.27	bd	$J_{7,6}$ = 3.7 $J_{7,11}$ = c	6,14,15
8-OH	8.37	bs		5,6,15
11	5.90	bs	$J_{11,7}$ = c	1α,1β,20S
14β	4.97	d	$J_{14,15}$ = 8.0	7,15,16Z
15	6.13	ddd	$J_{15,14}$ = 8.0 $J_{15,16Z}$ =17.3 $J_{15,16E}$ =10.5	14,16Z(-ve),16E
16Z	5.29[b]	dt	$J_{16Z,15}$ =17.3 $J_{16Z,16E}$= 1.7	(15,16Z)
16E	5.15	dd	$J_{16E,15}$ =10.5 $J_{16E,16Z}$= 1.7	7,15,16Z
18	1.10	s		3,6
20R	4.46	d	$J_{20R,20S}$= 9.8	20S
20S	2.98	dd	$J_{20S,5}$ = 2.2 $J_{20S,20R}$= 9.8	1β,11,14,20R

Notes: a) and b) pairs of signals irradiated together since superimposed.

c) unresolved coupling present.

The pro-R hydrogen (H-20R, 4,46δ) receives an n.O.e. on irradiation of the 2,3-epoxy group protons. Initially, this appears to suggest that the orientation of the epoxy group is α. However, on examining molecular models it becomes apparent that there is very little change in the spatial positions of the H-2 and H-3 protons between the α- and β-epoxy configurations and both isomers would be expected to show n.O.e.'s to H-20R. The α-epoxy isomer should show slightly bigger n.O.e. effects than the β-epoxy isomer, but in the absence of both possible compounds for close comparison, it was not possible to decide from the n.O.e. experiments which isomer we were dealing with. This revealed a limitation of the n.O.e. approach to solving stereochemical problems.

Fortunately, the stereochemistry of the epoxide group can be determined from the observed coupling constants. In compounds containing a 2,3-epoxy group with β-orientation (e.g. Sellowin A, Podolactone A, Podolactone C), the 1α- and 1β-protons both show characteristically small couplings (⩽2.5 Hz) to the 2α-proton. No naturally occurring dilactone having a 2,3-epoxy group with α-orientation has hitherto been isolated, but Hayashi et al[21] have prepared two examples by the epoxidation of 2,3-olefinic dilactones. In both these compounds, the observed couplings (in d_5-py) were $J_{1\alpha,2\beta}$=1.5 Hz, $J_{1\beta,2\beta}$=6.0-6.5 Hz. These observations are consistent with the geometries revealed by molecular models, which show that in the 2,3-β epoxide the 2α-proton sits approximately gauche to both the 1α- and 1β-protons; whereas in the 2,3-α epoxide the 2β-proton eclipses the 1β-proton and sits at a dihedral angle of about 120^0 to the 1α-proton, thus giving rise to two distinctly different coupling constants.

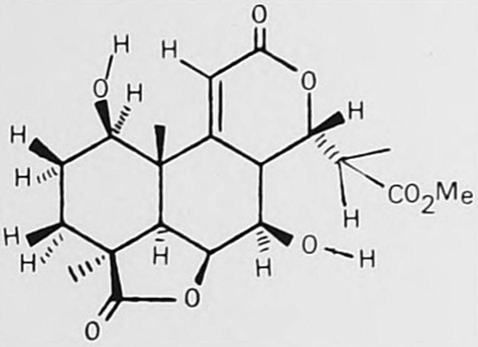
In compound A, the observed coupling constants (Table 1) of 3 Hz and 1.5 Hz are consistent with a 2,3-epoxy group having β-orientation, and hence the structure of compound A is that shown in (1).

During this work, we were made aware by Prof. Silva that a sample sent by him to Texas had been characterized by Prof. Watson as

Salignone A, using X-ray crystallography. Prof. Watson kindly provided the residue of his X-ray sample and on running its 400 MHz ^{1}H NMR spectrum it proved to be identical to the compound A which we had isolated. Thus, compound A is securely identified as Salignone A (1) and it can be seen that the use of high-field NMR together with decoupling and n.O.e. difference spectroscopy is a technique which rivals X-ray crystallography in its power for structural elucidation.

Compound B, mp 233-240°C

This compound was classified into sub-group B by its UV-spectrum, λ_{max} (MeOH) 224 nm (ε11,000). The 400 MHz ^{1}H NMR spectrum in d_5-pyridine is shown in Figure 4 and the results of decoupling and n.O.e.d.s. are summarized in Table 2. These results allow the structure to be assigned unambiguously as (2).



(2)

The -CH(Me)CO_2Me side-chain was readily recognized by the presence of a characteristic doublet at δ1.12 and a singlet at δ3.33 for the two methyl groups. An n.O.e. at the C-20 methyl on irradiation of H-14 places these two in a cis-relationship and hence the C-14 side-chain is α. The 7-OH group is defined as β by its n.O.e. effects on M-14 and Me-20. The 1-OH group gives an n.O.e. at Me-20, whilst the 1-H gives an n.O.e. to the 5α-H and hence the 1-OH group has a β-orientation.

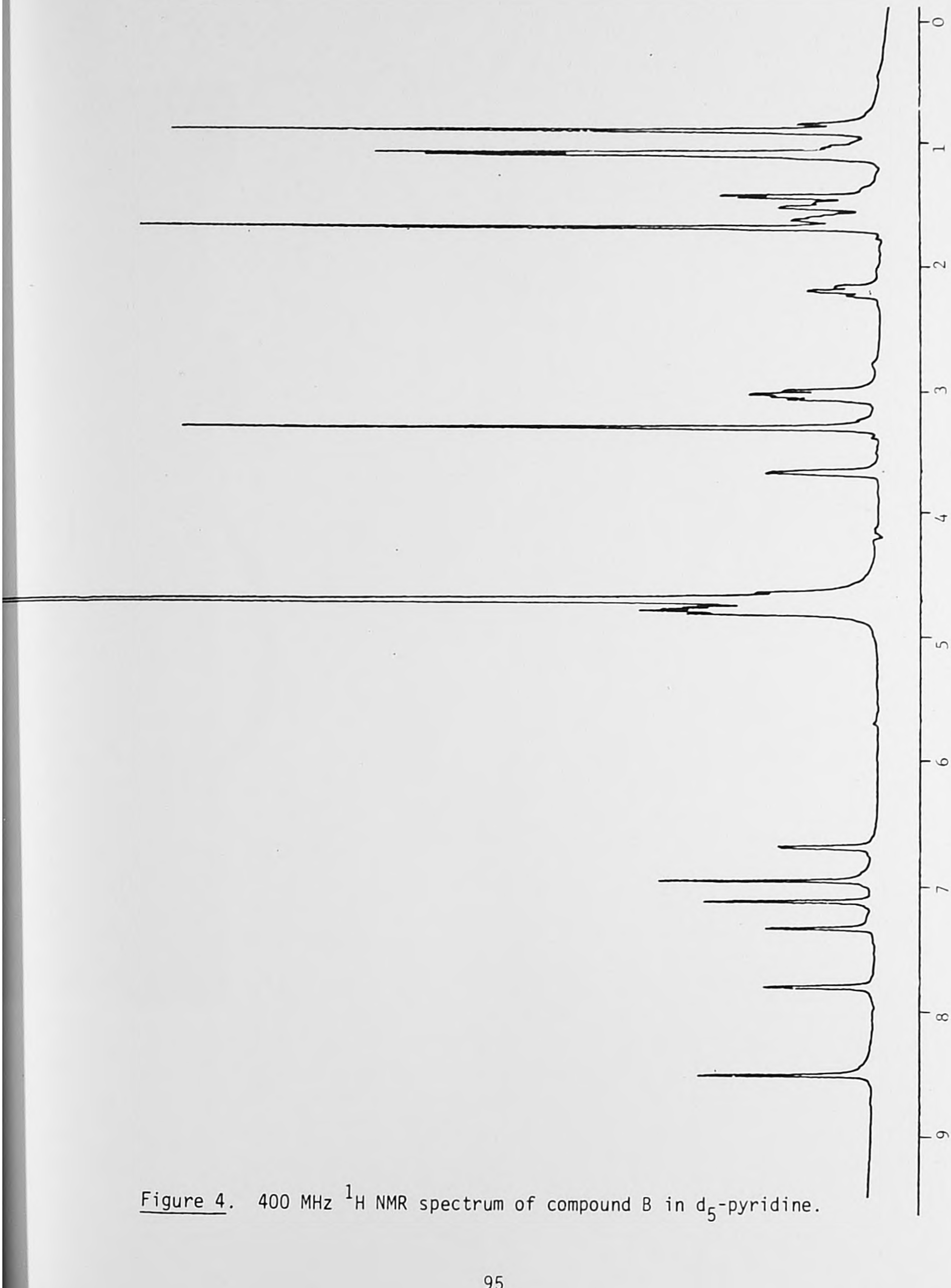
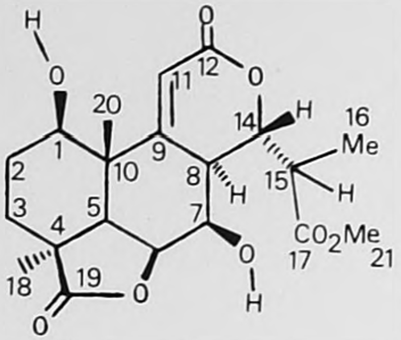


Figure 4. 400 MHz ^{1}H NMR spectrum of compound B in d_5-pyridine.

Table 2. 400 MHz ^{1}H NMR spectrum of compound B in d_5-pyridine

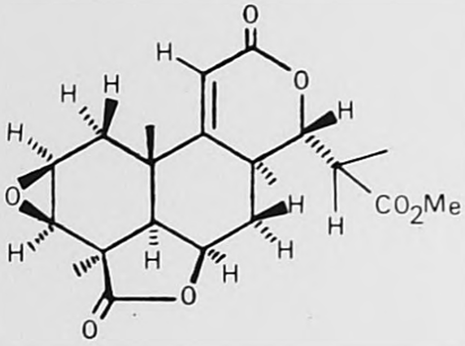


Proton	δ/ppm	multi-plicity	J/Hz	n.O.e.
1α	3.65	d	$J_{1\alpha,2\alpha} = 7$	2α,2β,5,11
1-OH	6.69	bs		1α,2β,11,20
2α	1.67	ddt	$J_{2\alpha,1\alpha} = 7$ $J_{2\alpha,2\beta} = 15$ $J_{2\alpha,3} = 5$	
2β	1.52	ddt	$J_{2\beta,2\alpha} = 15$ $J_{2\beta,3\alpha} = 3$ $J_{2\beta,3\beta} = 5$	1α,1-OH,3β
3α	1.03	m		
3β	2.20	dt	$J_{3,2} = 5$ $J_{3\beta,3\alpha} = 13$	3α,20
5α	1.45	d	$J_{5,6} = 6$	1α,2α,6,8,18
6α	4.78	m		5,18
7α	4.78	m		8,16
7-OH	7.80	d	$J_{7\text{-OH},7\alpha} = 2.44$	14,20
8α	3.05	ddd	$J_{8,7} = 3$ $J_{8,11} = 3$ $J_{8,14} = 11$	5
11	7.14	d	$J_{11,8} = 3$	1α,20
14β		m		7α,15,20,21
15	3.05	dq	$J_{15,14} = 2$ $J_{15,16} = 7$	14,16
16	1.12	d	$J_{16,15} = 7$	15/8α,14,21
18	0.90	s		5,6
20	1.70	s		1α,2β,3β,7-OH,11
21	3.33	s		

This structure has not been reported in the literature, but we have been informed by Watson and Silva that a compound of this structure (mp 230-233^{o}C) has been identified by X-ray crystallography in their parallel work on P. saligna, and was named Salignone B by them.

Compound C, mp 220-230^{o}C

This compound was classified into sub-group B by its UV-spectrum, λ_{max} (MeOH) 224 nm (ε12,500). Its 400 MHz ^{1}H NMR spectrum in d_5-pyridine and the results of decoupling and n.O.e.d.s. experiments are summarized in Figure 5 and Table 3. On the basis of this data, the structure (3) was assigned to this compound.



(3)

The presence of the -CH(Me)CO_2Me side-chain was again readily identified from the characteristic methyl singlet and doublet signals. The B-ring lacked oxygen substituents and showed a complex coupling pattern of the H-6, H-7 and H-8 protons. The presence of an epoxy group on the A-ring was deduced from the appearance of characteristic signals at δ3.04 and δ3.10. The location of the epoxy group at the 2,3-position follows from the observation of an n.O.e. between H-3 and the Me-18 group. The β-orientation of the 2,3-epoxy group was defined by the coupling constants (c.f. compound A), of 0 and 2.2 Hz between the 2α-H and the 1α-H and 1β-H respectively. Thus, the complete structure of compound C is that shown in (3) and this is a new compound.

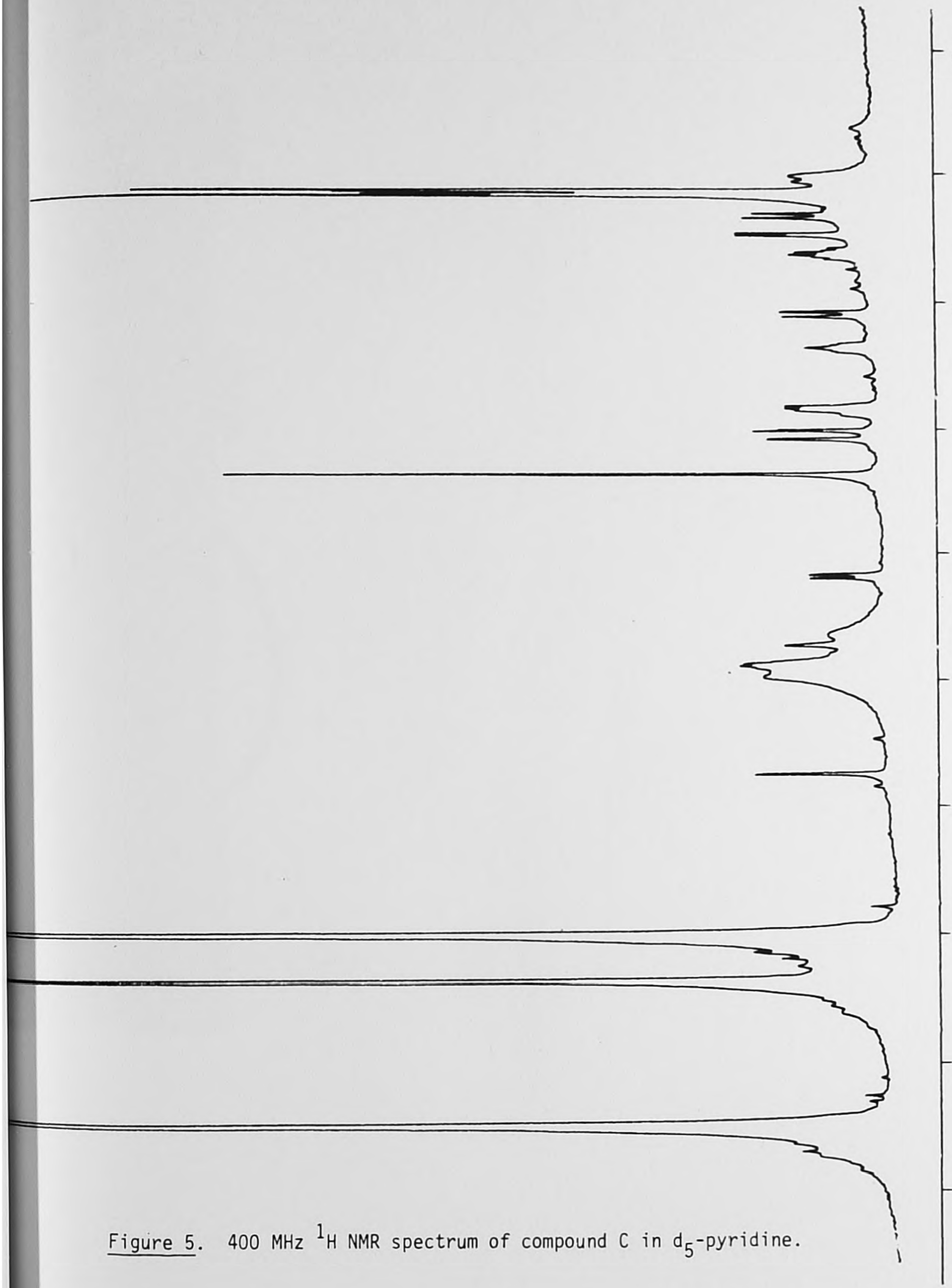
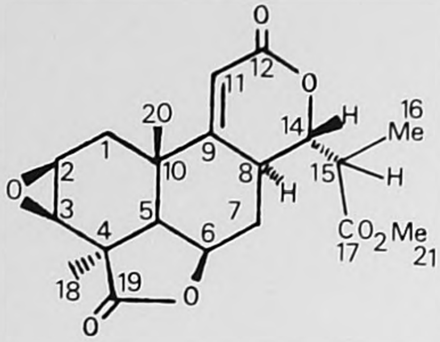


Figure 5. 400 MHz ^{1}H NMR spectrum of compound C in d_5-pyridine.

Table 3. 400 MHz ^{1}H NMR spectrum of compound C in d_5-pyridine.



Proton	δ/ppm	multi-plicity	J/Hz	n.O.e.
1α	1.35	d	$J_{1\alpha,1\beta}=13.0$ $J_{1\alpha,2\alpha}=0$	1β,2,11
1β	2.11	dd	$J_{1\beta,1\alpha}=13.0$ $J_{1\beta,2\alpha}=2.2$	1α,2,11,20
2α	3.10	dd	$J_{2\alpha,1\alpha}=0$ $J_{2\alpha,1\beta}=2.2$ $J_{2\alpha,3\alpha}=3.4$	1α,1β
3α	3.04	d	$J_{3\alpha,2\alpha}=3.4$	18
5α	1.48	d	$J_{5,6}=5.4$	6,8
6α	4.9	m	$J_{6,5}=5.4$ $J_{6,7\alpha}=4.4$ $J_{6,7\beta}=6.9$	
7α	2.35	m	$J_{7\alpha,6}=4.4$ $J_{7\alpha,7\beta}=13.0$ $J_{7\alpha,8}=8.8$ $J_{7\alpha,11}=1.0$	7β,(8 or 15 or both)
7β	1.63	m	$J_{7\beta,6}=6.9$ $J_{7\beta,7\alpha}=13.0$ $J_{7\beta,8}=12.0$	7α,14,20
8α	2.82[a]	m	$J_{8,7\alpha}=8.8$ $J_{8,7\beta}=12.0$ $J_{8,11}=2.0$ $J_{8,14}=10.5$	(5,7α,14,16)
11	5.75	dd	$J_{11,7\alpha}=1.0$ $J_{11,8}=2.0$	1β
14β	4.20	dd	$J_{14,8}=10.5$ $J_{14,15}=2.5$	7β,15
15	2.82[a]	m	$J_{15,14}=2.5$ $J_{15,16}=6.3$	(14,16)
16	1.18[b]	d	$J_{16,15}=6.3$	1β,3,5,6,7β,8,14
18	1.15[b]	s		
20	1.20[b]	s		
21	3.37	s		

Notes: a) and b). Groups of signals irradiated together since overlapping.

The 400 MHz ^{1}H NMR spectrum of this compound was also recorded in $CDCl_3$, giving very similar results (Fig. 6a,b and Table 4). No n.O.e. data was obtained for the $CDCl_3$ solution, but some decoupling experiments were carried out. Irradiation of proton 5α caused 6α to sharpen to a dd (7.5 and 10 Hz). Irradiation of 6α caused sharpening of 5α and 7β. Irradiation of 8α caused sharpening of protons (11,14, 7α and 7β). Irradiation of proton 11 caused proton 8 to simply and allowed the detection of a 6 Hz coupling in the latter ($J_{8,7\alpha}$) which was otherwise impossible to determine from the spectra.

A comparison of the $CDCl_3$ and d_5-pyridine spectra is interesting. Signals in the former all appear to lower field than in the latter. A possible explanation for this is that the pyridine is complexing to the various oxygen atoms and producing a shielding influence (c.f. typical shifts produced in benzene, especially for carbonyl compounds). These changes in solvation must be accompanied by conformational changes, especially around the B-ring, as indicated by the alterations in coupling constants around the 6, 7 and 8 positions.

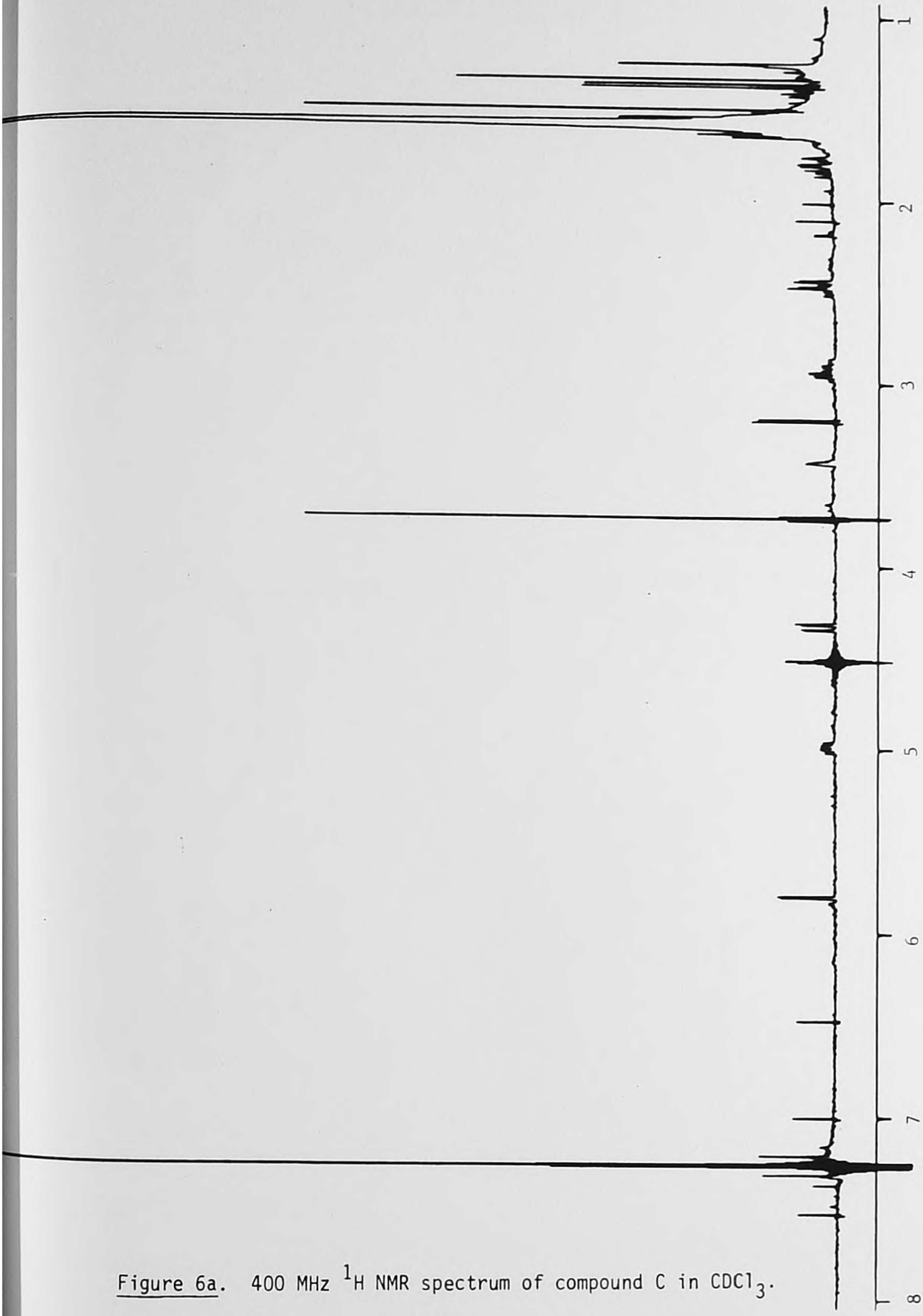


Figure 6a. 400 MHz ^1H NMR spectrum of compound C in $CDCl_3$.

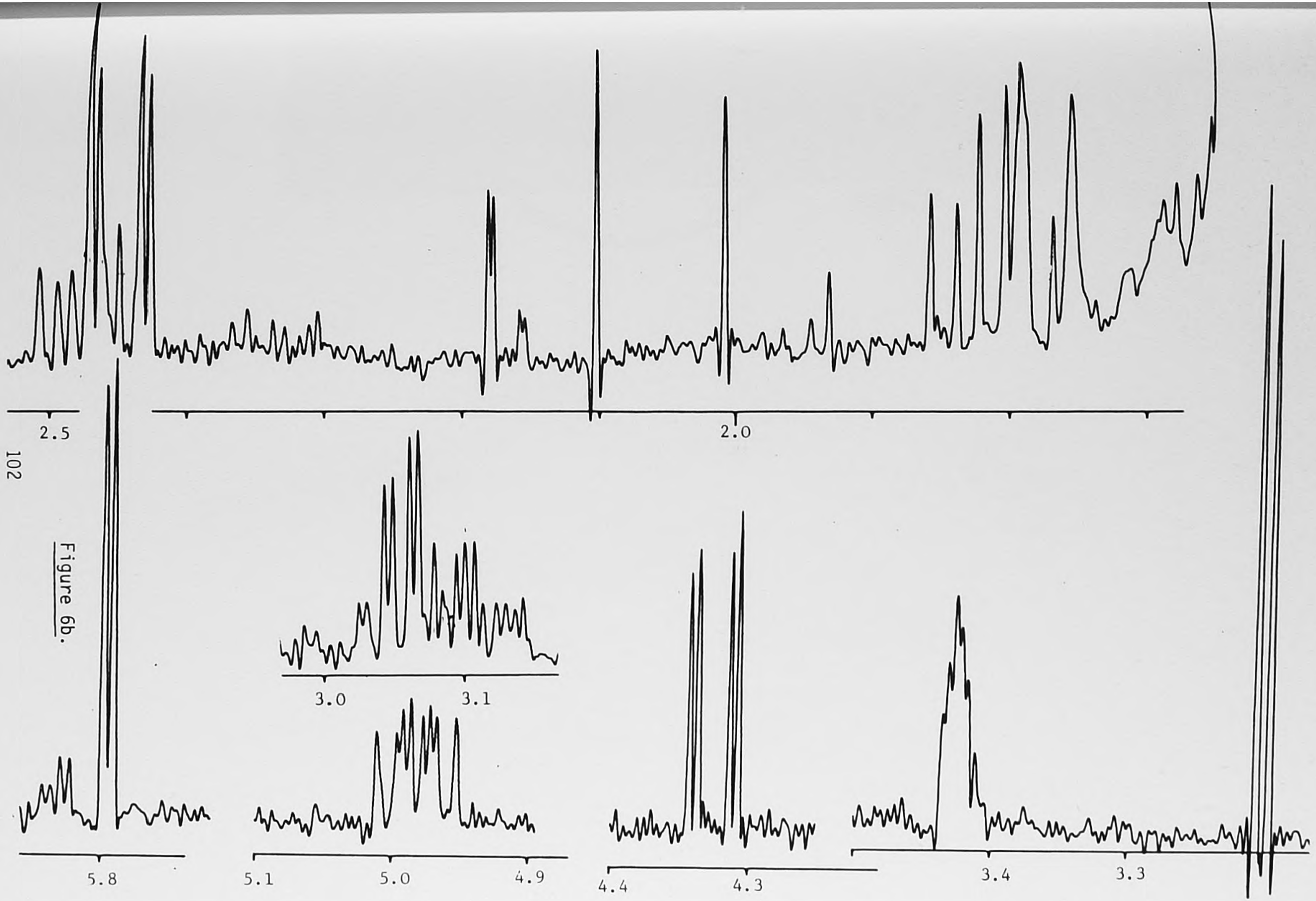
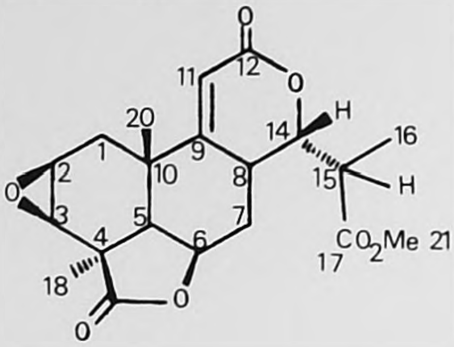


Figure 6b.

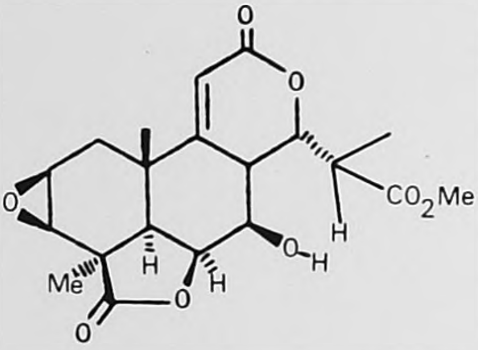
Table 4. 400 MHz ^{1}H NMR spectrum of compound C in $CDCl_3$.



Proton	δ/ppm	multi-plicity	J/Hz
1α	1.77	d	$J_{1\alpha,1\beta} = 14.5$ $J_{1\alpha,2} = 0$
1β	2.45	dd	$J_{1\beta,1\alpha} = 14.5$ $J_{1\beta,2} = 2.5$
2α	3.43	m	
3α	3.20	d	$J_{3,2} = 3.8$
5α	1.65	d	$J_{5,6} = 6$
6α	4.98	ddd	$J_{6,5} = 6$ $J_{6,7\alpha} = 10$ $J_{6,7\beta} = 7.5$
7α	2.48	ddd	$J_{7\alpha,6} = 10$ $J_{7\alpha,7\beta} = 14$ $J_{7\alpha,8} = 6$
7β	1.81	dt	$J_{7\beta,6} = 7.5$ $J_{7\beta,7\alpha} = 14$ $J_{7\beta,8} = 8$
8α	2.90	m	$J_{8,7\alpha} = 6$ $J_{8,7\beta} = 8$ $J_{8,11} = 2.5$ $J_{8,14} = 11.5$
11	5.79	d	$J_{11,8} = 2.5$
14β	4.33	dd	$J_{14,8} = 11.5$ $J_{14,15} = 2.5$
15	2.95	dq	$J_{15,14} = 2.5$ $J_{15,16} = 7$
16	1.37	d	$J_{16,15} = 7$
18	1.33	s	
20	1.50	s	
21	3.75	s	

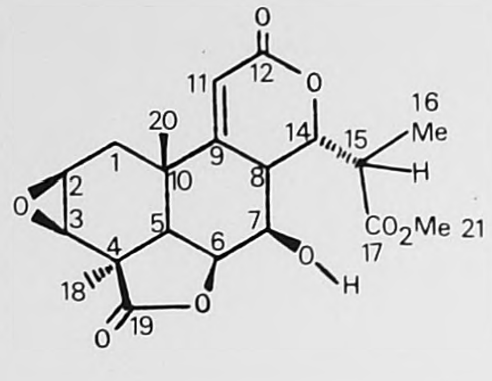
Compound D, mp 233-242°C

This compound, which showed λ_{max} (EtOH) 220 nm (ε11,000), had ^{1}H NMR signals which revealed it to be a close structural relative of compound C. Spectra were obtained in both d_5-pyridine (Figure 7) and $CDCl_3$ (Figure 8) and the latter analysed in detail (Table 5). The differences between the spectra of compound C and compound D could be accommodated by the presence of an additional C-7OH in compound D. Its β-orientation follows from the H-6 to H-7 and H-7 to H-8 coupling constants. The β-orientation of the 2,3-epoxy group is again shown from the small sizes of the coupling constants from 2-H to the 1α-H and 1β-H. The structure (4) for compound D is again a new one.



(4)

Table 5. 400 MHz ^{1}H NMR spectrum of compound D in $CDCl_3$.



Proton	δ/ppm	multi-plicity	J/Hz
1α	1.73		$J_{1\alpha,1\beta}$=15 $J_{1\alpha,2}$ = 0
1β	2.47		$J_{1\beta,1\alpha}$=15 $J_{1\beta,2}$ = 2.1
2α	3.41	m	
3α	3.20	d	$J_{3,2}$ = 3.6
5α	1.67	d	$J_{5,6}$ = 5.7
6α	4.87	dd	$J_{6,5}$ = 5.7 $J_{6,7\alpha}$ = 7.9
7α	4.78	dd	$J_{7\alpha,6}$ = 7.9 $J_{7\alpha,8}$ = 4.0
7-OH	2.50	bs	
8α	3.03	ddd	$J_{8,7\alpha}$ = 4.0 $J_{8,11}$ = 2.9 $J_{8,14}$ =11.4
11	5.83	d	$J_{11,8}$ = 2.9
14β	4.62	dd	$J_{14,8}$ =11.4 $J_{14,15}$= 2.1
15	3.10	dq	$J_{15,14}$= 2.1 $J_{15,16}$= 7.1
16	1.42	d	$J_{16,15}$= 7.1
18 20	1.55	s	
21	3.73	s	

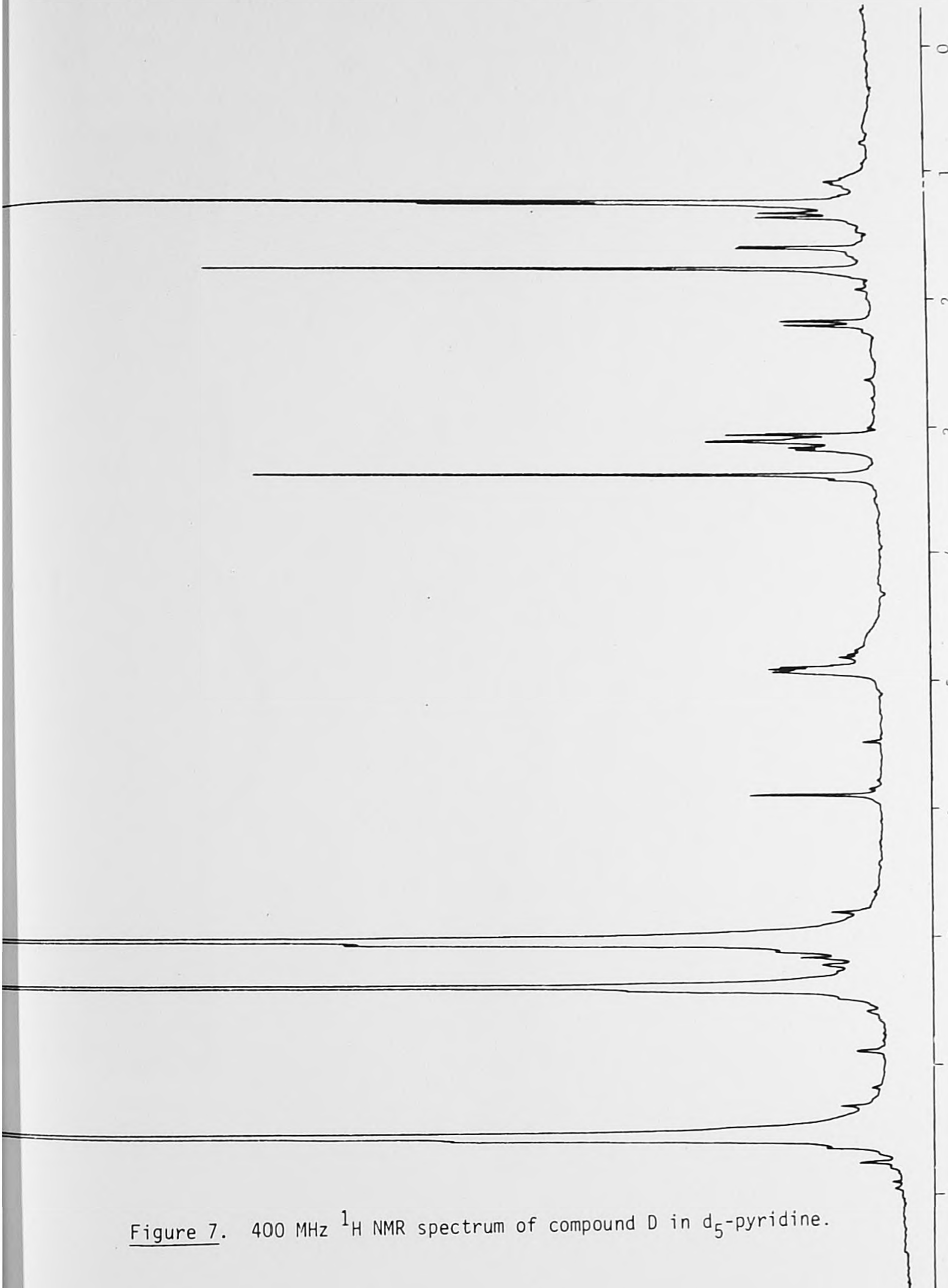


Figure 7. 400 MHz ^{1}H NMR spectrum of compound D in d_5-pyridine.

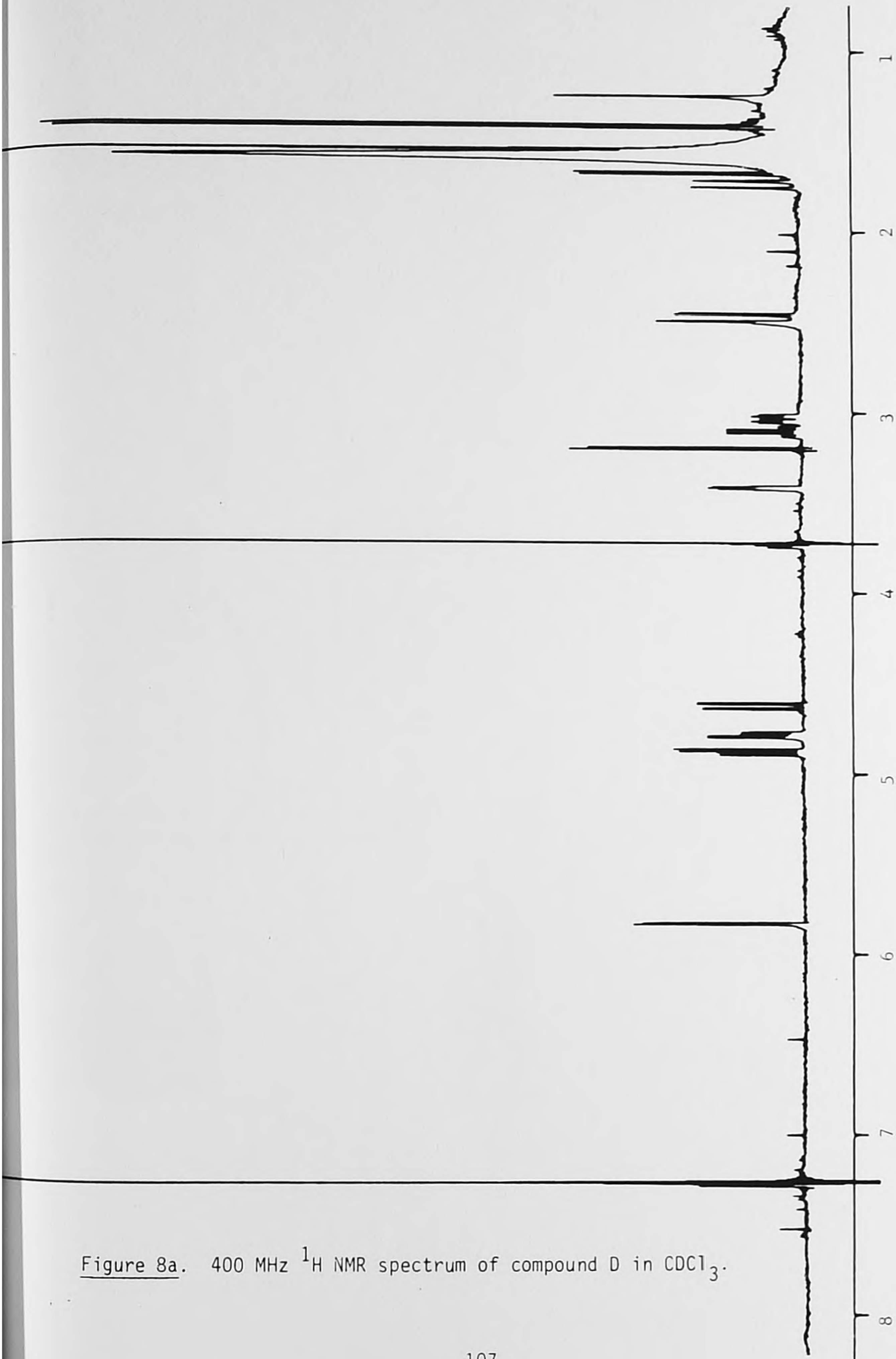
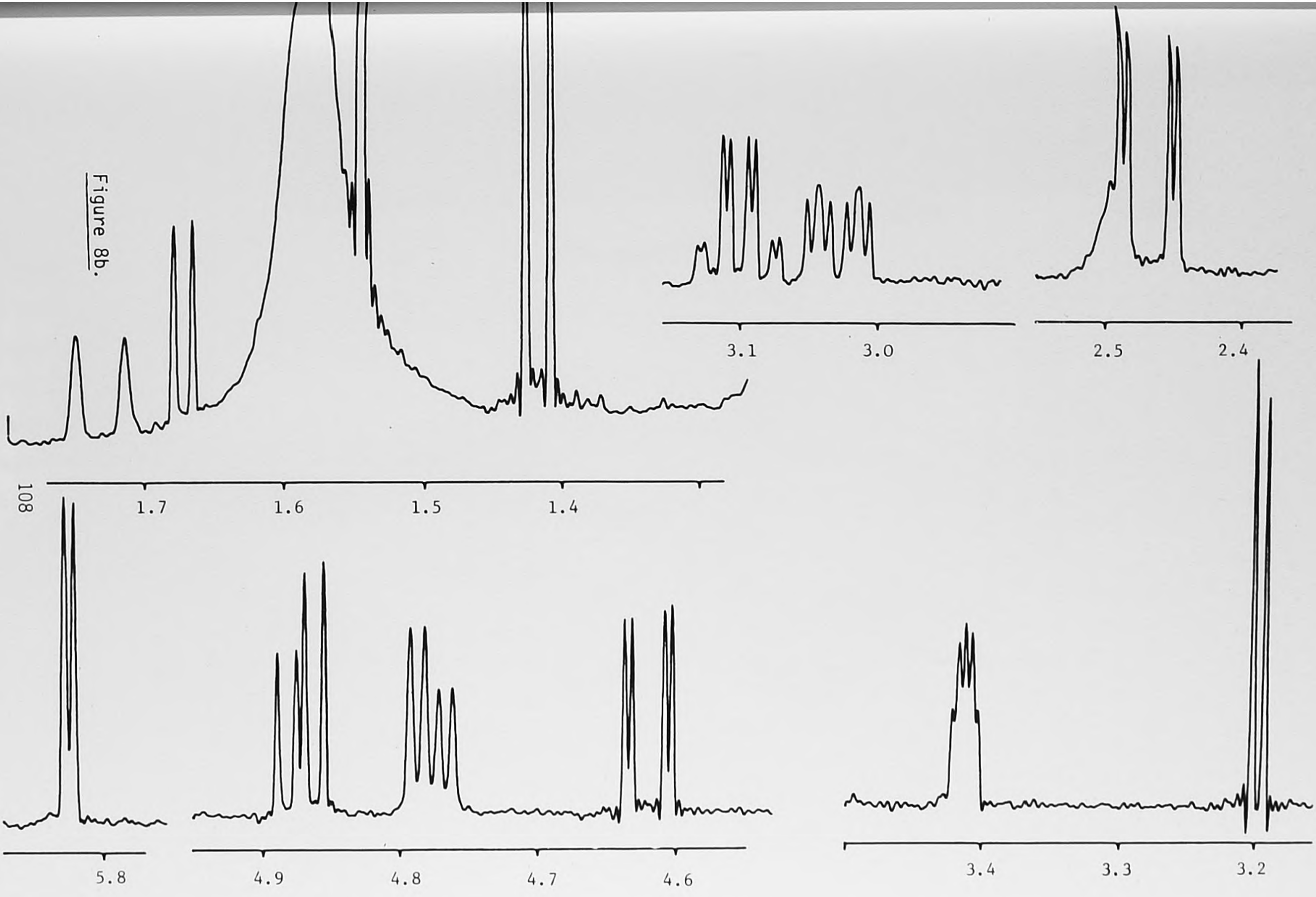


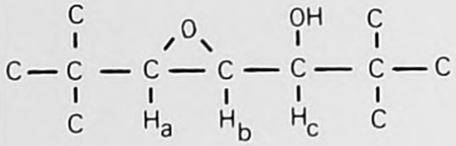
Figure 8a. 400 MHz ^{1}H NMR spectrum of compound D in $CDCl_3$.

Figure 8b.

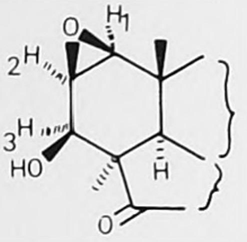


Compound E, mp 222-225°C

The ^{1}H NMR spectra of compound E in both d_5-pyridine and $CDCl_3$ (Figures 9 and 10 and Table 6) showed numerous similarities to the spectra of compound C, from which it was clear that compound E is also unsubstituted at C-7 and C-8 and also carries the -CH(Me)CO_2Me side-chain at C-14. Changes in the pattern of signals for the A-ring protons in compound E, compared with compound C, indicated a new pattern of oxygenation to be present: a 3-proton system could be seen (5) with H_a (δ3.33,d,J=4 Hz), H_b (δ3.39,bd,J=4 Hz and second, small coupling $\leq$2 Hz) and H_c (δ4.07,bs,J$\leq$2 Hz).

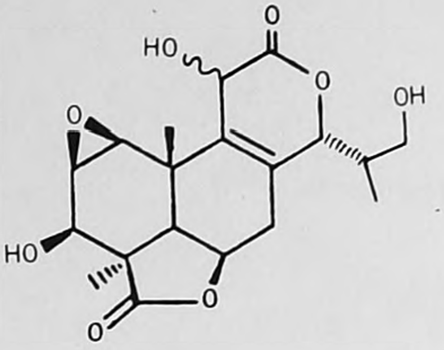


(5)



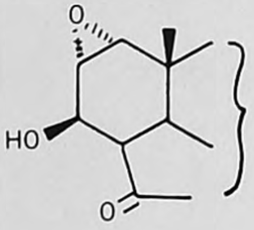
(6)

All of the A-ring hydroxy-epoxides so far reported in the literature have a 1,2-β-epoxy, 3β-hydroxy arrangement (6). A close examination of the NMR data reported for about a dozen compounds of this type (c.f. Tables 1-3, Chapter 1) shows that they all have very similar patterns for the A-ring: H_1 (δ3.54-3.71,d,J=4-5 Hz), H_2 (δ3.38-3.77, dd,J=4-5 Hz and 6-7 Hz), H_3 (δ4.30-4.69,d,J=6-7 Hz). Only one compound, inumakilactone C, deviates slightly from these ranges, having H_1 (δ3.36), H_2 (δ3.29) and H_3 (δ4.30), which are all slightly low, but still retains the two characteristic couplings of 4 Hz and 6 Hz. It is significant that inumakilactone C has an unusual C-ring structure (7), which may account for the slight change in chemical shifts around ring A.

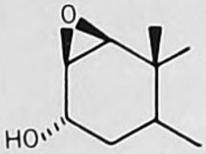


(7)

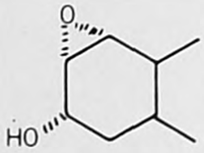
Compound E differs significantly from these compounds in that both the H_a and H_c chemical shifts are low and in the very small size of the J_{bc} coupling constant. Thus, it becomes necessary to consider the alternative structures (8)-(14) to account for this.



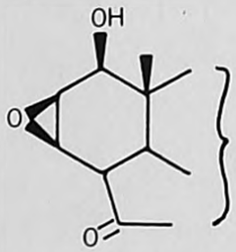
(8)



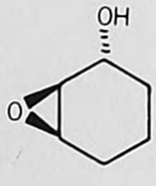
(9)



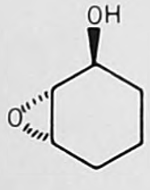
(10)



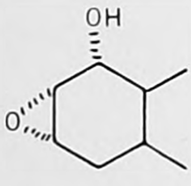
(11)



(12)



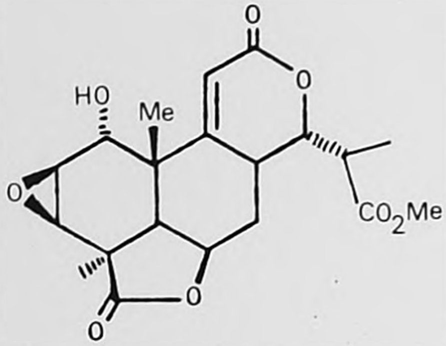
(13)



(14)

Structures (10), (11) and (14) can be dismissed at once. Molecular models show that for these, as in the "normal" pattern (6), the <u>cis</u> arrangement of hydrogens produces a very small dihydral angle between the C<u>H</u>(OH) and adjacent C<u>H</u> of the epoxide, from which a large coupling constant (∿6 Hz) would be expected.

The similarity in chemical shift of H_a in compound E to H-3 in compounds C and D suggests the presence of a β-orientated 2,3-epoxy group and this is further substantiated by the close similarity in chemical shifts of the Me-18 and Me-20 in these three compounds. This suggests the structure (12) to be the correct one and leads to the formulation of compound E, overall, as structure (15). This is a new compound.



(15)

Unfortunately, there was insufficient material available for n.O.e. difference spectroscopy, which would undoubtedly have greatly assisted in confirming this structural assignment.

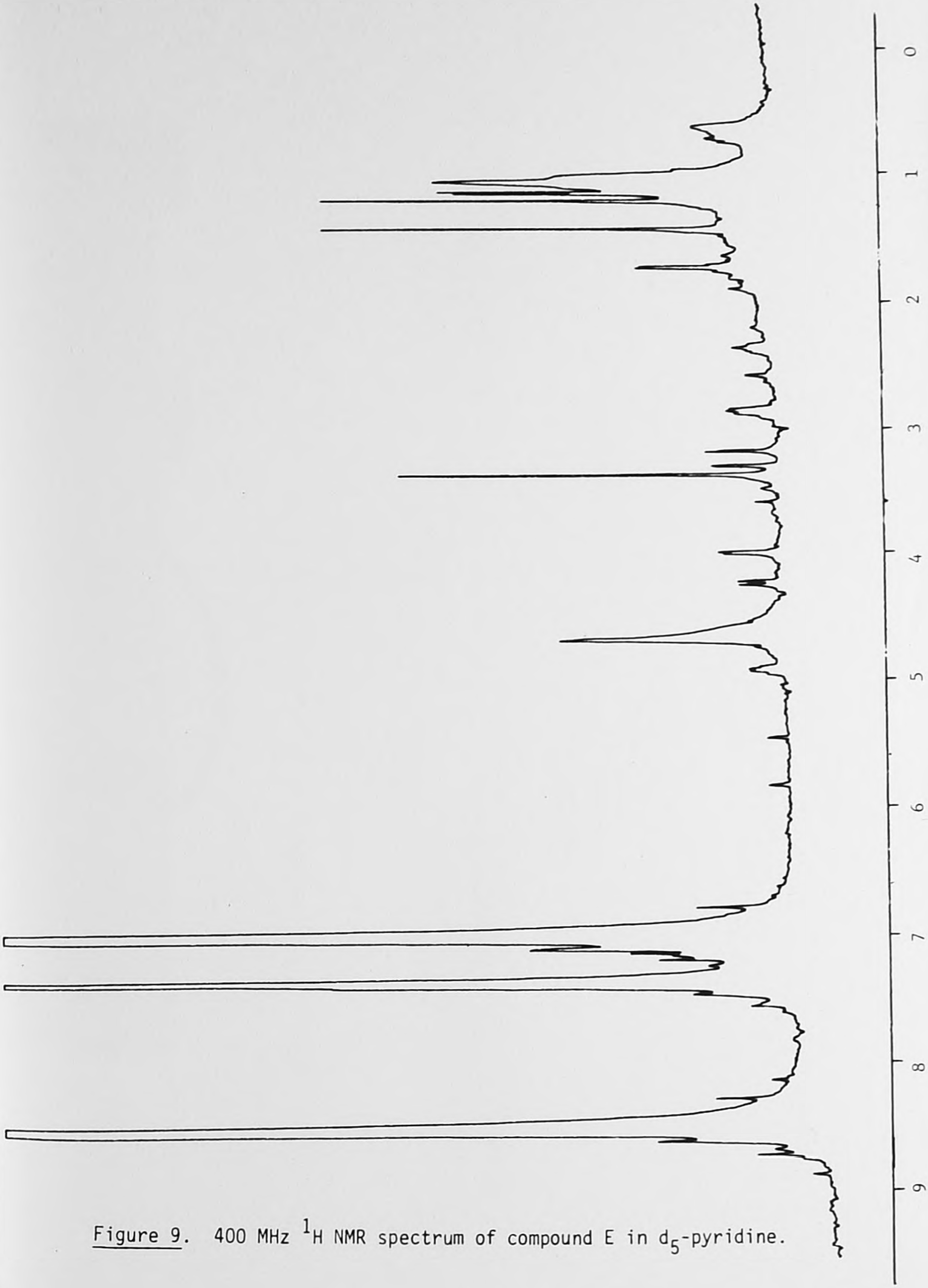
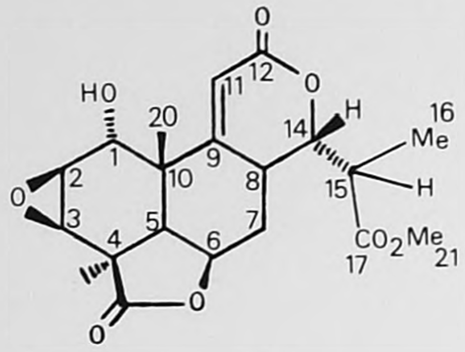


Figure 9. 400 MHz ^{1}H NMR spectrum of compound E in d_5-pyridine.

Table 6. 400 MHz ^1H NMR spectrum of compound E in $CDCl_3$.



Proton	δ/ppm	multi-plicity	J/Hz
1β	4.07	bs	$J_{1,2} \leq 2$
2α	3.39	bd	$J_{2,1} \leq 2$ $J_{2,3} = 4$
3α	3.33	d	$J_{3,2} = 4$
5α	Hidden by H_2O		
6α	4.95	m	
7α	2.45	m	
7β	2.34	m	$J = 8$ $J = 15$
8α	2.85	m	
11	6.72	d	$J_{11,8} \leq 3$
14β	4.31	dd	$J_{14,8} = 12$ $J_{14,15} = 2$
15	2.94	m	
16	1.36	d	$J_{16,15} = 6$
18	1.25	s	
20	1.49	s	
21	3.73	s	

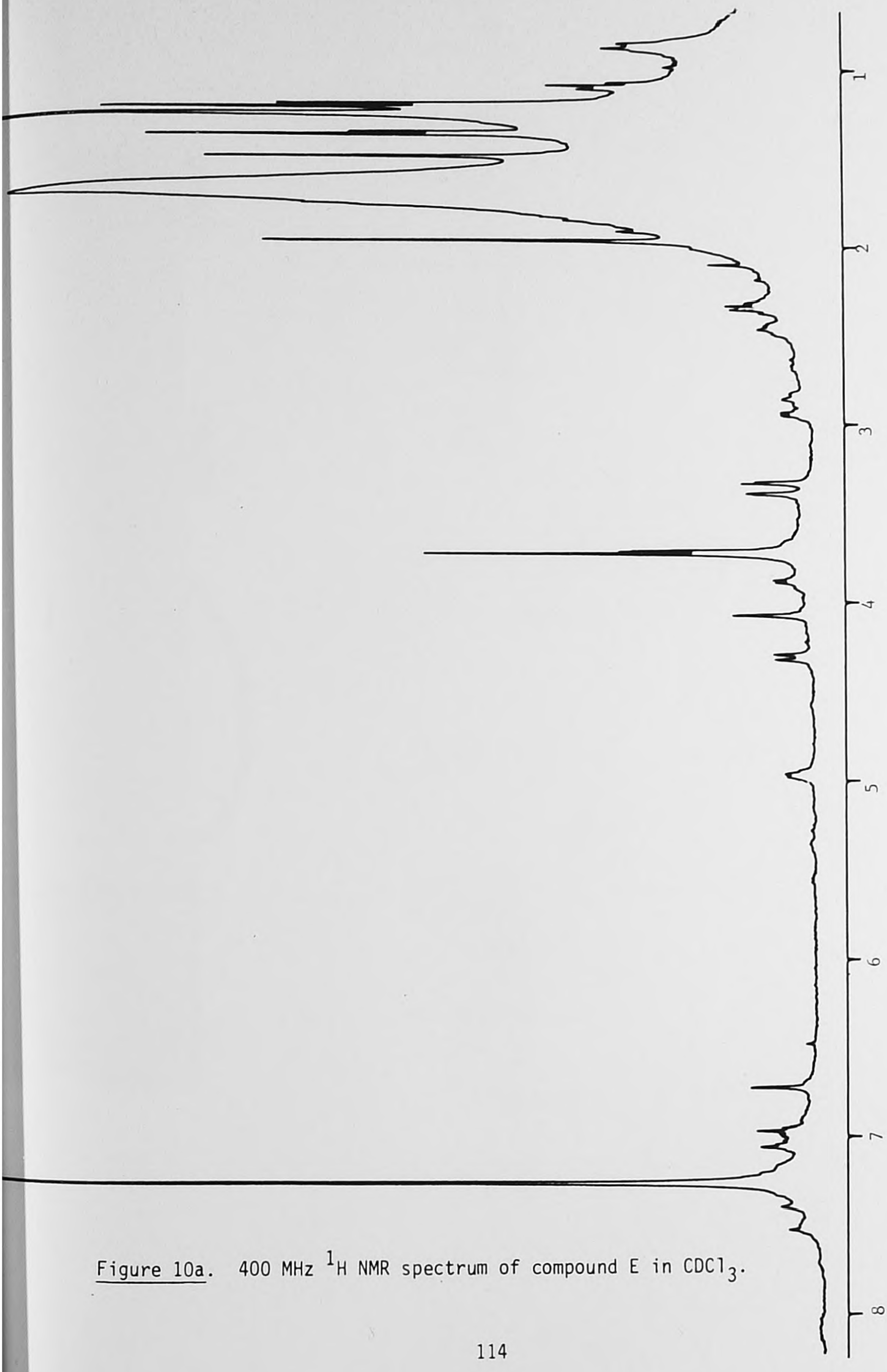


Figure 10a. 400 MHz ^1H NMR spectrum of compound E in $CDCl_3$.

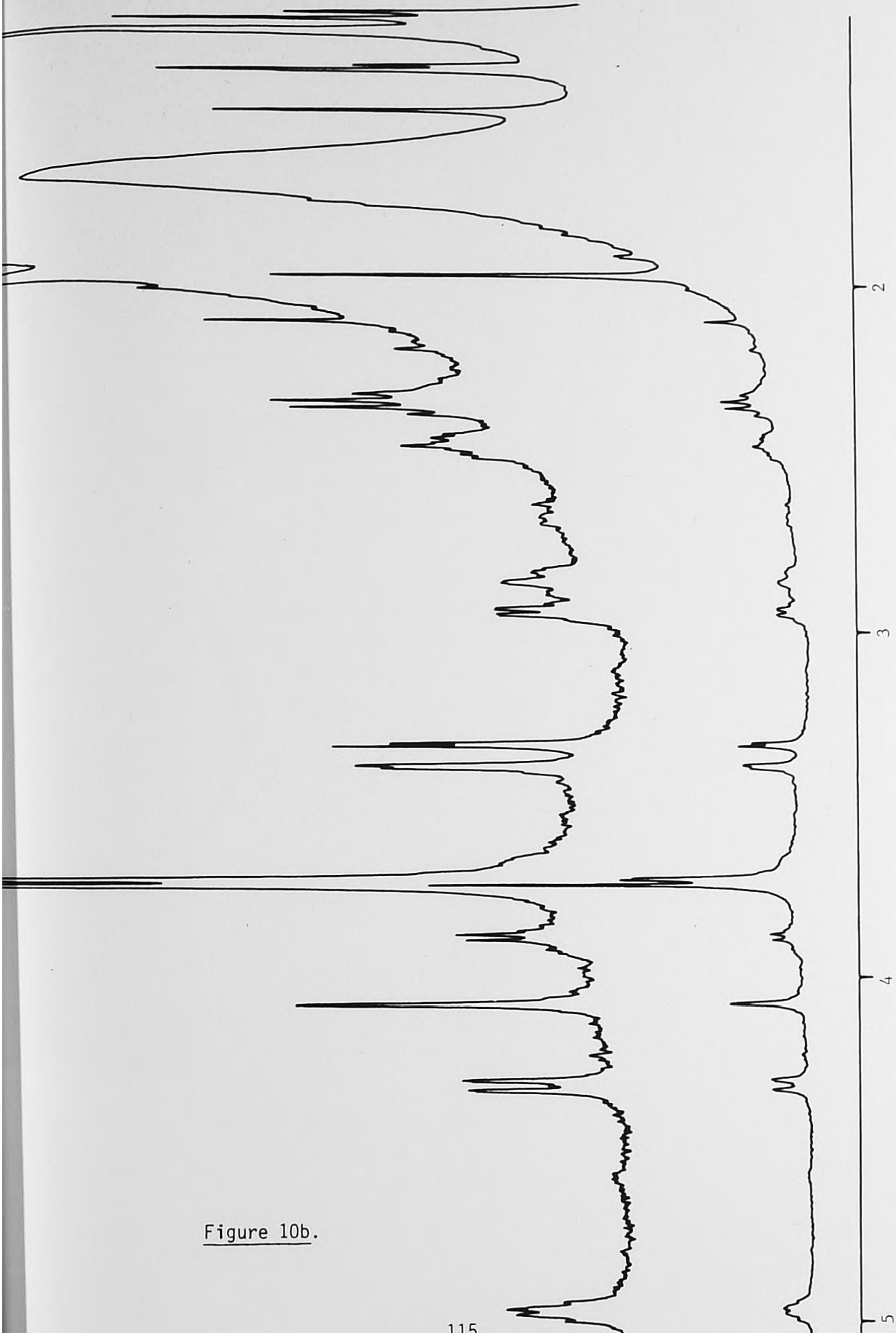
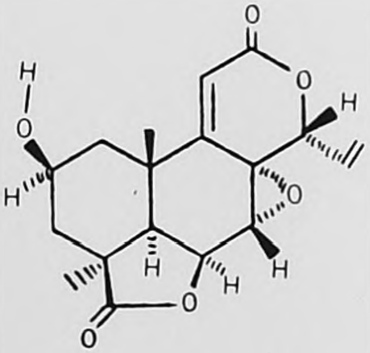


Figure 10b.

Compound F, mp 270°C (Dec)

The 400 MHz ^{1}H NMR spectra of this compound were obtained in d_5-pyridine and $CDCl_3$ (Figures 11 and 12), the latter being analysed in detail (Table 7). The presence of a vinyl group at C-14 was at once recognized (c.f. compound A). H-14 appeared as a simple doublet which indicated that C-8 did not carry a hydrogen. From this, and the appearance of the 7β-proton as a low-field doublet, the presence of the 7,8-epoxy group found in many of the sub-group B type was deduced. The pattern of signals for the A-ring protons (4H between δ1.9 and 2.4; 1H multiplet at 4.06) closely resembled that reported for 1-deoxy-2β-hydroxy-nagilactone F and indicated the presence of a 2β-hydroxyl group. In double irradiation experiments, irradiation of the signal at δ4.06 caused alterations in the patterns for all four of the other protons, confirming that the C<u>H</u>OH group was centrally located between the two methylene groups. Irradiation at δ1.94 and sharpening of the peak at δ2.18, and vice versa, with sharpening also at δ4.06 but without effect on the multiplets at 2.31 and 2.38. Thus, the structure (16) is clearly established for compound F and this is another new compound. Since only ca. 40 μg was available for these NMR experiments, it was not possible to obtain n.O.e. difference spectra.



(16)

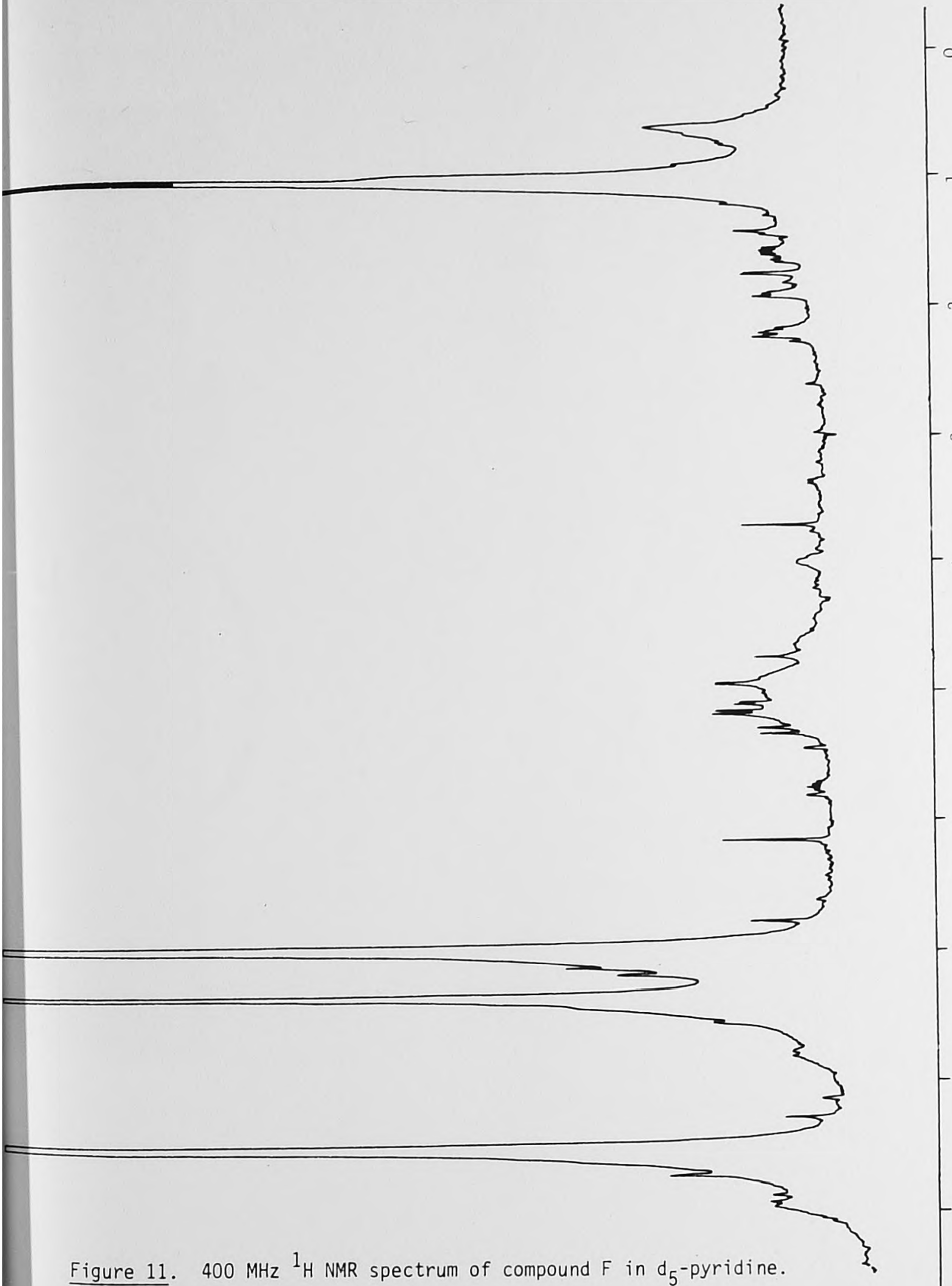


Figure 11. 400 MHz ^1H NMR spectrum of compound F in d_5-pyridine.

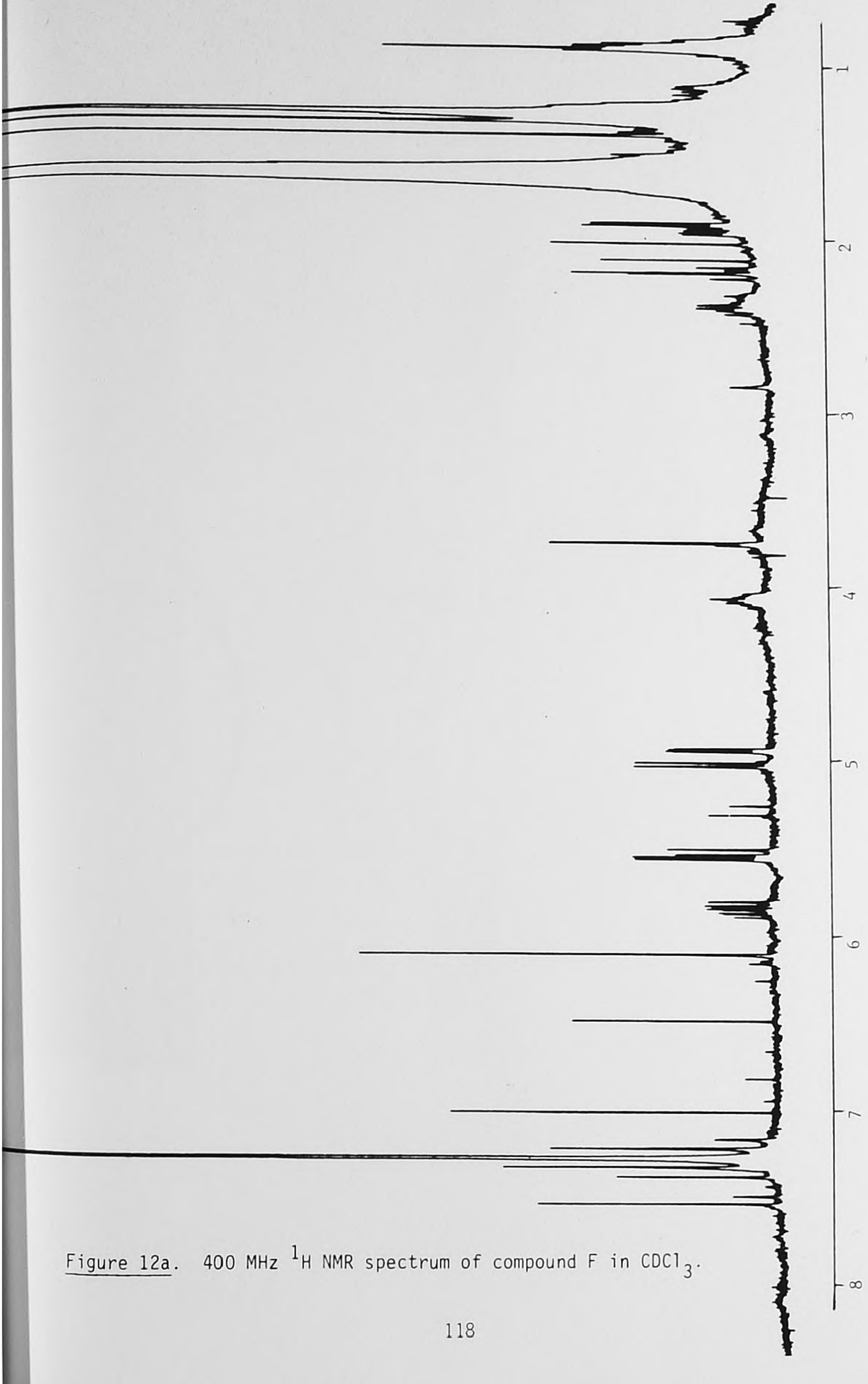


Figure 12a. 400 MHz ^1H NMR spectrum of compound F in $CDCl_3$.

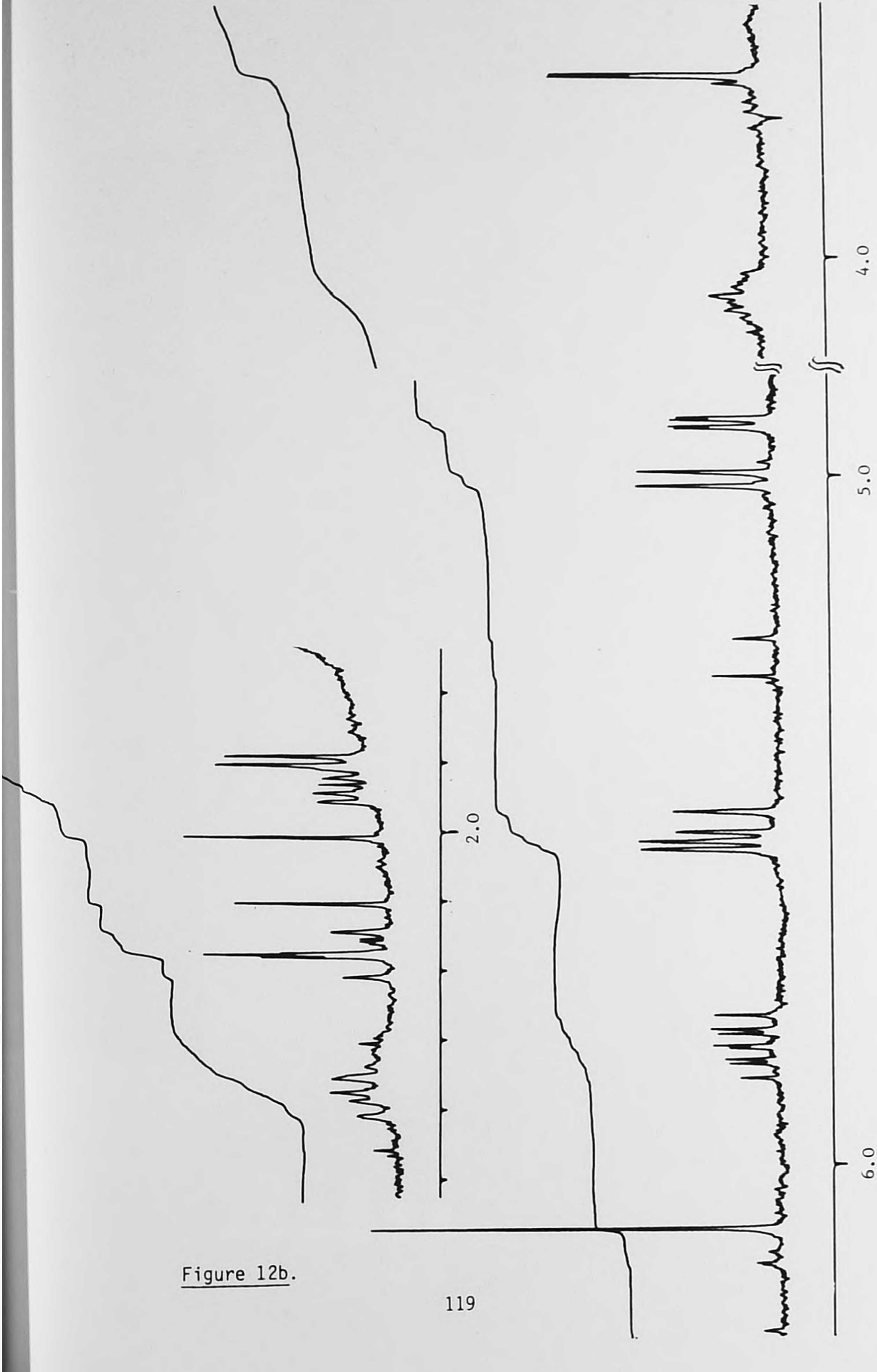
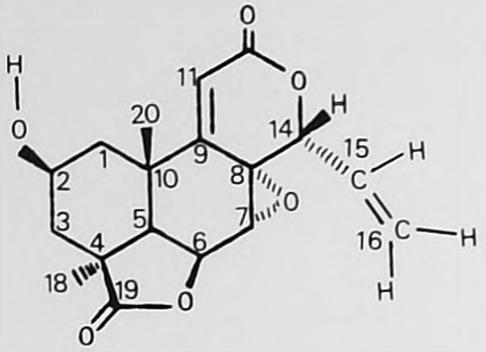


Figure 12b.

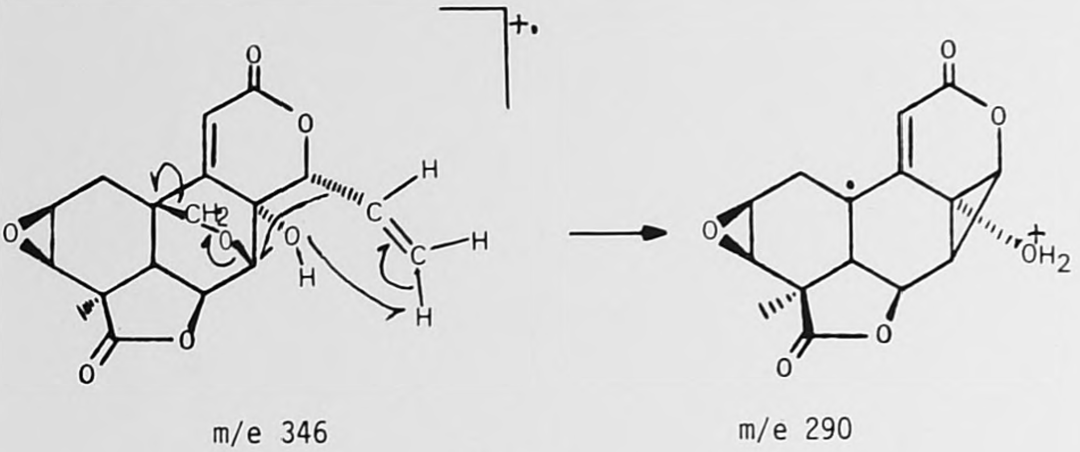
Table 7. 400 MHz ^1H NMR spectrum of compound F in $CDCl_3$



Proton	δ/ppm	multi-plicity	J/Hz
1α	2.31	dd	$J_{1\alpha,1\beta}=14$ $J_{1\alpha,2}=10$
1β	2.38	dd	$J_{1\beta,1\alpha}=14$ $J_{1\beta,2}=10$
2α	4.06	m	
3α	1.95	dd	$J_{3\alpha,2\alpha}=5$ $J_{3\alpha,3\beta}=14$
3β	2.18	dd	$J_{3\beta,2\alpha}=10$ $J_{3\beta,3\alpha}=14$
5α	1.90	d	$J_{5,6}=5$
6α	4.93	dd	$J_{6,5}=5$ $J_{6,7}=2$
7β	3.73	d	$J_{7,6}=2$
11	6.09	s	
14β	5.00	d	$J_{14,15}=8$
15	5.83	ddd	$J_{15,14}=8$ $J_{15,16Z}=10$ $J_{15,16E}=17$
16Z	5.53	d	$J_{16Z,15}=10$
16E	5.51	d	$J_{16E,15}=17$
18	1.31	s	
20	1.39	s	

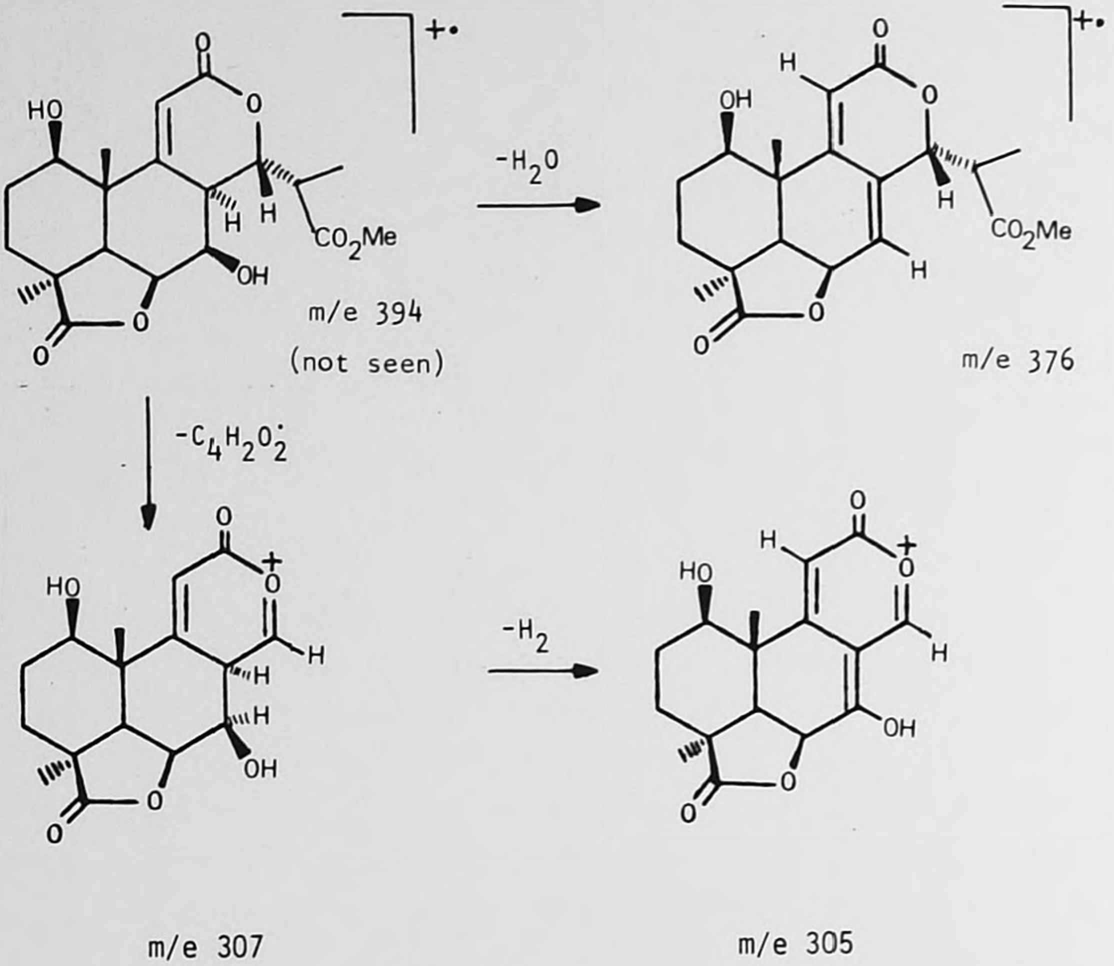
4.4 Mass spectral studies

Mass spectrometry has so far proved to be of rather limited value as a structural tool in the case of the dilactones. Only one of the compounds isolated, Salignone A, gave a molecular ion (m/e 346; 1.0%) under normal EI conditions (70 eV). The base peak for this compound was at m/e 290, the even mass value suggesting a rearrangement process. A suggested fragmentation is shown in Scheme 4 .



Scheme 4

Salignone B did not show a molecular ion (required m/e 394), but did show a peak at m/e 376 (M-18), which might be expected for the diol. The base peak appeared at m/e 307 (M-87), corresponding to loss of the -CH(Me)CO_2Me side-chain from the molecular ion and directly confirming the presence of two hydroxyl groups on the nucleus. A further peak at m/e 305 (14%) can be explained by a further loss of H_2 to give the pyrillium cation (Scheme 5).



Scheme 5

Compound C did not produce a molecular ion, even at 20 eV. It showed as the highest mass fragment an ion m/e 289, corresponding to loss of the -CH(Me)CO_2Me (M-87).

Several other samples of dilactones isolated from HPLC were also examined at 70 eV and 20 eV, but in no case could a molecular ion be observed. Usually, the largest fragment apparently corresponded to loss of the side-chain.

These disappointing results prompted us to examine the use of one of the new "soft" ionization techniques in the hope of observing molecular ions more readily. During the last decade, field desorption (FD) mass spectrum has proved to be a powerful method for obtaining molecular ions from even the most sensitive molecules. It relies on the use of a very large, local electric field gradient to remove a electron from a molecule on the surface of an emitter wire. Following ionization, the charged species (often M+1 due to ion-molecule collisions in the surface film) is rapidly repelled from the charged surface of the emitter. The major drawbacks of FD which have been widely recognized are the difficulty of producing good emitter wires and the problem that, often, too little fragmentation is observed, reducing the information content of the spectrum.

Very recently, a new ionization technique called fast atom bombardment (FAB) has been developed[22,23]. This is closely related in some ways to FD, in that ionization is induced in a surface film and is followed by rapid expulsion of the charged species from the surface. It differs in that the ionization is induced by argon atom bombardment of a film of the compound in glycerol by an "ion sputtering" mechanism. This is technically easier to arrange than FD, gives rise to a more persistent ion beam and also it usually produces a spectrum with a considerable amount of fragmentation information, as well as a strong pseudo-molecular ion (often M+1).

In a preliminary exploration of the utility of FAB for norditerpene lactones, three of the compounds characterized by us (compounds A, B and C) were submitted to Cambridge where their FAB mass spectra were kindly run by [redacted].

Terpenes A and B gave disappointing results, showing ions at m/e 391 and no peaks which could be related in an obvious way to the known structures. Possibly, the amounts of material submitted were too small and only contaminants were being detected.

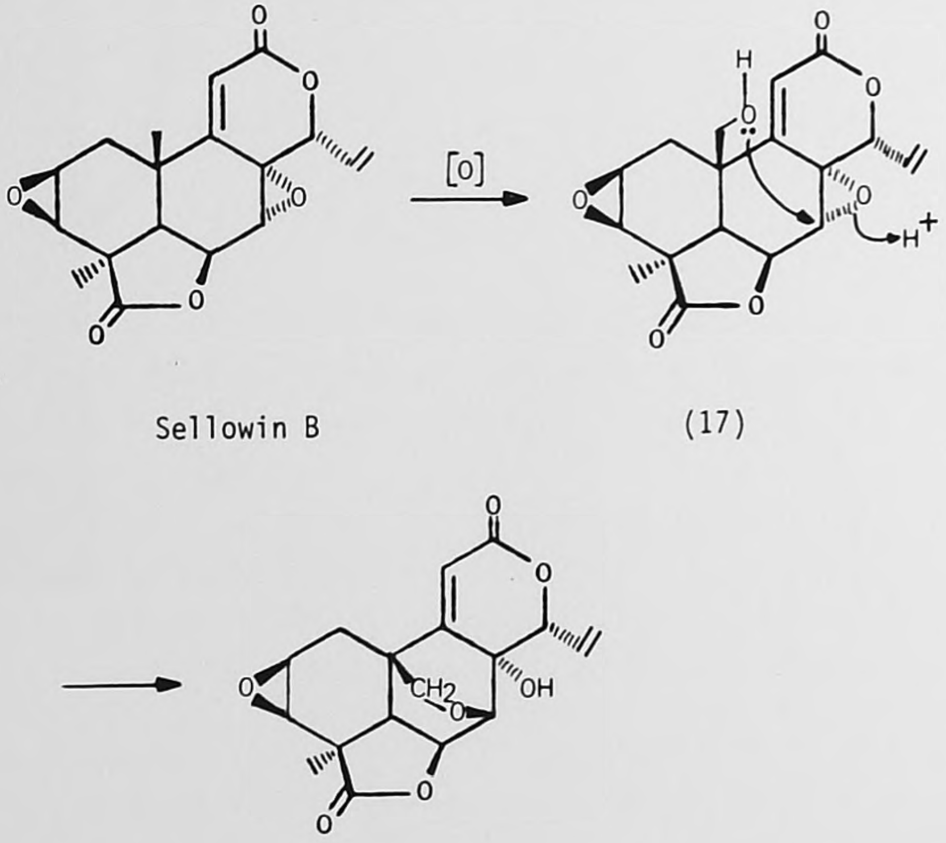
More encouragingly, compound C, which had failed to produce a molecular ion on EI MS, gave a strong ion at m/e 377 (M+1). It also showed prominent ions at m/e 327 and 283. The first of these must be a contaminant and the other one may well be, since they lack any obvious interpretation as fragment ions.

It is to be hoped that larger quantities of norditerpene lactones will become available in the future, allowing a systematic study of the application of soft ionization techniques to be made.

4.5 Summary and conclusions

Six dilactones isolated from P. saligna have been characterised by ^1H NMR and other spectroscopic techniques in this work. Only one of these, Salignone A, has been reported in the literature, coincident with our own structure determination.

Salignone A is unusual in being the first example of a dilactone from Podocarpaceae to have a bridging oxymethylene group across C-10 to C-7. A plausible biosynthesis of this compound would involve the oxidation of the Me-20 of Sellowin B (which has not so far been detected in P. Saligna itself) to give an intermediate (17), which could then cyclise as shown (Scheme 6).



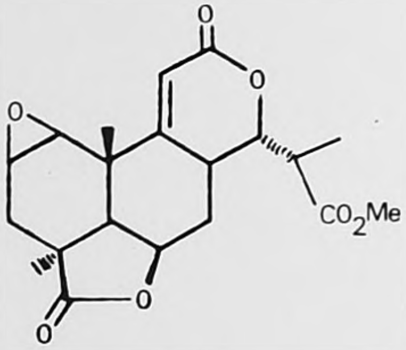
Sellowin B

(17)

Salignone A

Scheme 6

Compound C is a new structure, not previously reported in the literature. It is interesting to note, however, that Sammes, Silva et al, reported the isolation of a compound ("Lactone A") from P. saligna in 1972 and assigned to it a provisional structure (18), based solely on mass spectral evidence. The location of the A-ring epoxy group was suggested simply by analogy with other literature structures then available, many of which later turned out to be erroneous[2]. In the light of later structural revisions[2], the epoxy group would probably have been suggested as 2,3-β. Thus, it is tempting to suggest that our compound C is the "revised" structure for "Lactone A". However, the melting point reported for the latter (258-260°) differs significantly from our own (220-230°C) and a direct comparison of samples has not been possible as yet.

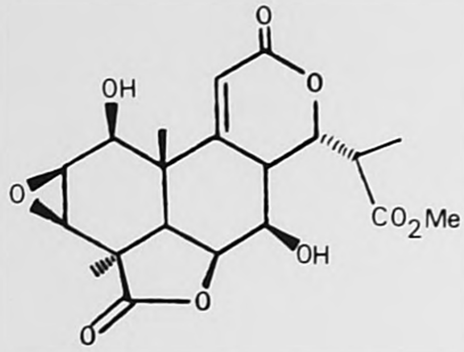


(18)

Compound D differs from compound C only in having an additional hydroxyl group at C-7 and it is interesting to speculate as to how the two are related biosynthetically. Several possibilities can be considered, e.g.:

(i) D is formed from C via hydroxylation
(ii) C is formed from D via reduction
(iii) both are formed from a common precursor having a 7,8-double bond by separate processes of oxidation or reduction.

Although the precise details of the arrangement of oxygens in compound E await final confirmation, it is clear that a hydroxy-epoxide is present and that the oxygenation pattern differs from the "normal" one. It is interesting that one other dilactone of unusual oxygenation pattern in ring A has been identified by Silva and Watson, but not yet published. This compound is Salignone C (19), the structure of which was established by X-ray methods.



(19)

Compound F represents another new structure, without having any particularly remarkable features.

It is instructive to survey the overall structural relationships of the compounds we have isolated from the leaves and stems of P. saligna. All belong to the sub-group B type and all have either a $-CH=CH_2$ or $-CH(Me)CO_2Me$ side-chain. Other, less oxidized side-chains were obtained by Silva and Watson in their partly unpublished structures (Table 2, Chapter 1) of Salignones A-H, but in all cases these also belong to sub-group B.

4.6 <u>References</u>

1. W.H. Watson, paper presented at 12th International Symposium on the Chemistry of Natural Products, Tenerife, Canary Islands, Spain, 21-27th September, 1980.
2. S.K. Arora, R.B. Bates, and P.C.C. Chou, <u>J. Org. Chem.</u>, 1976, <u>41</u>, 2458.
3. D.S. Bhakuni, M. Bittner, P.G. Sammes, and M. Silva, <u>Rev. Latinoamer.</u> 1974, <u>Quim.</u> <u>5</u>, 163-168.

4. S. Itô and M. Kodama, Heterocycles, 1976, 4, 595.
5. K.S. Brown, Jr. and W.E. Sanchez L., Tetrahedron Lett., 1974, 675.
6. Y. Hayashi, T. Matsumoto, M. Uemura, and M. Koreeda, Org. Mag. Res., 1980, 14, 86.
7. Y. Hayashi, Y. Yuki, T. Matsumoto, T. Sakan, Tetrahedron Lett., 1977, 3637.
8. Y. Hayashi, T. Matsumoto, Y. Yuki, and T. Sakan, Tetrahedron Lett., 1977, 4215.
9. H. Kakisawa, M. Sato, T. Tuo, and T. Hayashi, J. Chem. Soc. (CC), 1973, 802.
10. Y. Hayashi, T. Matsumoto and T. Sakan, Heterocycles, 1978, 10, 123.
11. J.W. Dorner, R.L. Cole, J.P. Springer, R.H. Cox, H. Cutler, and D.T. Wicklow, Phytochem., 1980, 19, 1157.
12. S. Ito, M. Kodama, M. Sunagawa, T. Takahashi, H. Imamura, and O. Honda, Tetrahedron Lett., 1968, 2065.
13. S. Ito, M. Kodama, M. Sunagawa, M. Koreeda and K. Nakanishi, Chem. Comm., 1971, 855.
14. S. Ito, M. Sunagawa, M. Kodama, H. Honma and T. Takahashi, Chem. Comm., 1971, 91.
15. S. Ito, M. Kodama, M. Sunagawa, H. Honma, Y. Hayashi, S. Takahashi, H. Ona, T. Sakan, and T. Takahashi, Tetrahedron Lett., 1969, 2951.
16. L.D. Hall and J.K.M. Sanders, J. Am. Chem. Soc., 1980, 102, 5703.
17. L.D. Hall and J.K.M. Sanders, J. Org. Chem., 1981, 46, 1132.
18. S.A. Matlin and L. Chan, Tetrahedron Lett., 1981, 4025.
19. J.D. Mersh and J.K.M. Sanders, Tetrahedron Lett., 1981, 4029.
20. J.D. Mersh and J.K.M. Sanders, Org. Mag. Res., in press.
21. Y. Hayashi, T. Matsumoto, T. Hyono, and T. Sakan, Chem. Letters, 1977, 1461.
22. D.J. Surman and J.C. Vickerman, Chem. Comm., 1981, 324.
23. M. Barber, R.S. Bordoli, R.D. Sedgwick, and A.N. Tyler, Chem. Comm., 1981, 325.

SECTION 2

SYNTHETIC APPROACHES TO NORDITERPENE DILACTONES

CHAPTER 5. LITERATURE SURVEY OF THE TOTAL SYNTHESIS OF DITERPENES

5.1 Introduction

Although the norditerpene dilactones have been known since the 1960's, and despite the very extensive reports of their varied and interesting biological activity, to date there has been no general route developed for the total synthesis of these compounds. Consequently, in the later stages of this research programme on the isolation and characterization of naturally occurring dilactones, we turned our attention to the possibility of developing a flexible and convenient total synthesis which would provide access to a large number of structural types in reasonable quantities for biological evaluation.

This chapter surveys some of the general methods available for the construction of diterpenes, beginning with some of the more straightforward, less oxygenated members and leading up to the heavily oxidized and degraded members related to the norditerpene dilactones. In view of the vast amount of literature available on the synthesis of the simpler diterpenes, this section is intended to be illustrative rather than an exhaustive survey.

Many synthetic approaches to different polycyclic diterpenoids of many degrees of structural and functional complexity have been tried. Synthetic efforts aimed at the diterpenoids have employed various permutations of the order in which the three rings A, B, C are assembled: i) AC → ABC, ii) AB → ABC, iii) BC → ABC and iv) C → BC → ABC.
We will show in the following survey some examples illustrative of all these sequences and types of reactions used before by different authors.

In the case of the synthesis of diterpenoid resin acids some examples will also be given of methods of introducing the methyl and carboxyl substituents at the quaternary 4-position in the correct configuration relative to the C-10 angular methyl.

5.2 Literature survey of diterpene synthesis

The general route A → B → C to diterpenoid synthesis used by Meyer *et al*[1,2,3,4] is schematically depicted in fig. 1:

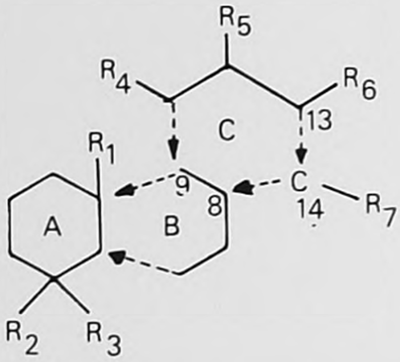
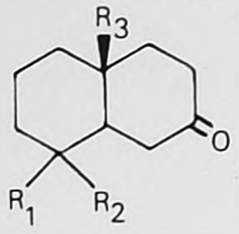


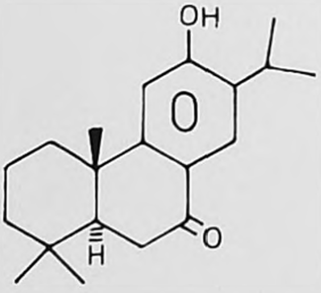
Figure 1

Ring A was envisioned to originate as a trisubstituted cyclohexanone carrying the three ultimate A/B substituents (R_1, R_2 and R_3). These three substituents were chosen so that as many as possible of the diterpenoid A/B extranuclear oxidation patterns could be obtained by later synthetic transformation of the same set of R_1, R_2 and R_3. The B-ring was added in a manner which would activate C-8 and C-9 for attachment of the C-ring or side-chains.
The appropriate bicyclic intermediates chosen by these authors were 4,4,10-trisubstituted-7-decalones (1).

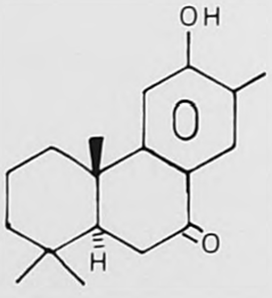


(1)

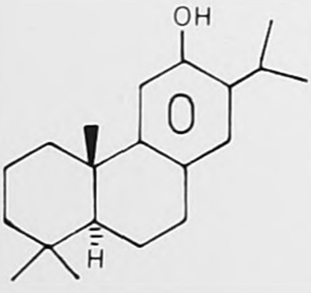
The C-8 and C-9 substituents were subsequently introduced as fragments which could be connected by 13,14-bond formation if a C-ring was desired or modified without 13,14-bond formation if bicyclic derivatives were the goal. These fragments would also contain groups R_6 and R_7 which could become C-ring substituents or additional rings. Sugiol (2), nimbiol (3), ferruginol (4), dehydroabietic acid (5) and carnosic acid (6) are among the compounds synthesized following this general route.



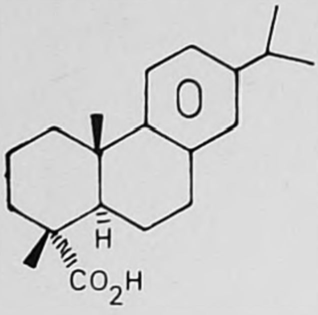
(2)



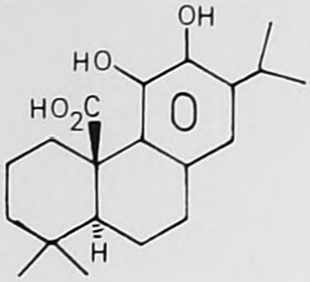
(3)



(4)



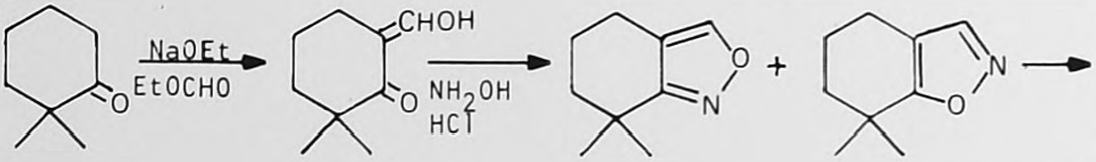
(5)



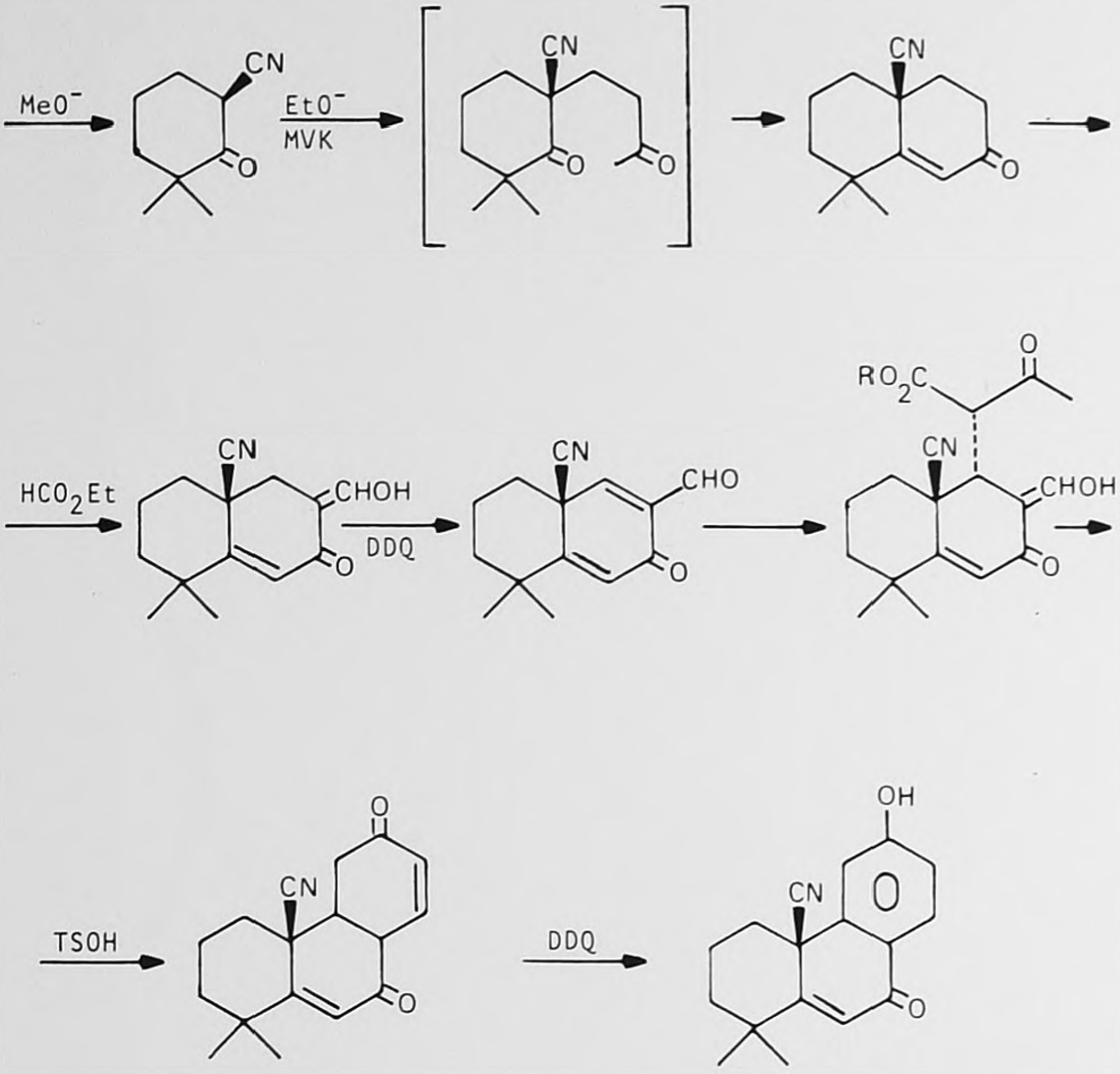
(6)

Three examples of application of this general synthetic route followed by Meyer et al are given below (schemes 1, 2, 3, 4).

i) Total synthesis of 10-cyano-12-hydroxy-7-oxo-17-norpodocarpa-5,8,11,13-tetraene[1] (7), scheme 1.



Scheme 1



(7)

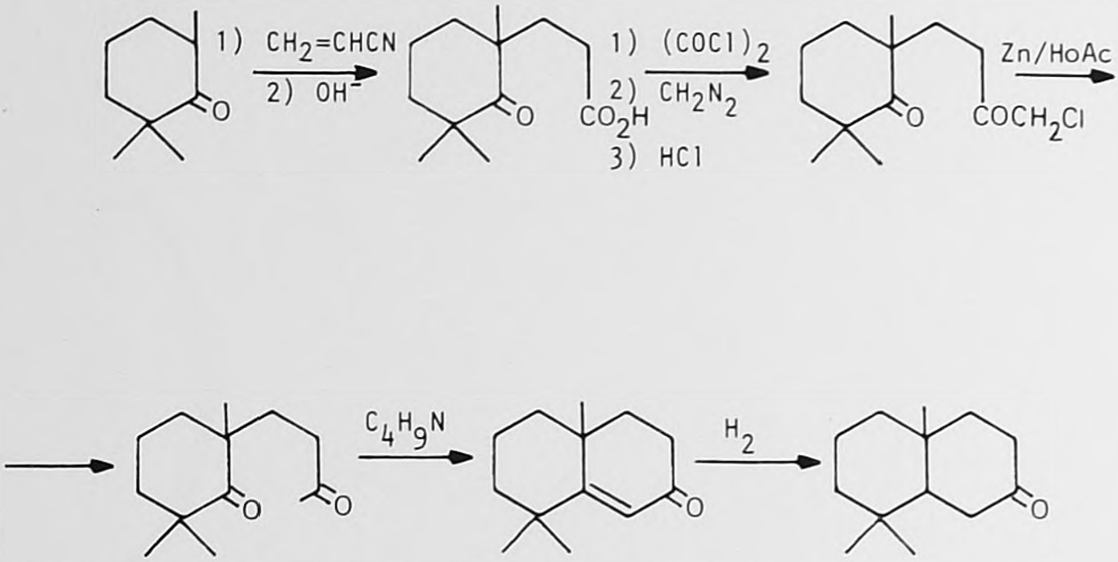
DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

MVK = methyl vinyl ketone.

Scheme 1 (Contd)

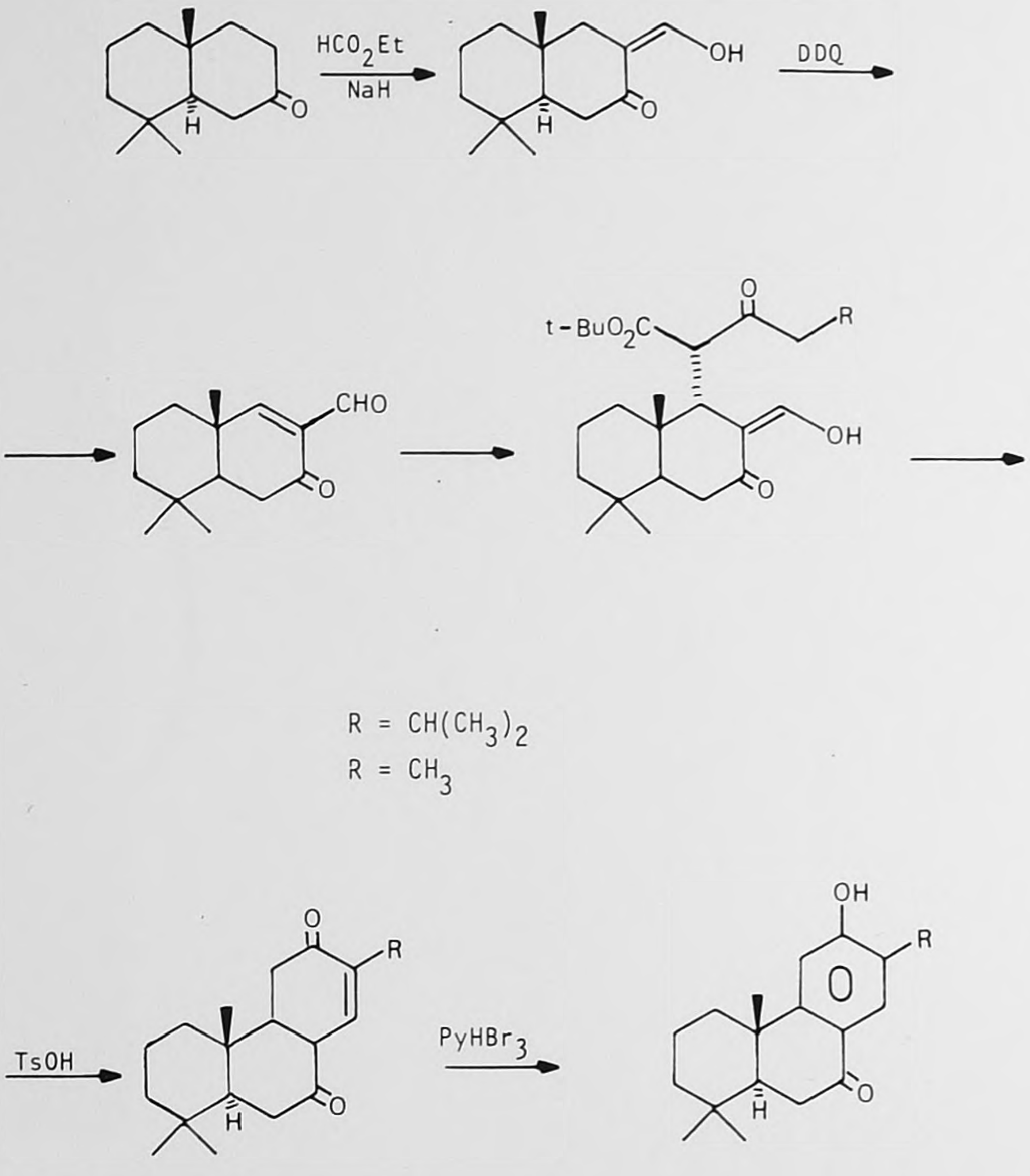
ii) Total synthesis of dl-sugiol (2), dl-nimbiol (3) and dl-ferruginol (4)[2], schemes 2 and 3.

The first part of the synthesis concerned the preparation of 4,4,10-trimethyl-7-decalone (8,8a) (scheme 2), followed by the synthesis of the three diterpenoids from 4,4,10-trimethyl-trans-7-decalone (8), scheme 3.



(8) trans
(8a) cis

Scheme 2

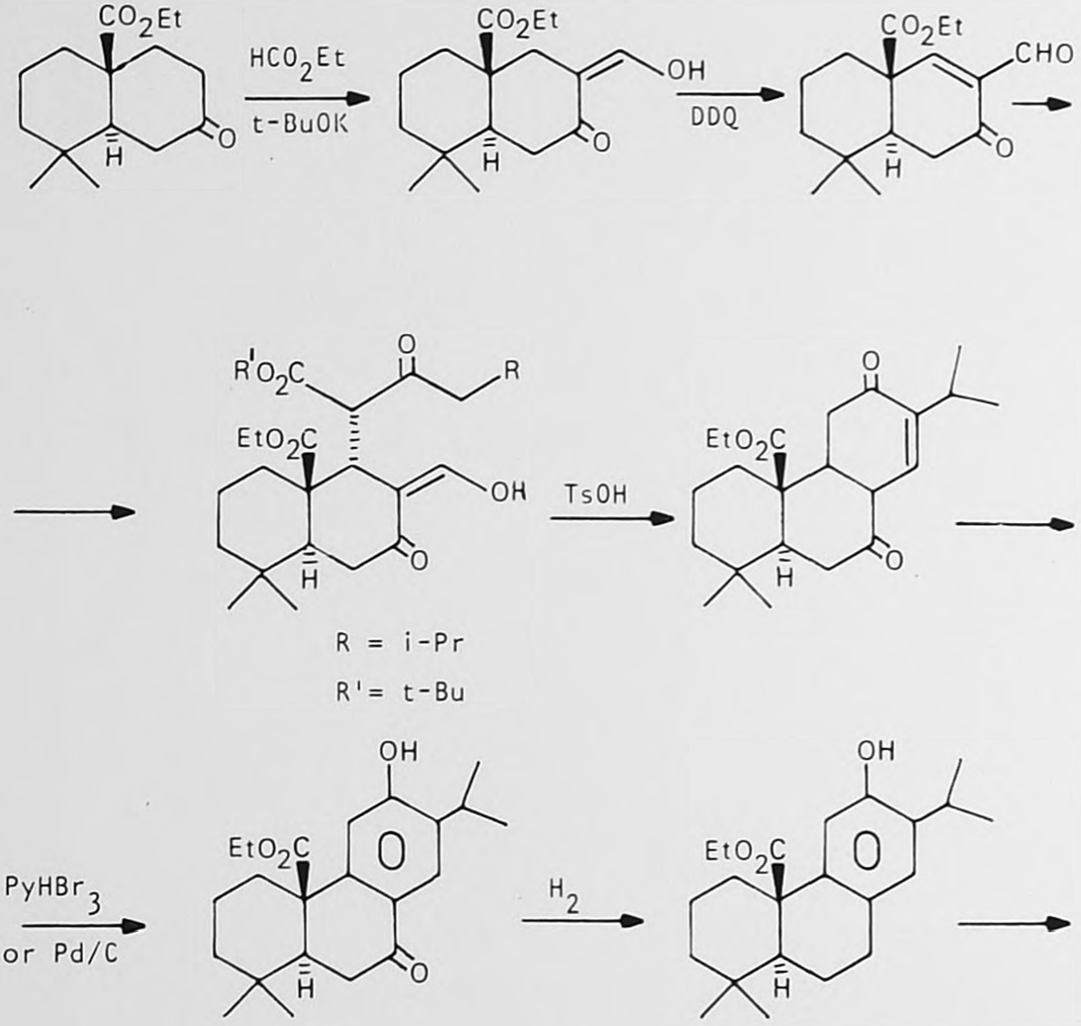


R = $CH(CH_3)_2$
R = CH_3

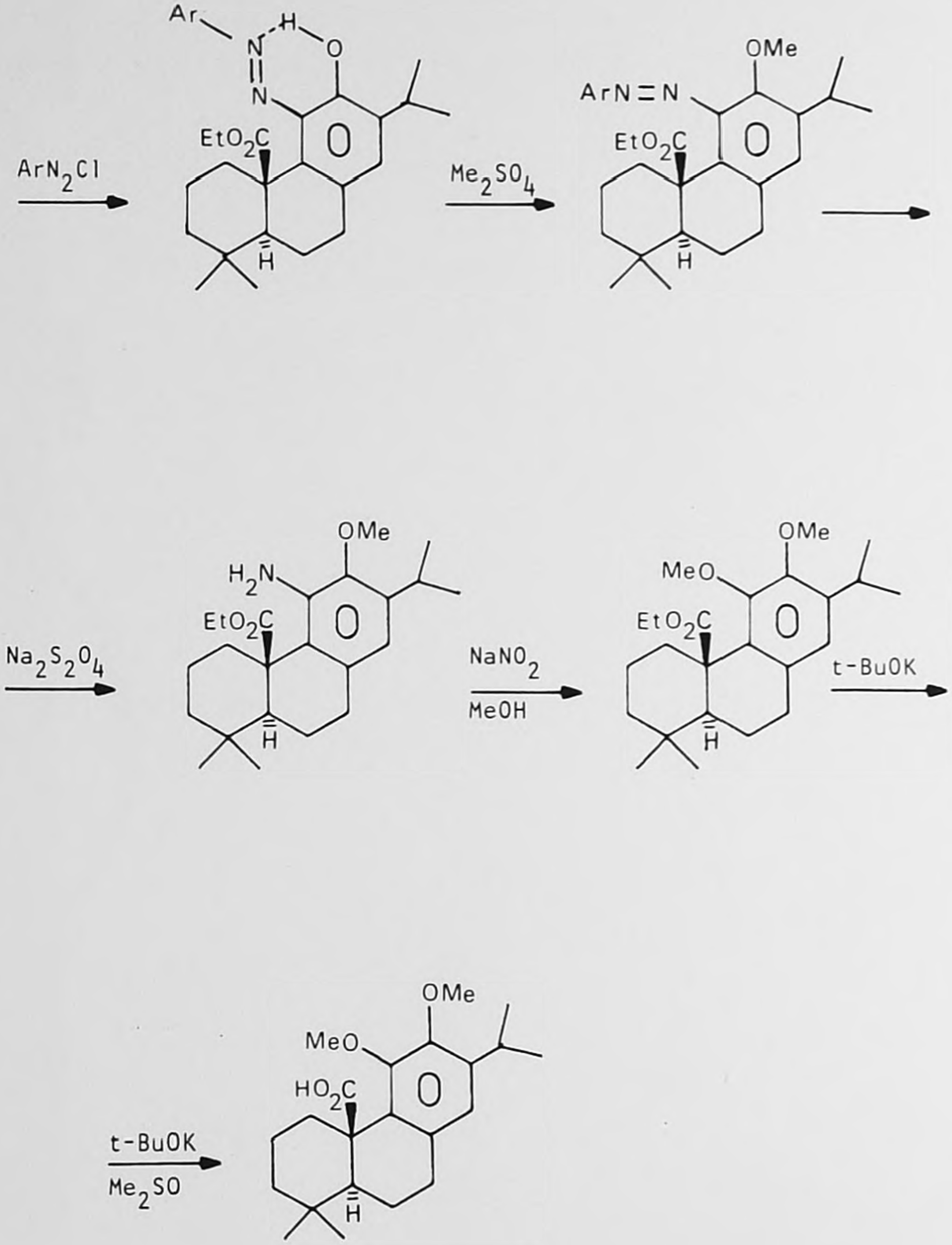
Scheme 3

Hydrogenolysis of dl-sugiol afforded dl-ferruginol (4).

iii) Synthesis of (±) carnosic and dimethyl ether[3] (9).



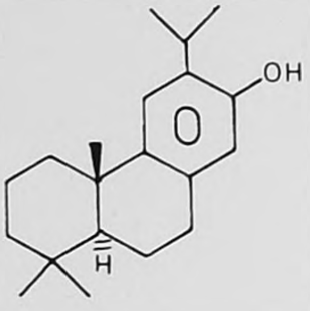
Scheme 4



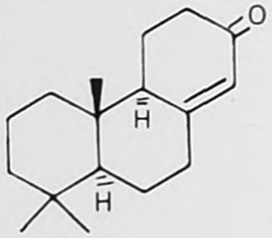
(9)

Scheme 4 (Contd)

An example of the AC → ABC approach is demonstrated in the total synthesis of (+) ferruginol (4), (+) sempervirol (10) and (+) Podocarpa-8(14)-en-13-one (11) by Matsumoto and Usui[5], scheme 5.

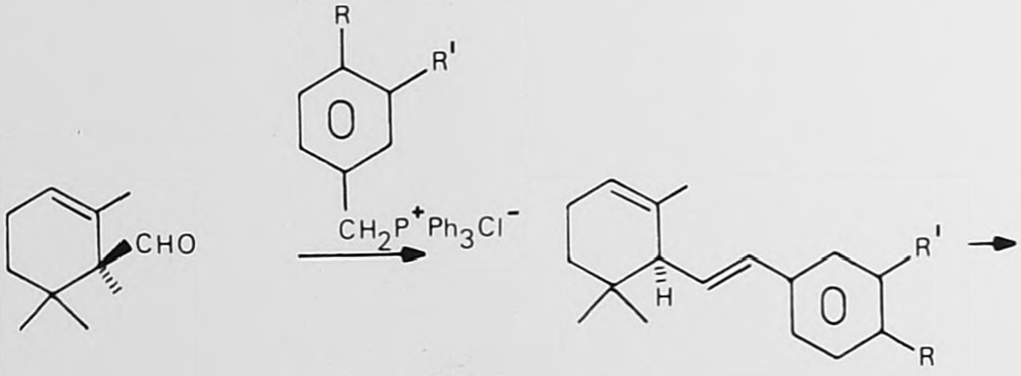


(10)



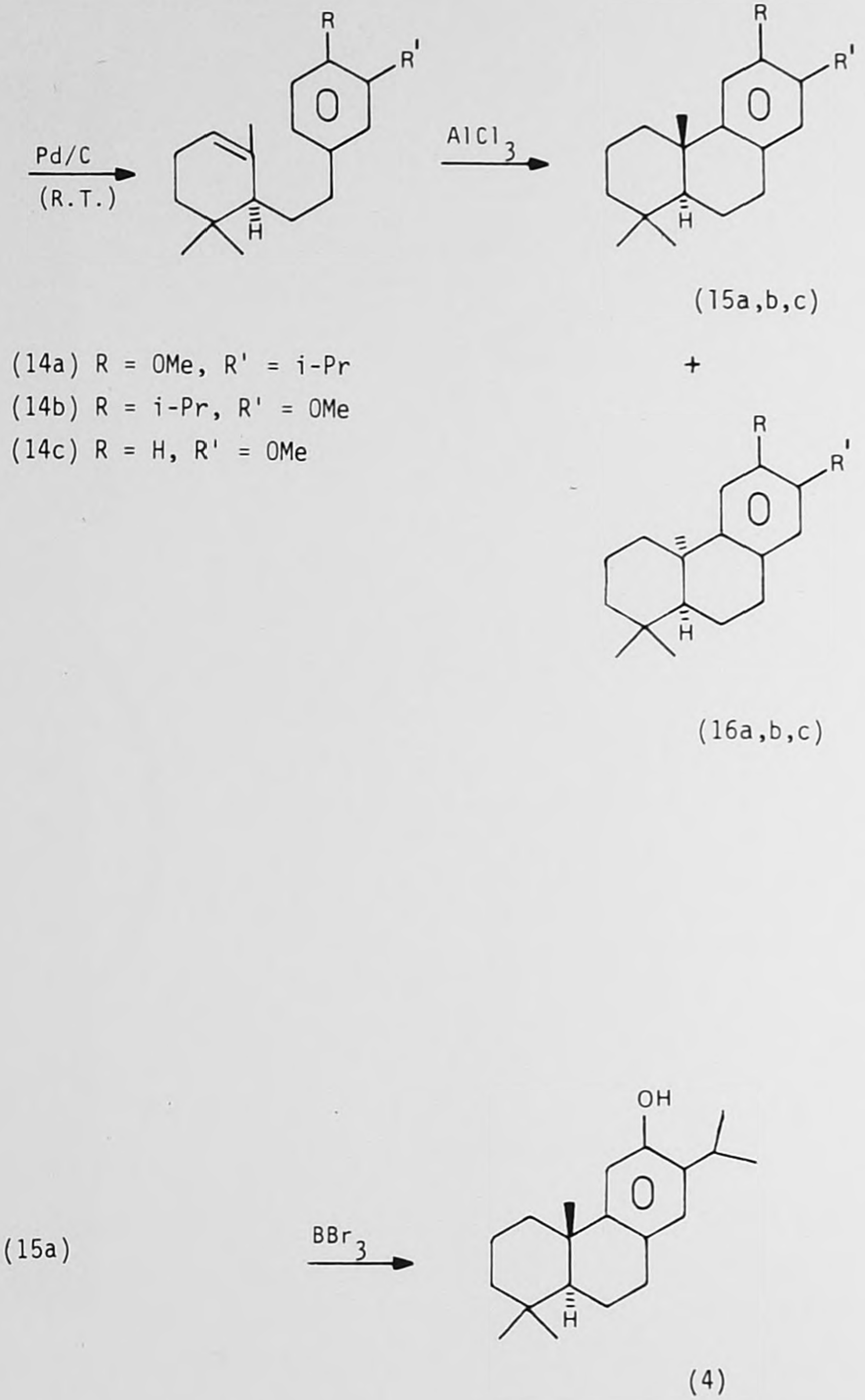
(11)

In this case the authors have employed a Wittig reaction as a key step in the general synthetic route.



(12)

(13a) R = OMe, R' = i-Pr
(13b) R = i-Pr, R' = OMe
(13c) R = H, R' = OMe



(15a,b,c)

\+

(16a,b,c)

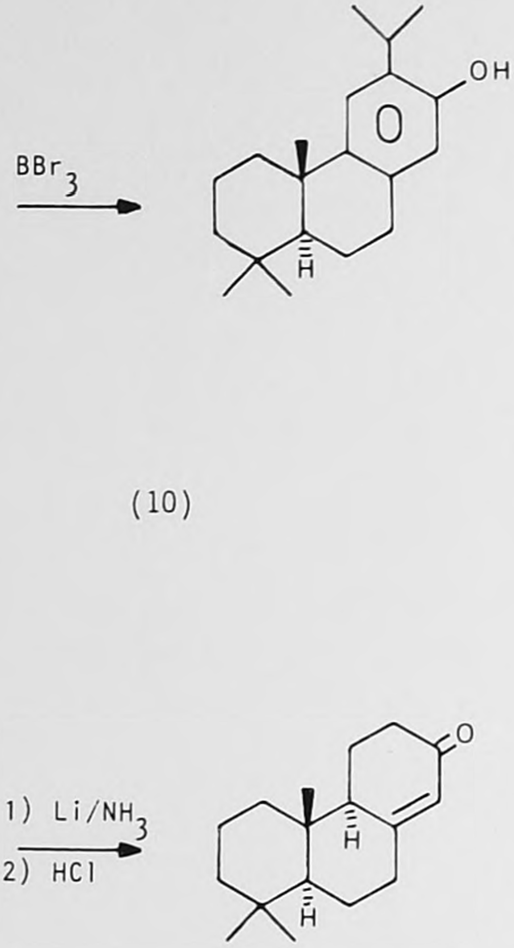
(14a) R = OMe, R' = i-Pr
(14b) R = i-Pr, R' = OMe
(14c) R = H, R' = OMe

(15a)

(4)

Scheme 5

(15b)



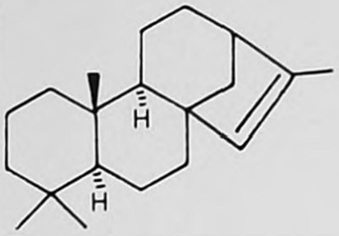
(10)

(15c)

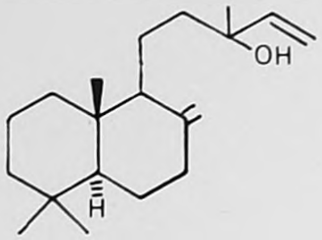
(11)

Scheme 5 (Contd)

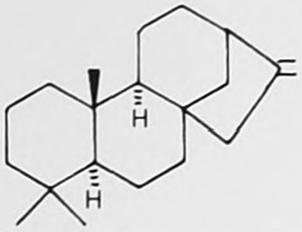
(+)-Podocarpa-8(14)-en-13-one (11) was shown to be a versatile intermediate for diterpene synthesis. This compound has already been transformed into several diterpenoids such as isophyllocladene (17), manool (18), phylocladene (19) and others.



(17)

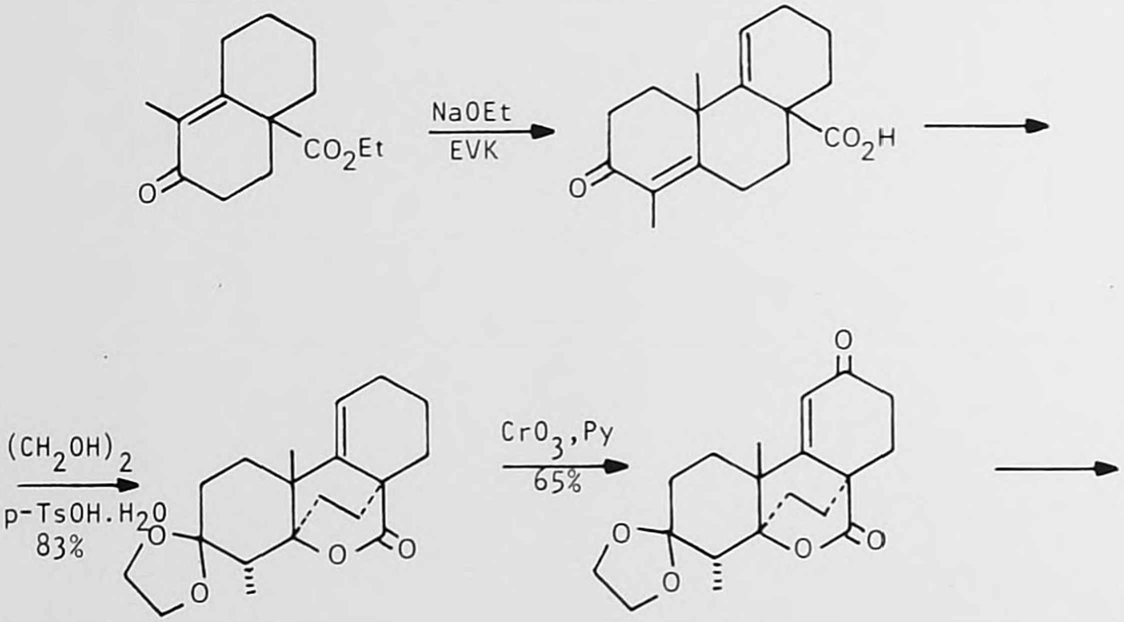


(18)

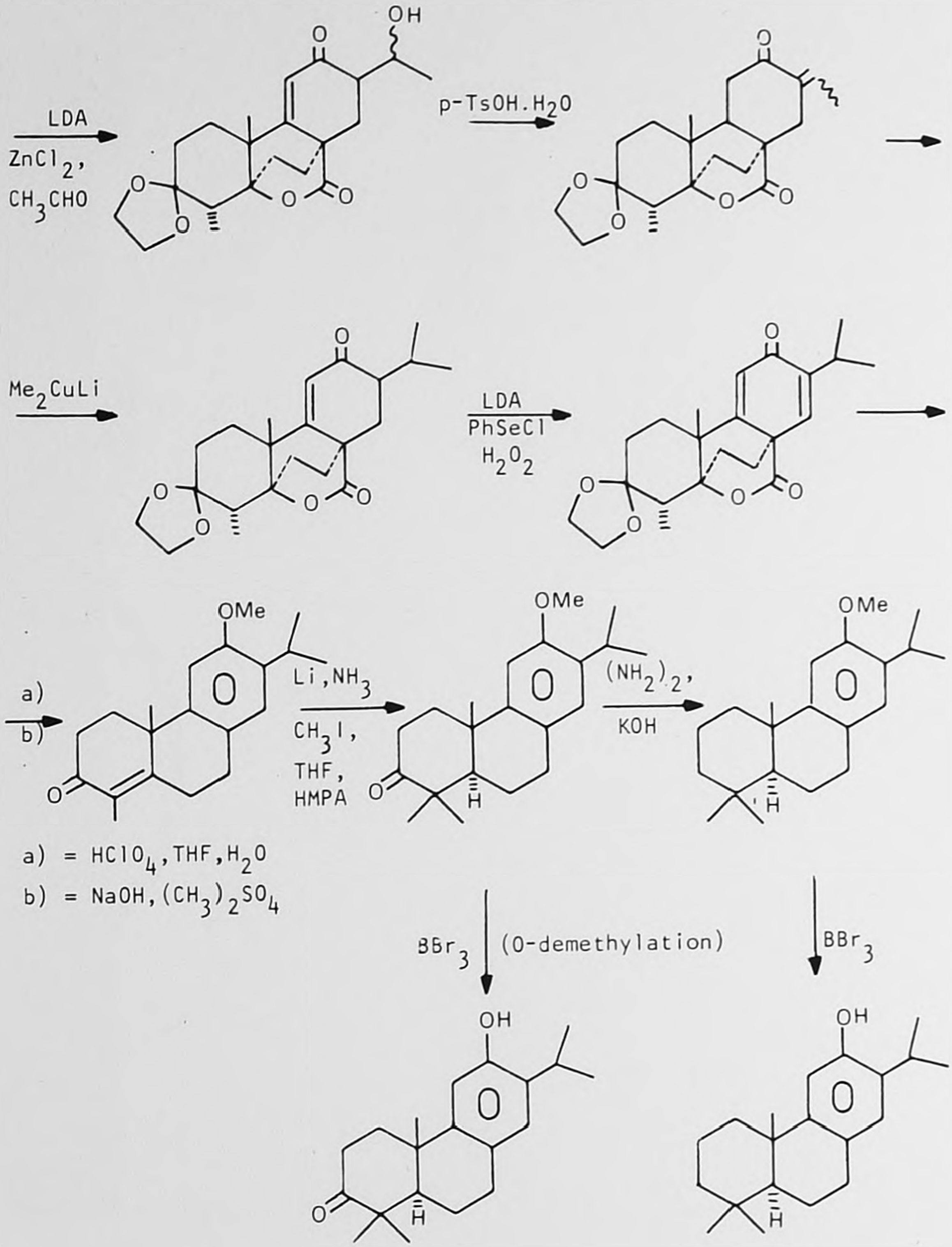


(19)

Snitman *et al*[6] reported another total synthesis of (+) ferruginol (4) together with (±) hinokione (20) in which the tricyclic ring system was assembled in the order C → BC → ABC, scheme 6.



Scheme 6

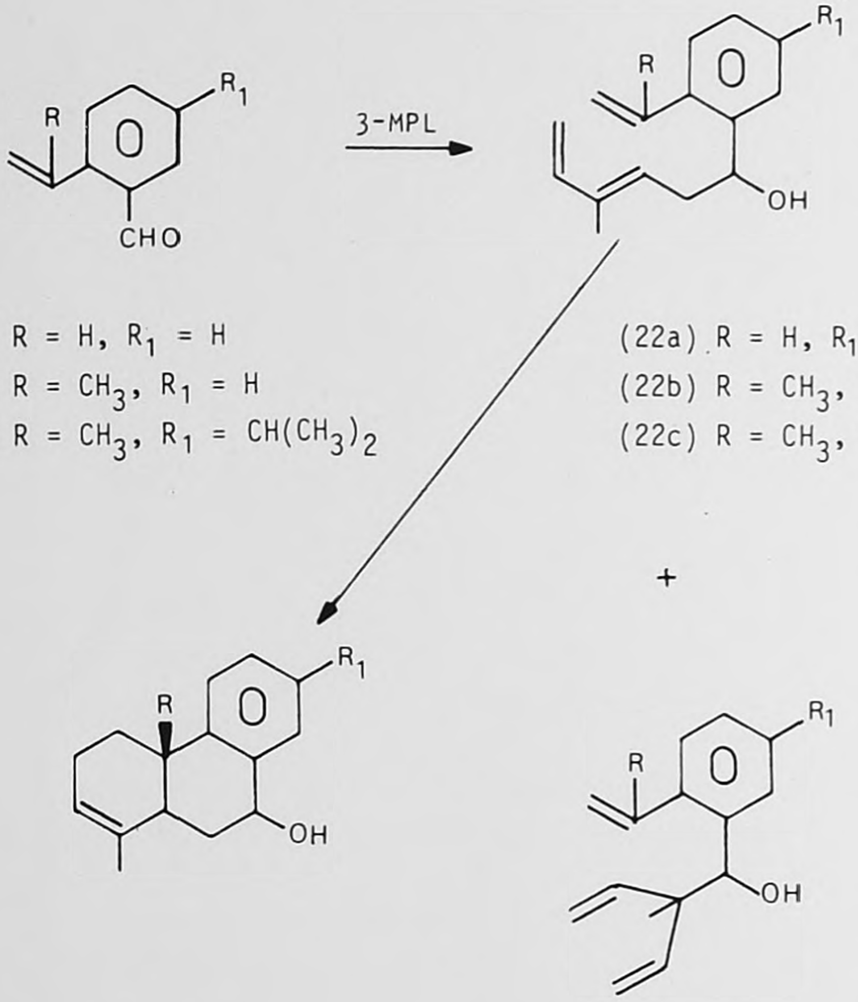


(20)

(4)

Scheme 6 (Contd)

An intramolecular Diels-Alder approach to diterpene skeleton synthesis which involved the simultaneous formation of two six-membered rings and an angular methyl group was described by Wilson and Mao[7]. The requisite dienes for the Diels-Alder reactions were readily obtained via the addition of 3-methylpentadienyllithium (3-MPL) to the aldehydes (21a), (21b) and (21c) to give (22a), (22b) and (22c) and (23a), (23b) and (23c).



(21a) R = H, R_1 = H
(21b) R = CH_3, R_1 = H
(21c) R = CH_3, R_1 = $CH(CH_3)_2$

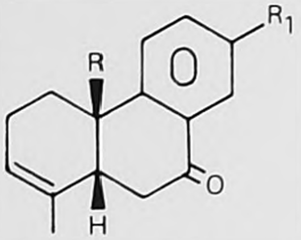
(22a) R = H, R_1 = H
(22b) R = CH_3, R_1 = H
(22c) R = CH_3, R_1 = $CH(CH_3)_2$

(24a) (72% yield)
(24b)
(24c)

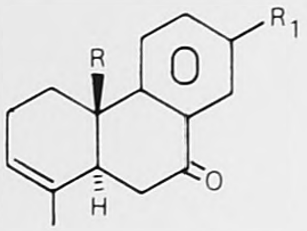
(23a) R = H, R_1 = H
(23b) R = CH_3, R_1 = H
(23c) R = CH_3, R_1 = $CH(CH_3)_2$

(22a) cyclized readily at 100°C to give (24a).

(22b) and (22c) didn't cyclize so easily, because of the presence of the angular CH_3 group. However, the corresponding trimethylsilyl ethers underwent intramolecular Diels-Alder reaction at 180°/24 h to give the expected adducts (silyl ethers) which were hydrolyzed (KF/CH_3OH) and oxidized (Jones) to give (25b) and (25c) and (26b) and (26c). 24(a) was also oxidized (Jones) to give 25(a) and 26(a).



(25a) $R = H, R_1 = H$
(25b) $R = CH_3, R_1 = H$
(25c) $R = CH_3, R_1 = CH(CH_3)_2$



(26a) $R = H, R_1 = H$
(26b) $R = CH_3\ \ R_1 = H$
(26c) $R = CH_3, R_1 = CH(CH_3)_2$

	cis/trans	yield
Ratios:	25a/26a - 4:1	from (22a) - 72%
	25b/26b - 2:1	from (22b) - 43%
	25c/26c - 1:1	from (22c) - 77%.

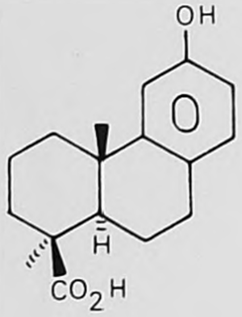
It is important to point out that there was not a requirement for an activating substituent on the diene or the dienophile.

In the case of the synthesis of diterpenoid resin acids such as podocarpic acid (27), dehydroabietic acid (28), and others the problem of stereoselective introduction of methyl and carboxyl substituents at the quaternary 4-position in the correct configuration was attacked by several authors in several ways:

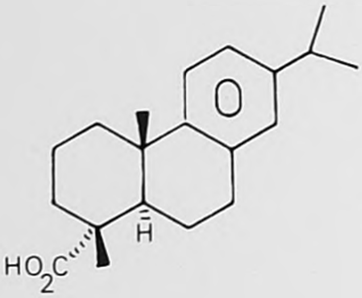
(i) through stereoselective alkylation of a bi- or tricyclic 3-keto or Δ^4-3-keto derivatives, e.g. total synthesis of d-podocarpic acid[8] (27), schemes 7 and 8 of dl-dehydroabietic acid[9,10] (28), scheme 9 and of (±) callitrisic acid (29) and (±) podocarpic acid[11], scheme 10.

(ii) through generation of the angular C-10 asymmetric center by closure of the 9,10-bond in an aromatic alkylation process after the C-4 substitution pattern has been established, e.g. synthesis of desoxypodocarpic acid[12] (30), scheme 11.

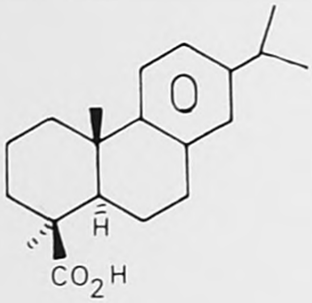
(iii) through 1,4 stereoselective addition of HCN to a 4-methyl-Δ^3-2-keto intermediate (31): e.g. stereoselective total synthesis of podocarpic acid[13], scheme 12.



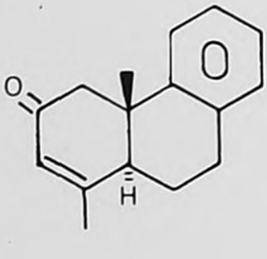
(27)



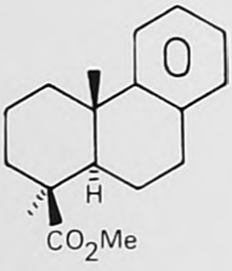
(28)



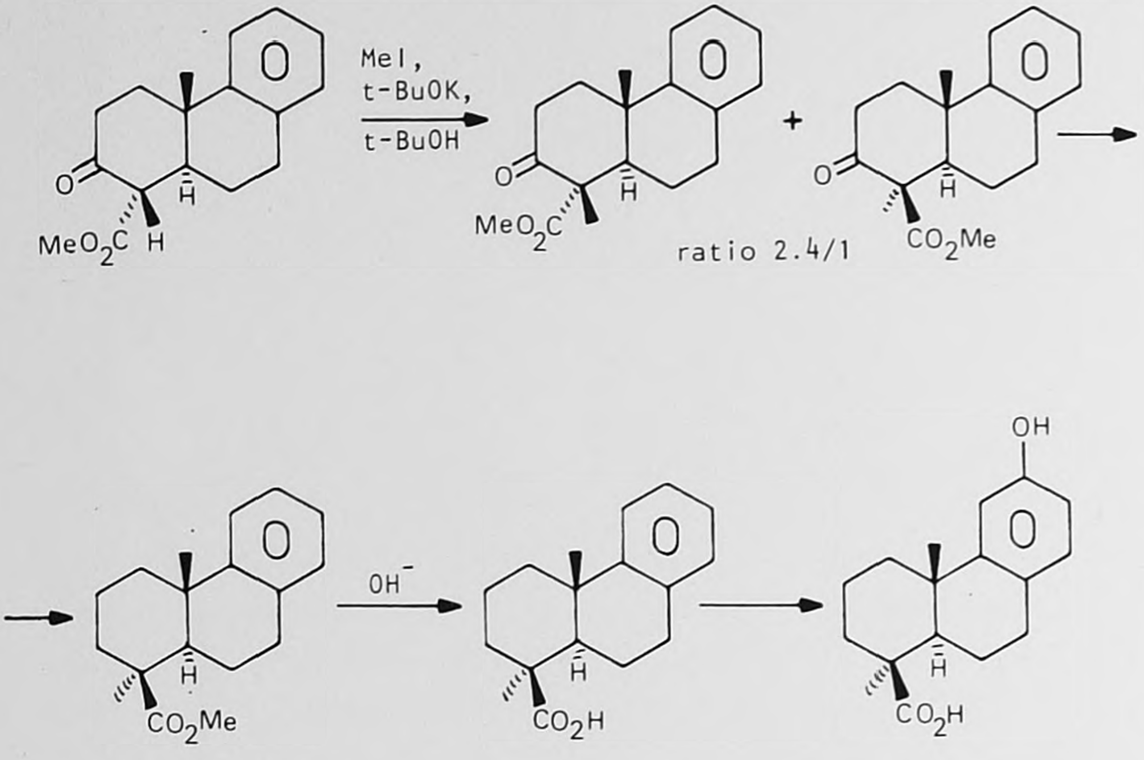
(29)



(31)



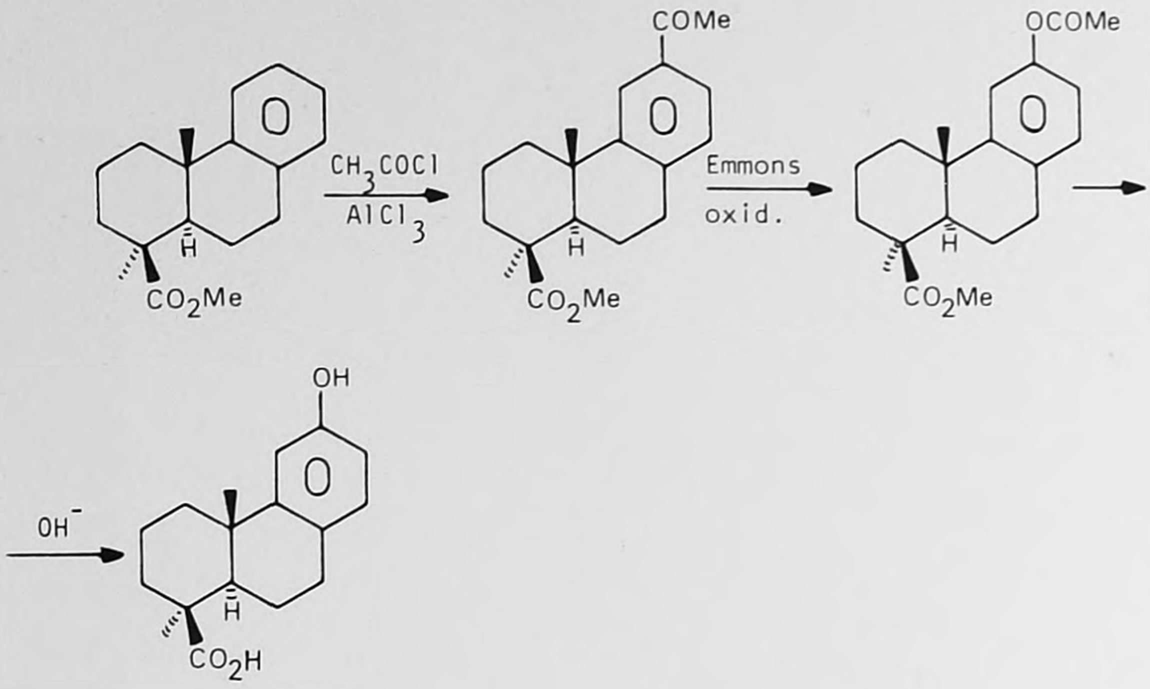
(30)



Scheme 7

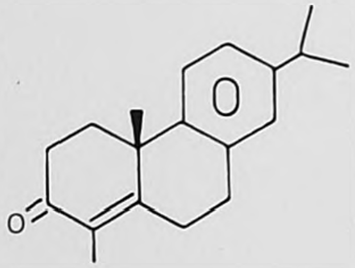
Exposure of (32) to cinchonine and crystallization of the mixture from aqueous methanol yielded an insoluble salt that treated with mild hydrochloric acid liberated d-desoxypodocarpic acid (32).

The conversion of d-desoxypodocarpic acid to podocarpic acid was previously described by Wenkert and Jackson[14] as follows:

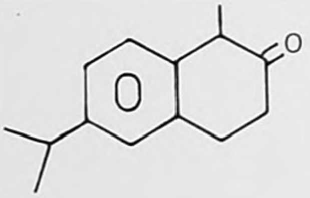


Scheme 8

Stork and Schulenberg[9,10] found the tricyclic ketone (33) a good intermediate for the introduction of the desired asymmetry at C_4 and C_5 and it thus became the first synthetic objective in the total synthesis of dl-dehydroabietic acid (28).

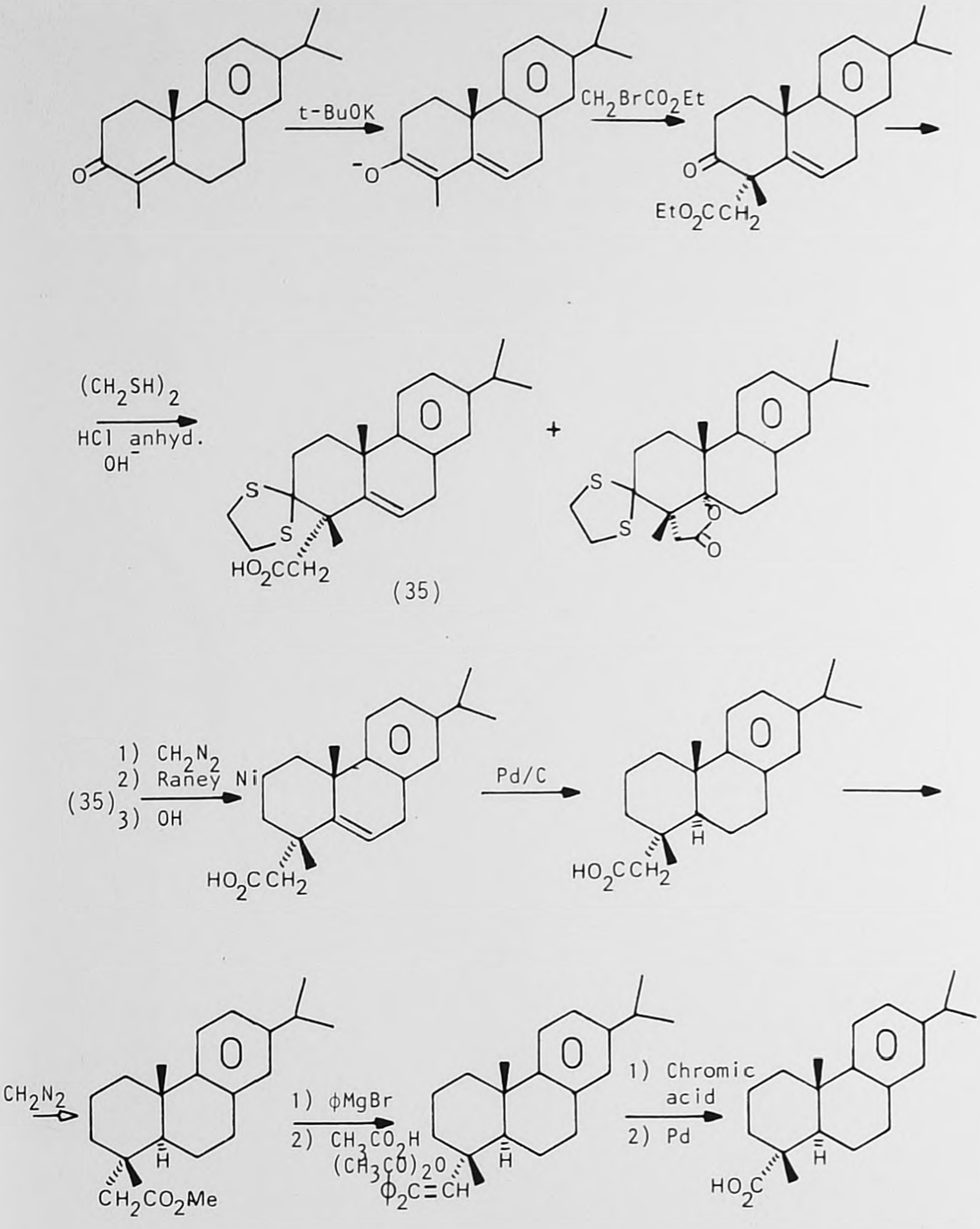


(33)



(34)

This ketone was synthesized from 1-methyl-6-isopropyl-2-tetralone (34) by addition of ethyl vinyl ketone in the presence of aqueous methanolic potassium hydroxide with 74% yield.

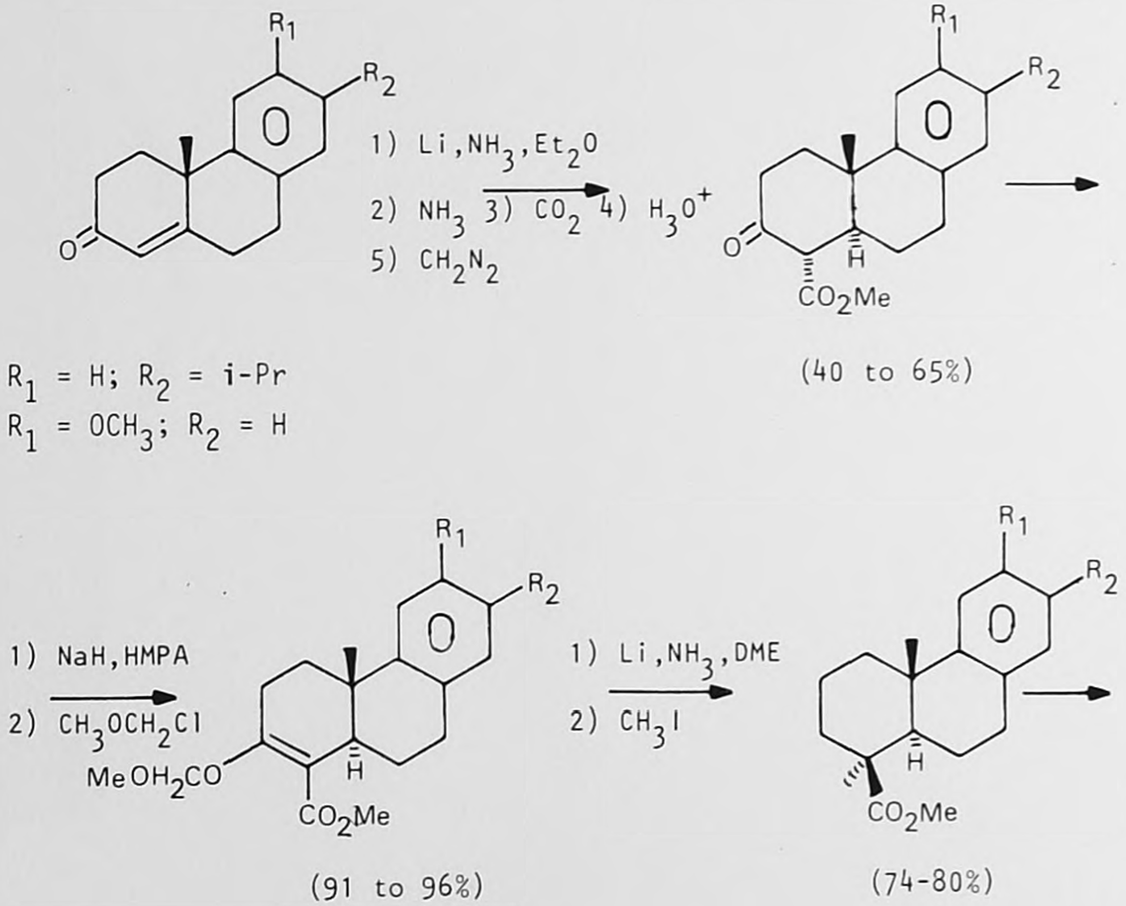


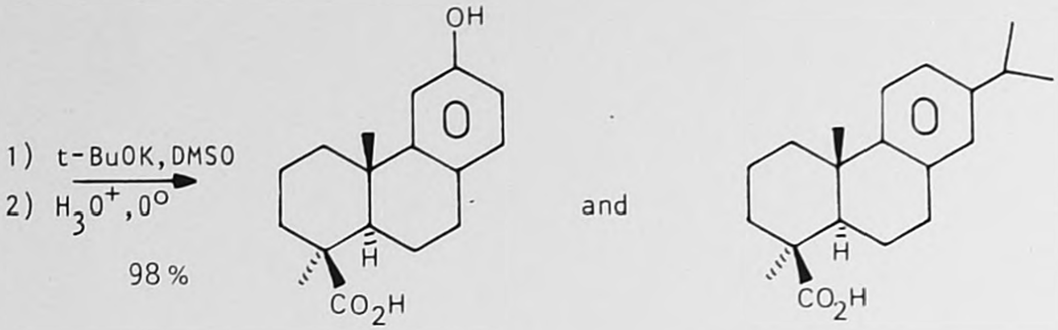
t-BuOK
CH2BrCO2Et
O
-O
EtO2CCH2
(CH2SH)2
HCl anhyd.
OH-
S
S
HO2CCH2
(35)
+
O
1) CH2N2
2) Raney Ni
3) OH
(35)
HO2CCH2
Pd/C
H
HO2CCH2
CH2N2
H
CH2CO2Me
1) φMgBr
2) CH3CO2H
(CH3CO)2O
φ2C=CH
H
1) Chromic acid
2) Pd
H
HO2C

Scheme 9

Welch <u>et al</u>[11] in their approach to a general synthetic procedure for the construction of podocarpane type natural products utilized a similar intermediate to the one used by Stork and Schulenberg[10]. However, the route followed a different course. They utilized a highly stereoselective reductive elimination-alkylation reaction for establishing the axial stereochemistry of the carbomethoxyl functional group.

(±) callitrisic acid and (±) podocarpic acid were synthesized by this procedure (scheme 10).

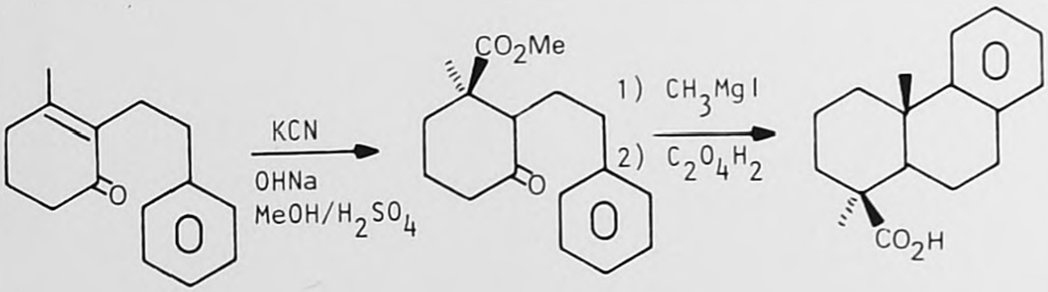




(27) (29)

Scheme 10 (Contd)

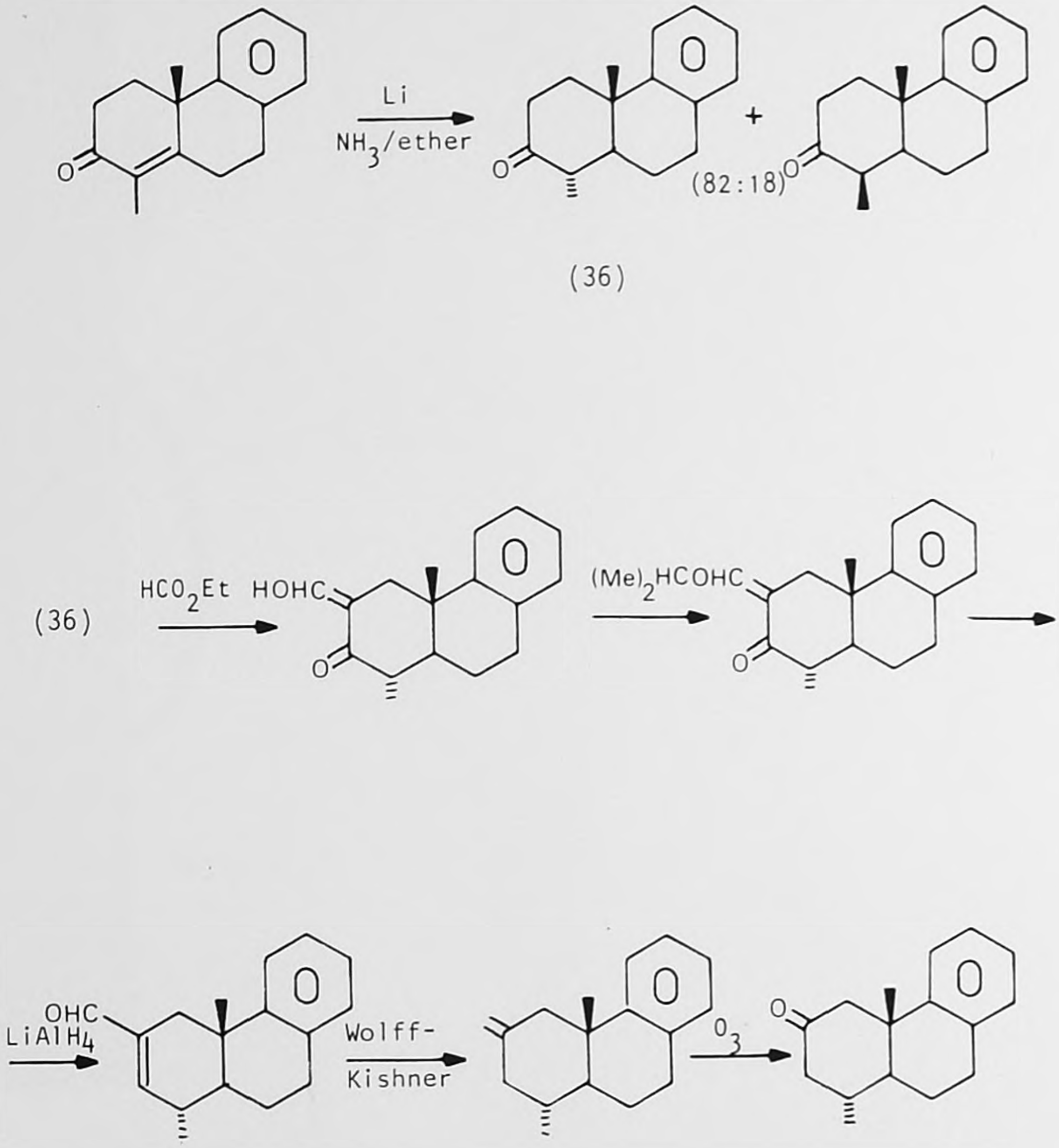
Ghatak et al[12] synthesized two enantiomers of desoxypodocarpic acid following the general procedure of generation of the angular C-10 asymmetric center by closure of the 9,10-bond in an aromatic alkylation after the C-4 substitution pattern has been established (scheme 11).

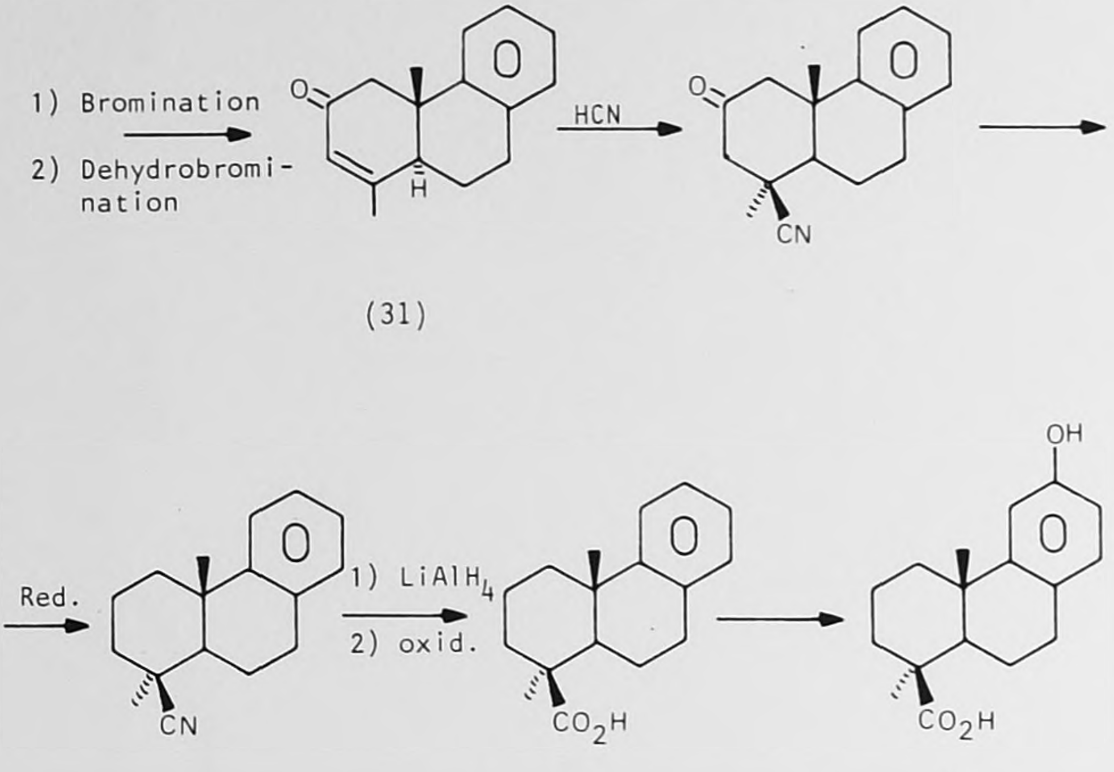


(30)

Scheme 11

Stereoselective total synthesis of podocarpic acid by Meyer and Maheshwari[13] is shown in scheme 12.



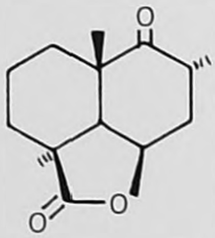


(31)

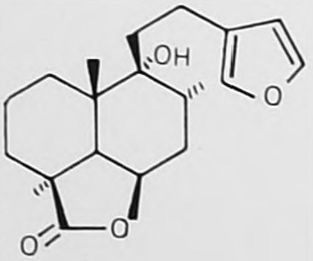
(27)

Scheme 12

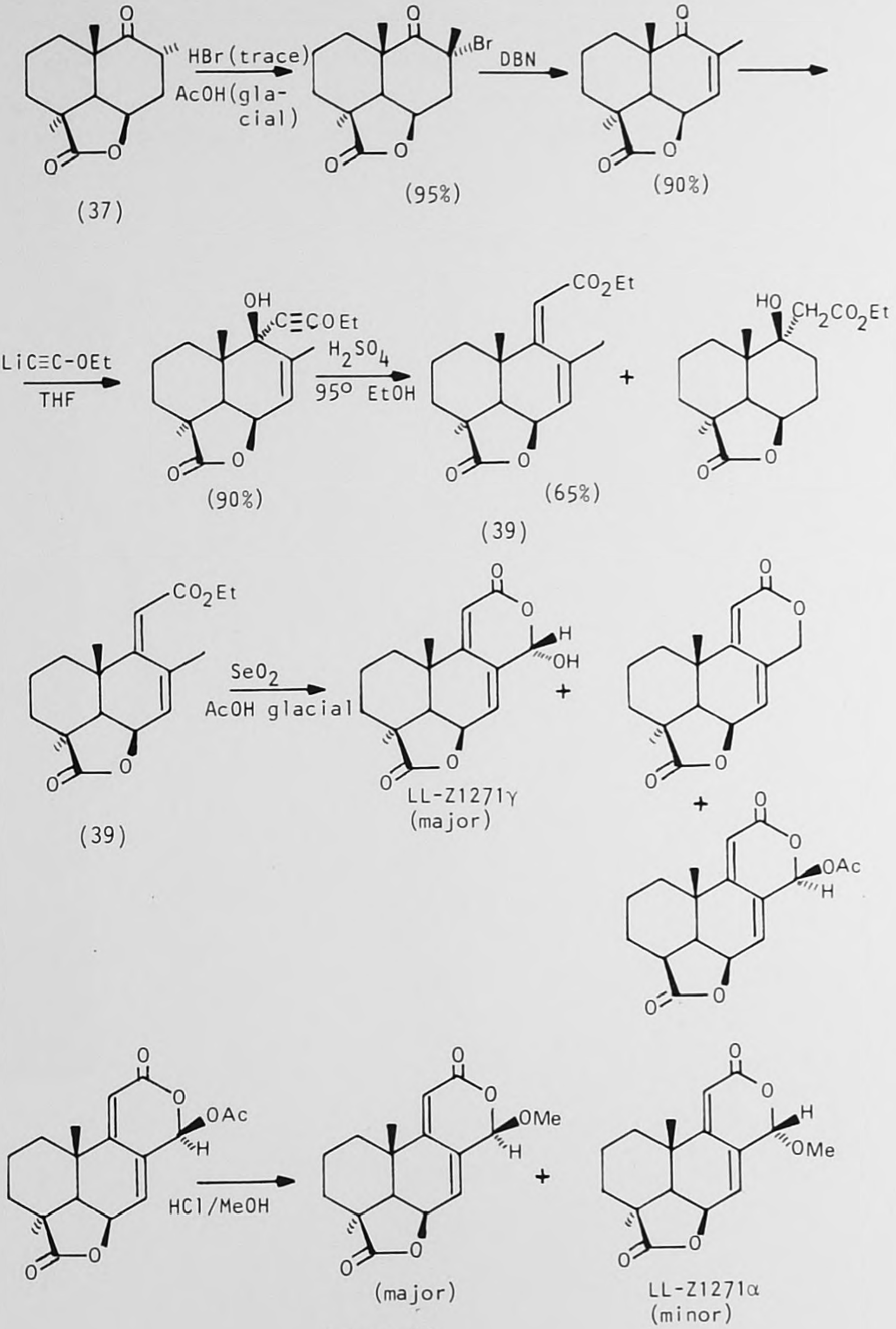
The synthesis of the diterpenoid antibiotic LL-Z1271α (50 chap. 1) was described by Adinolfi *et al*[15]. The starting material used was (+) ketolactone (37) easily available by degradation of marrubiin (38).



(37)

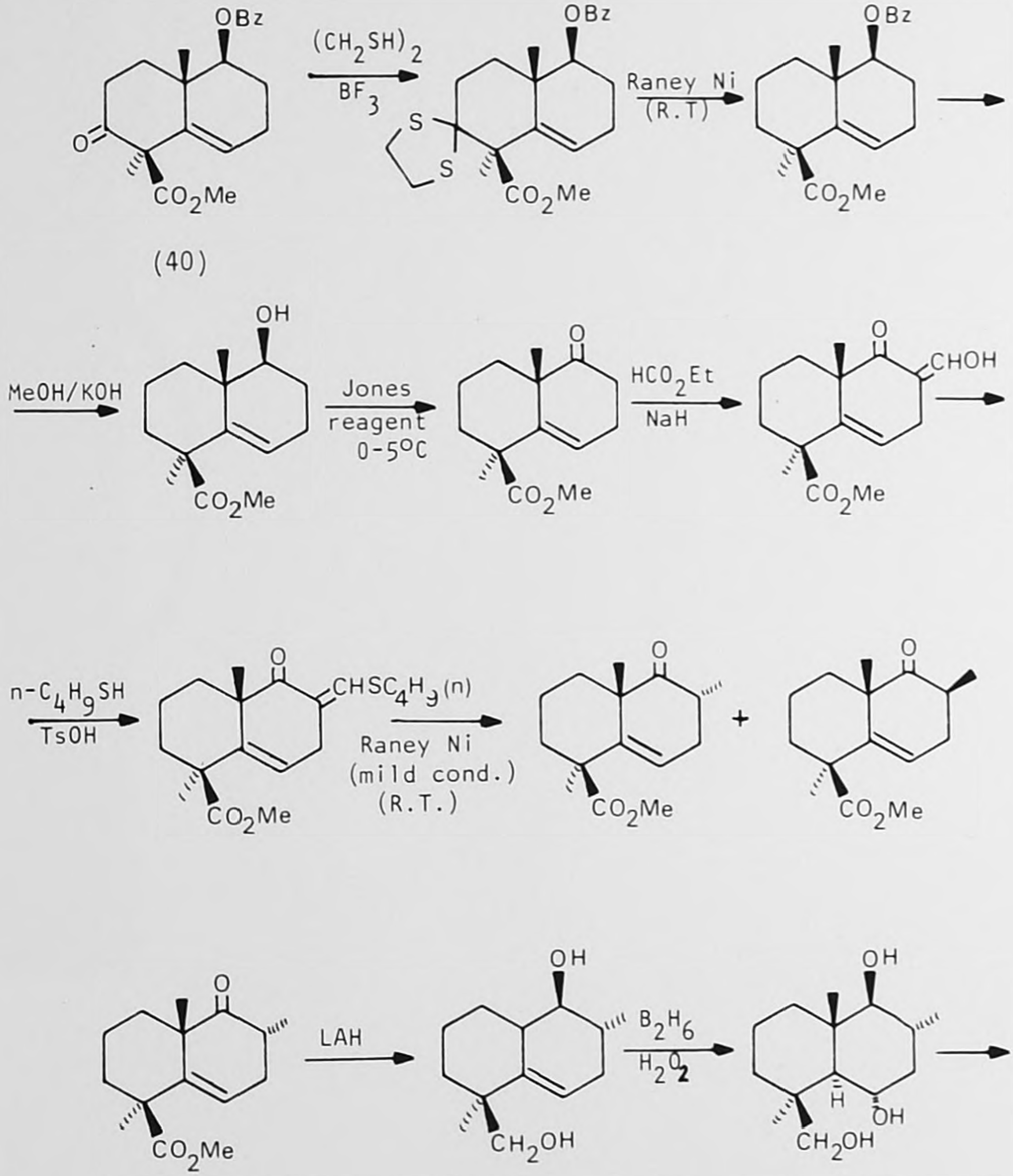


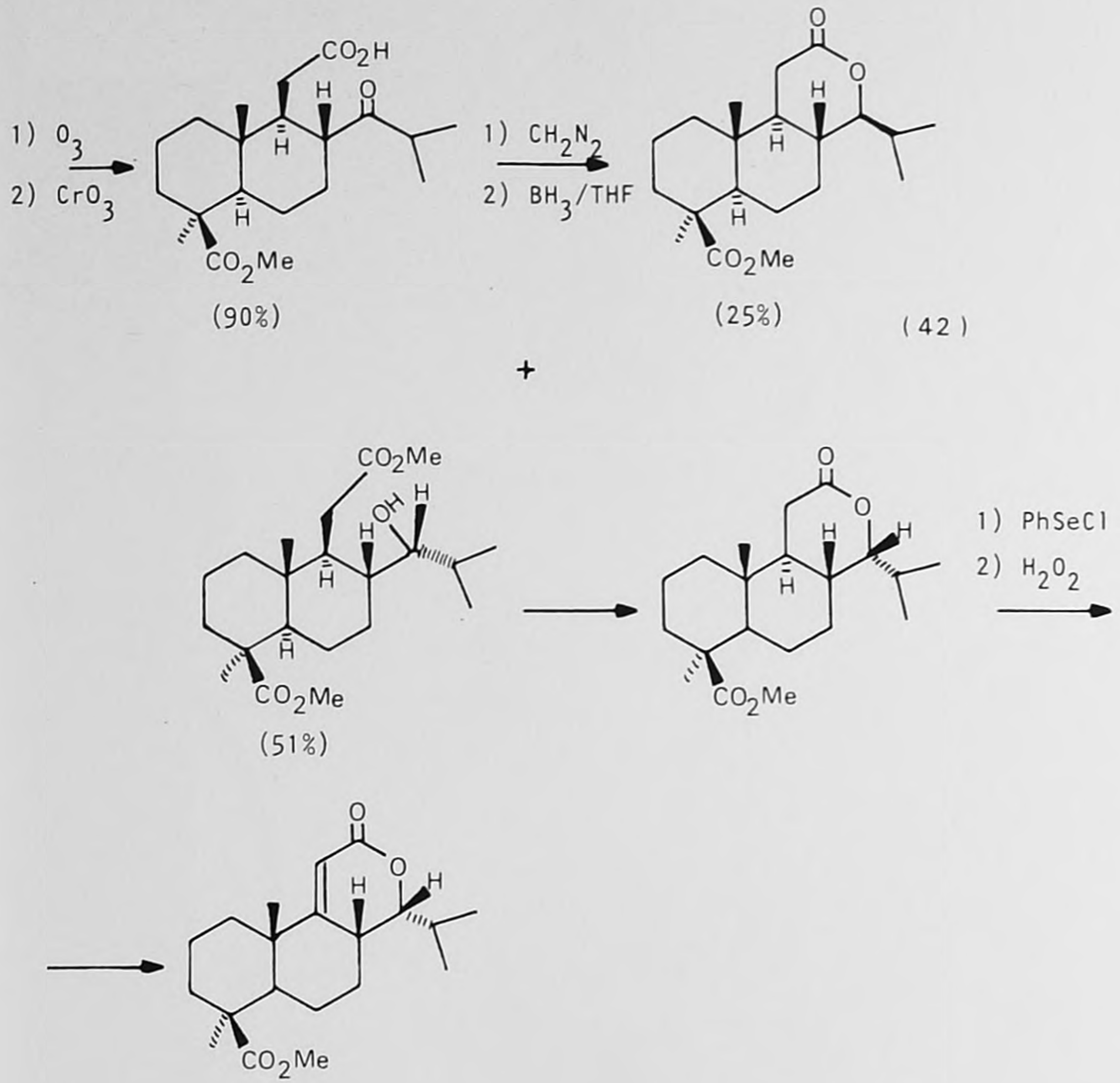
(38)


(37)
HBr(trace)
AcOH(glacial)
Br
(95%)
DBN
(90%)
LiC≡C-OEt
THF
OH
C≡COEt
(90%)
H2SO4
95° EtOH
CO2Et
(65%)
(39)
HO
CH2CO2Et
(39)
SeO2
AcOH glacial
OH
H
LL-Z1271γ
(major)
OAc
H
HCl/MeOH
OMe
(major)
LL-Z1271α
(minor)

Scheme 13

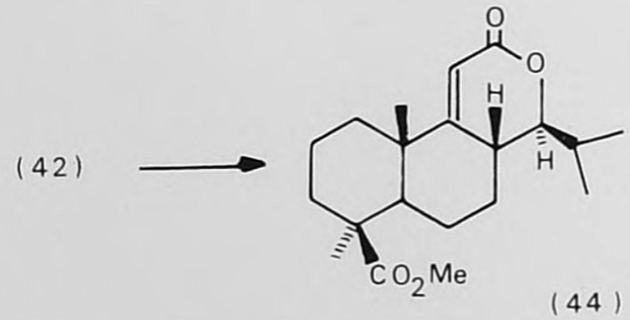
Adinolfi[16] also reported the synthesis of the ketolactone (37) from the keto ester (40) which has the carbomethoxyl group correctly oriented with respect to the angular methyl, an oxygenated function at C_9 and the 5,6 double bond suitably placed for the introduction of the other oxygenated function at C_6, scheme 14.



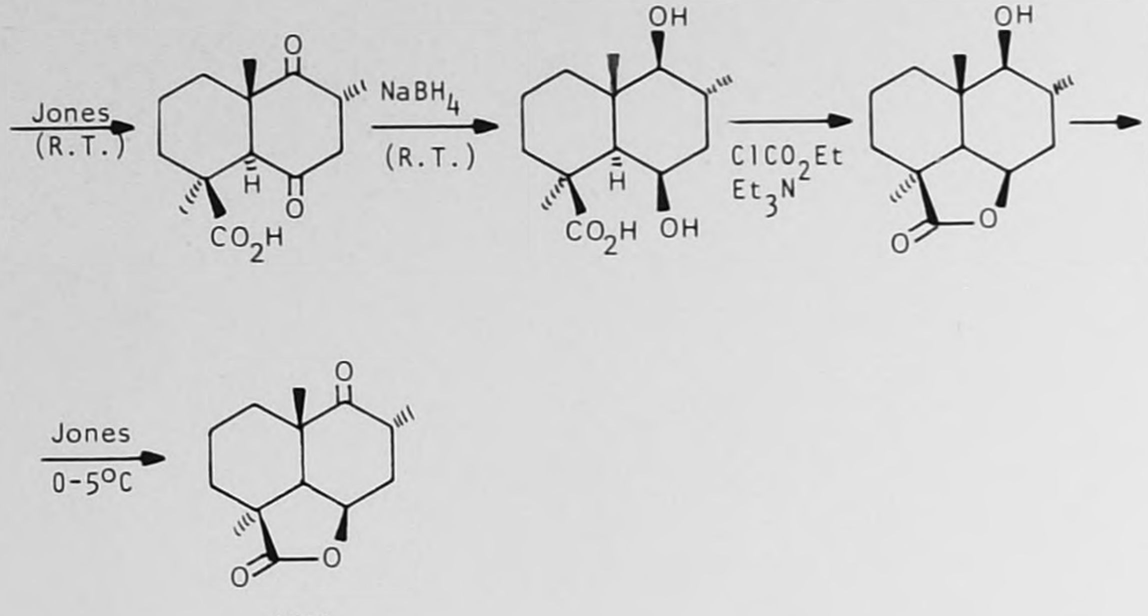

1) O3
2) CrO3
CO2H
H
O
H
H
CO2Me
(90%)
1) CH2N2
2) BH3/THF
O
O
H
H
H
CO2Me
(25%)
+
CO2Me
H
OH
H
H
H
CO2Me
(51%)
O
O
H
H
H
CO2Me
1) PhSeCl
2) H2O2
O
O
H
H
CO2Me

(42)

(43)


(42)
O
O
H
H
CO2Me

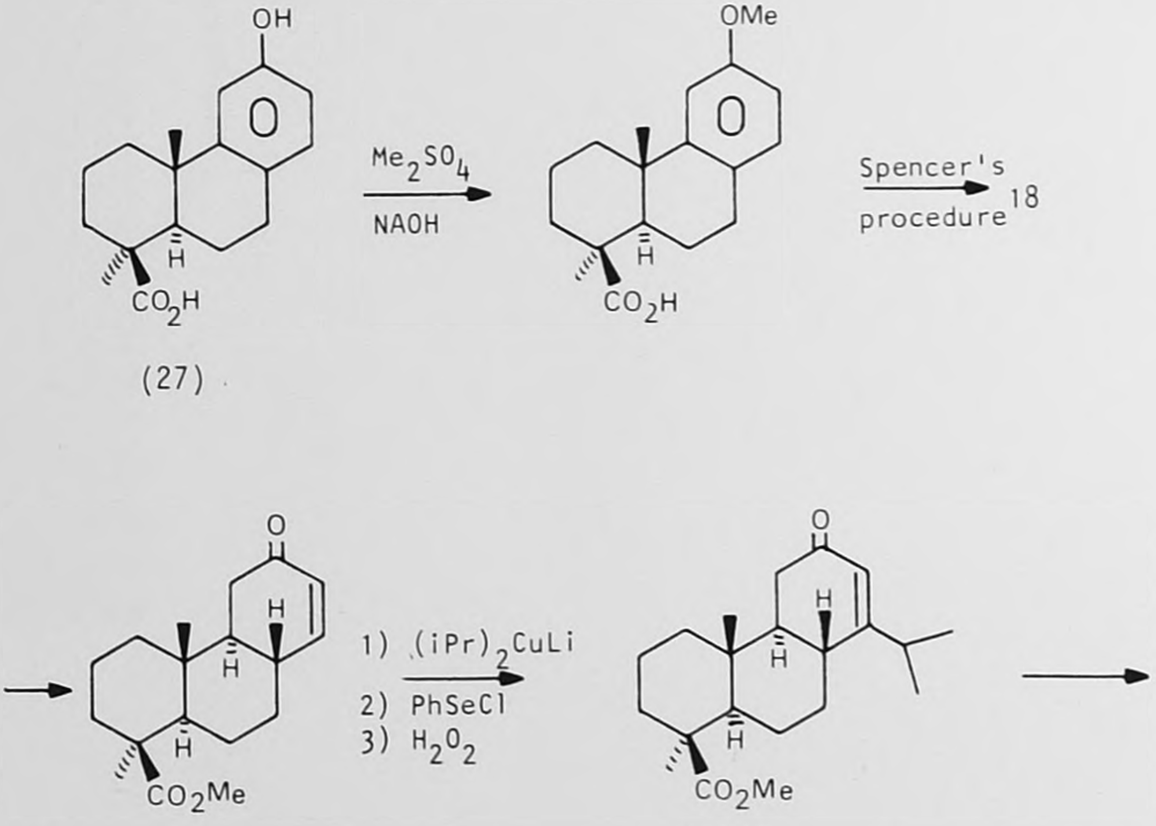
(44)



(37)

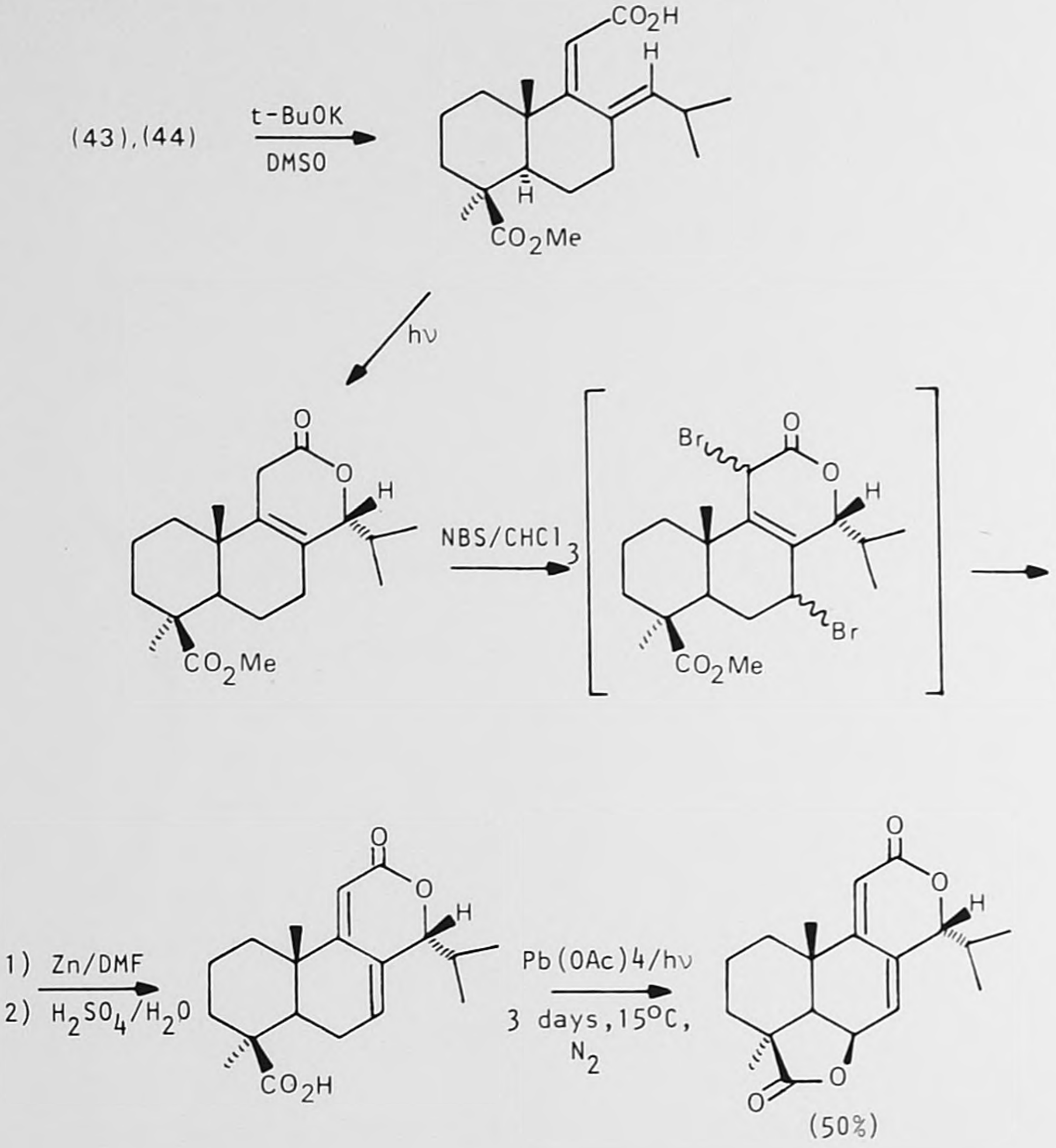
Scheme 14

Hayashi *et all*[17] achieved for the first time the total synthesis of the biologically active nagilactone F (41)(42, chap. 1), from (+) podocarpic acid, scheme 15.



(27)

(55%)


(43),(44)
t-BuOK
DMSO
CO2H
H
H
CO2Me
hν
O
O
H
CO2Me
NBS/CHCl3
Br
O
O
H
Br
CO2Me
1) Zn/DMF
2) H2SO4/H2O
O
O
H
CO2H
Pb(OAc)4/hν
3 days,15°C,
N2
O
O
H
O
O
(50%)

(41)

Scheme 15

5.3 References

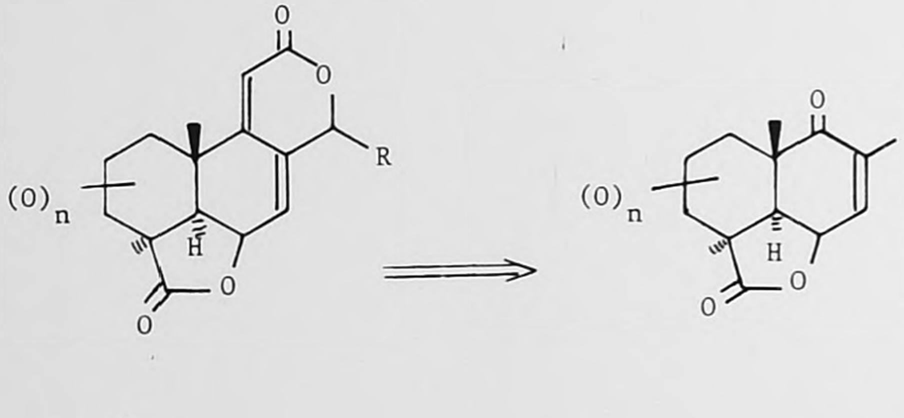
1. W.L. Meyer, R.W. Huffman, and P.G. Schroeder, *Tetrahedron*, 1968, **24**, 5959.
2. W.L. Meyer, G.B. Clemans, and R.A. Manning, *J. Org. Chem.*, 1975, **40**, 3686.
3. W.L. Meyer, R.A. Manning, E. Schindler, R.S. Schroeder, and D.C. Shew, *J. Org. Chem.*, 1976, **41**, 1005.
4. W.L. Meyer, and C.W. Sigel, *J. Org. Chem.*, 1977, **42**, 2769.
5. T. Matsumoto, and S. Usui, *Bull. Chem. Soc. Japan*, 1979, **52**, (1), 212.
6. D.L. Snitman, R.J. Himmelsbach, and D.S. Watt, *J. Org. Chem.*, 1978, **43**, 4758.
7. S.R. Wilson, D.T. Mao, *J. Org. Chem.*, 1979, **44**, 3093.
8. E. Wenkert and A. Tahara, *J. Am. Chem. Soc.*, 1960, **82**, 3229.
9. G. Stork, and J.W. Schulenberg, *J. Am. Chem. Soc.*, 1956, **78**, 250.
10. G. Stork, and J.W. Schulenberg, *J. Am. Chem. Soc.*, 1962, **84**, 284.
11. S.C. Welch, C.P. Hagan, J.H. Kim, and P.S. Chu, *J. Org. Chem.*, 1977, **42**, 2879.
12. U. Ranjanghatak, D.K. Datta, and S.C. Ray, *J. Am. Chem. Soc.*, 1960, **82**, 1728.
13. W.L. Meyer, and K.K. Maheshwari, *Tetrahedron Lett.*,1964, 2175.
14. E. Wenkert, and B.G. Jackson, *J. Am. Chem. Soc.*, 1958, **80**, 217.
15. M. Adinolfi, L. Mangoni, G. Barone, and G. Laonigro, *Tetrahedron Lett.*, 1972, 695.
16. L. Mangoni, M. Adinolfi, G. Laonigro, and R. Caputo, *Tetrahedron*, 1972, **28**, 611.
17. Y. Hayashi, T. Matsumoto, T. Hyono, N. Nishikawa, M. Uemura, M. Nishizawa, M. Togami, and T. Sakan, *Tetrahedron Lett.*, 1979, 3311.

18. T.A. Spencer, R.A.J. Smith, D.L. Storm, and R.M. Villarica, J. Am. Chem. Soc., 1971, 93, 485.

CHAPTER 6. PROPOSAL FOR A NOVEL SYNTHETIC APPROACH TO NORDITERPENE DILACTONES

6.1 Retrosynthetic Analysis

The structures of the family of norditerpene dilactones from plant and fungal sources can be represented in general terms by the composite structure (1), representing a subgroup C type with A-ring oxygenation and R = O-alkyl or alkyl substituents. Subgroup C is considered to be the most direct synthetic target, since the 7,8-double bond should be readily amenable to movement into the C-ring, or to hydrogenation, epoxidation, hydroxylation or reduction, which would thus provide routes to all of the other known structual types. Disconnecting the 9,11-double bond and placing the minimum (1C) residue at C-14 then generates a potential precursor structure (2).



(1) (2)

As a synthetic target, the distinctive requirements of (2) which merit consideration are:

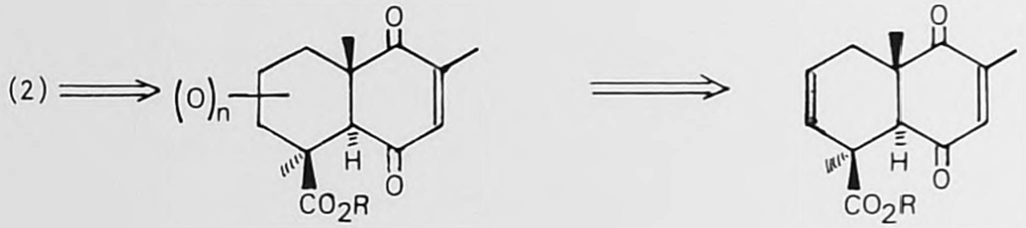
(i) a suitable method for construction of a decalin framework with *trans* stereochemistry

(ii) a method of generating the *cis* relationship between the angular methyl at C-10 and the γ-lactone bridge

(iii) a method for incorporating functional groups from which the γ-lactone can be formed

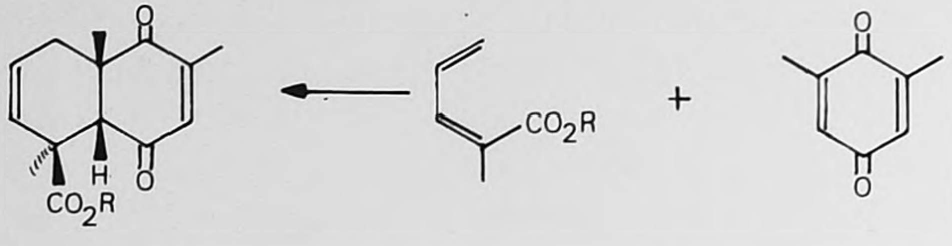
(iv) methods which will give all of the other visible functionalities in the molecule, including at least one functional group which will provide total and flexible control over the final oxidation pattern at C_1, C_2 and C_3 (such a functionality might be a double bond or a C_2-carbonyl group, as a minimum).

Continuing the retro-synthetic analysis from (2) with these considerations in mind, cleavage of the γ-lactone bridge and oxidation at C-6 leads to the enedione (3). If the oxygenation potential of the A-ring is now more specifically represented by a 2,3-double bond, this gives the precursor (4).



(3) (4)

At this point, it can be recognised that (5) is formally the result of a cycloaddition reaction between a diene (6) and xyloquinone (2,6-dimethylbenzo-1, 4-quinone)(7). Compound (5) has a <u>cis</u> ring fusion, but the presence of the 6-keto group means that on treatment with base this would be expected to undergo a facile epimerization to thermodynamically more stable <u>trans</u> arrangement of (4).



(5) (6) (7)

The remaining, and crucial questions in this potential synthetic route are therefore concerned with the cycloaddition (Diels-Alder) reactions between (6) and (7). In particular, it needs to be established whether:

(i) the required regioselectivity can be obtained which places the ester group *distant*, rather than *ortho*, to the angular methyl

(ii) the required *endo* stereoselectivity can be obtained which places the ester group of the *Z*-olefin (6) *cis* to the angular methyl group derived from the xyloquinone

(iii) an adequate synthetic yield of the required adduct can be obtained, given that Diels-Alder reactions involving terminally disubstituted olefins such as (6) are notoriously sluggish.

In answering these questions in detail, it is necessary to review what is known about the Diels-Alder reaction and a summary of this important synthetic method is therefore presented below.

6.2 Review of Diels-Alder reactions

Diels-Alder reactions observe the following general rules derived from experimental results:

a) the stereochemistry of reactants is retained in the products[1].

b) in general the adduct with the endo configuration is usually much preferred over the exo adduct[1]

c) while the concerted nature of the reaction is well established, it is believed that the two σ bonds do not form completely simultaneously[2,3]

d) the reaction rate increases with increasing electron-donating groups in the diene and with electron-attracting groups in the dienophile[1]

e) terminally trans substituted dienes react faster than cis terminally substituted, e.g. the bulky groups hinder approach of the reactants with correct orientation each other[1]

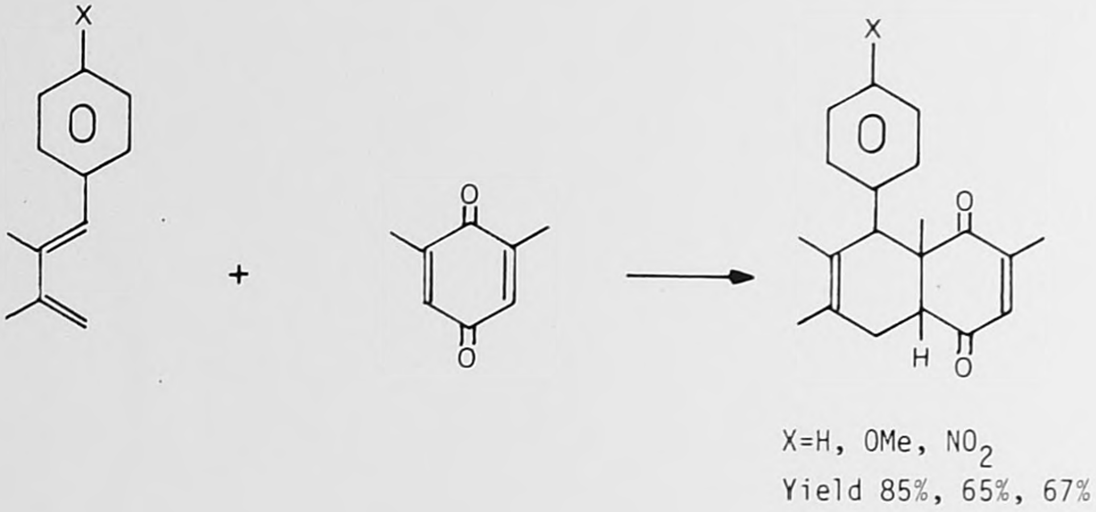
f) the reaction is little influenced by polar factors, such as changing the solvent from a non-polar to a polar one[1]. Despite this Lewis acids exert a strong catalysing effect, increasing the rate, the stereoselectivity and regioselectivity of the reaction[4,5,6,7].

g) the regioselective effect of electron-donating and electron withdrawing substituents on the diene taking part in the Diels-Alder reaction was studied by Schmidt et al[3,8]. They reported additions of 1,4 unsymmetrically disubstituted butadienes to 2,6-disubstituted benzoquinones and proved that the ortho-directive influence of terminal substituents follows the order $OCH_3 > CH_3 > H$ and $COOCH_3 > OCOCH_3 > CH_3$.

   Furthermore, the influence of non-terminal substituents compared to terminal substituents in the diene appears to be insignificant.

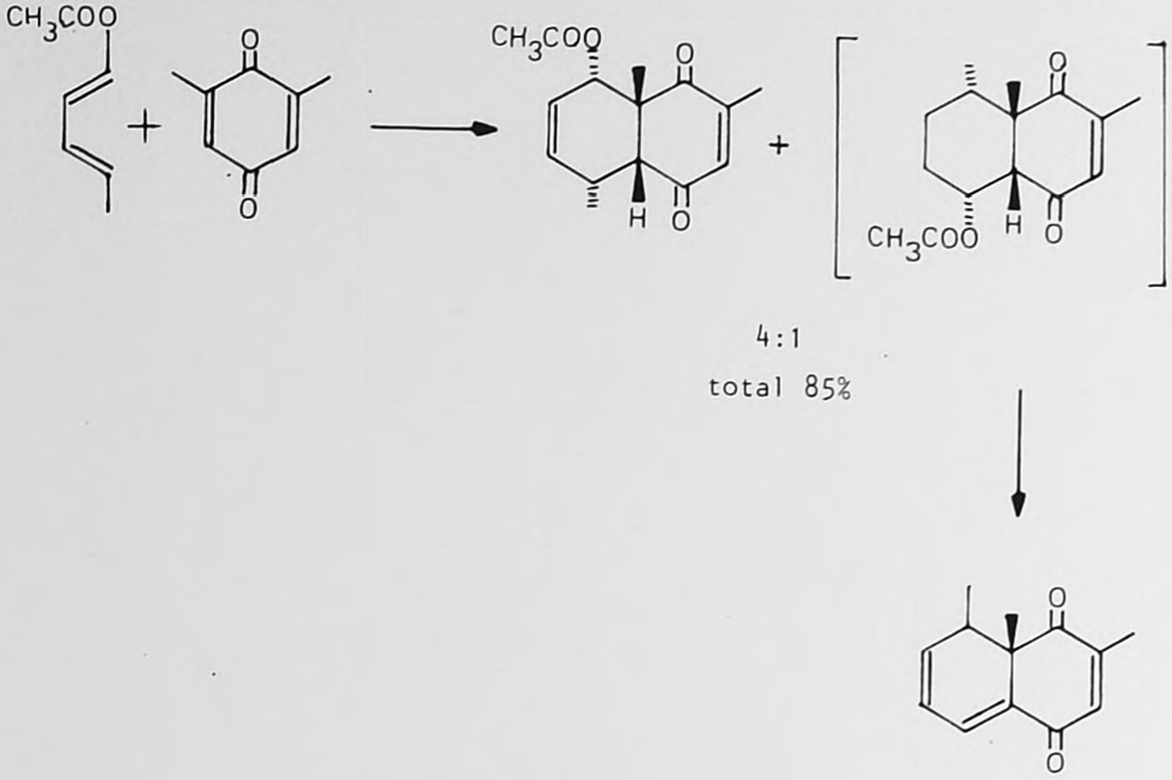
h) the ortho-directive effect is reversed when Lewis acids catalysts are used in the cycloadditions of xyloquinone and toluquinone to substituted butadienes and dialkyl dienes[5,6].

Some of the most representative work in the literature, upon which the above generalisations are based, will be presented here. Ansell and Clements[9] reported a number of Diels-Alder reactions between dienes and substituted benzoquinones. The results show, in agreement with many other studies, that there is generally a strong preference for an "ortho" orientation of the substituents derived from the diene terminus and the benzoquinone (e.g. scheme 1).

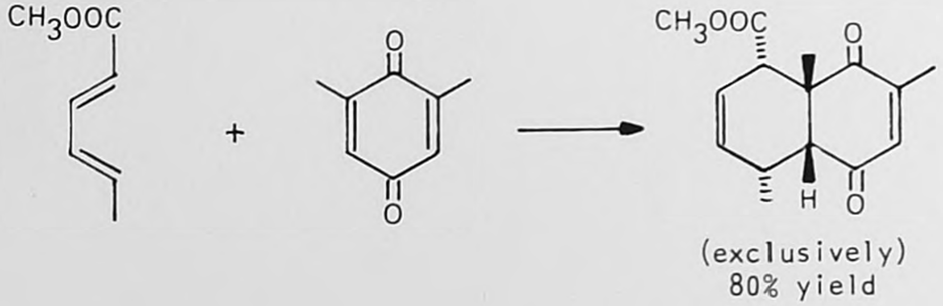


Scheme 1

Diels-Alder reactions of xyloquinone with 1-acetoxy-1,3-pentadiene (8) and with methyl sorbate (9) were studied by Schmidt[3], the results showing that the ortho-directive influence of an electron-donating group (acetoxy), an electron-withdrawing group (carbomethoxy) and a methyl group follows the order $COOCH_3 > OCOCH_3 > CH_3$, schemes 2 and 3.

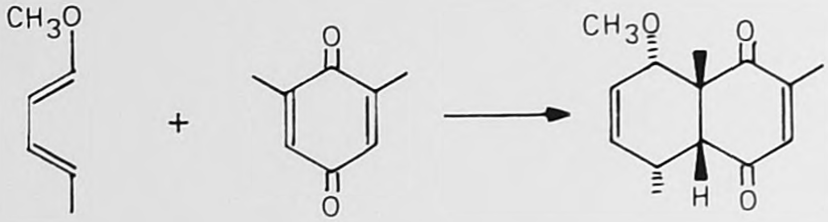


Scheme 2



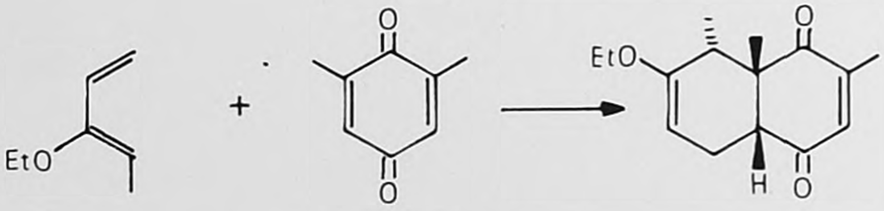
Scheme 3

Schmidt et al[8] have also demonstrated that the competitive ortho-directing influence of a terminally located methoxy is stronger than the effect of a terminal methyl group in a disubstituted diene (scheme 4).



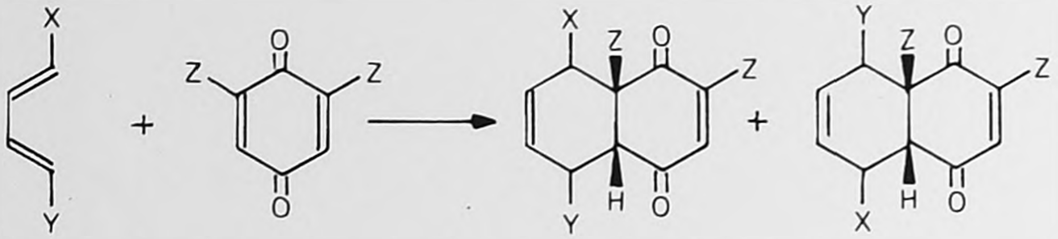
Scheme 4

These authors also found, through the addition reaction of 3-ethoxy-1,3-pentadiene to xyloquinone that the influence of non-terminal substituents is insignificant compared to the terminal substituents in the disubstituted diene (scheme 5)(in the case of simple 2-substituted dienes, the 2-substituent would be expected to be located para to the angular methyl in the cycloadduct).



Scheme 5

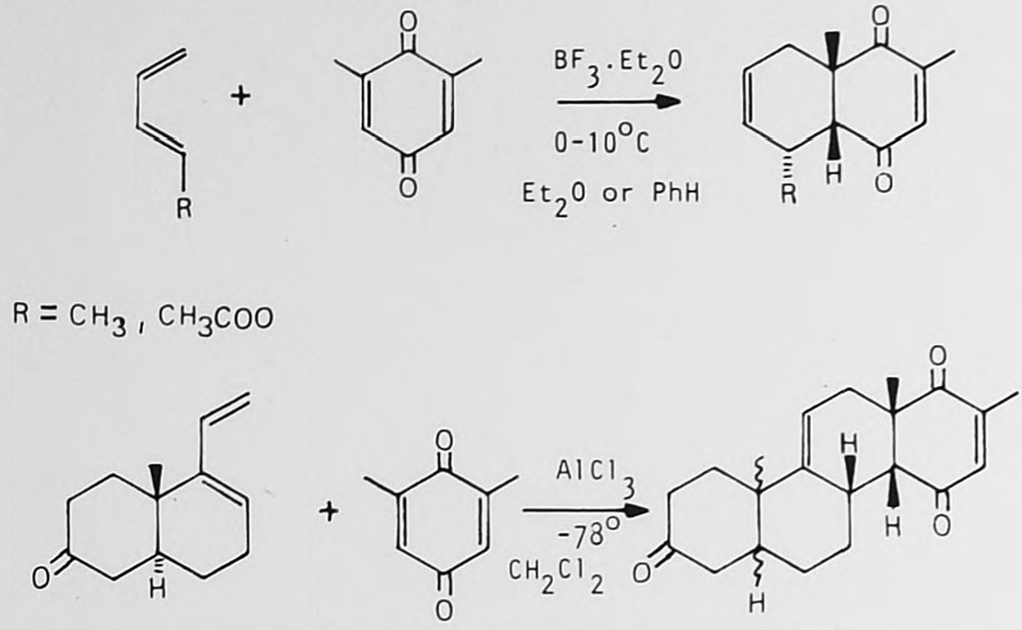
Schmidt and Liu[2] have carried out kinetic studies of the Diels-Alder reactions represented by scheme 6, and concluded that the results appeared to indicate that the Diels-Alder addition is a concerted process in which the first σ-bond develops in advance of the other, between those centres at which the overlap of orbitals is the least hindered sterically. Furthermore it is preferably initiated by that terminal of the diene which possesses the higher electron density.



Scheme 6

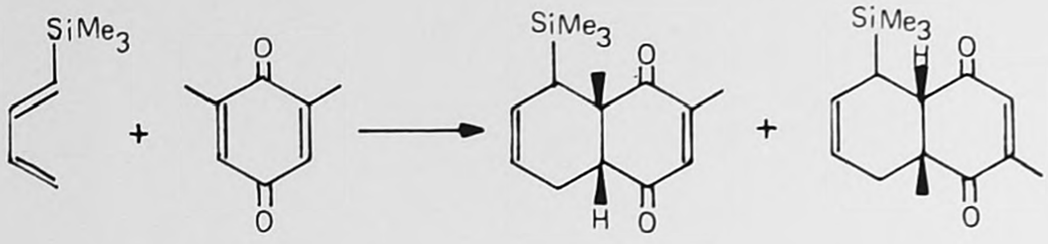
Titov[10] has reviewed the directive effects of substituents on the orientation of Diels-Alder reactions, his conclusions supporting the findings of Schmidt et al.

In 1960, Yates and Eaton[4] reported a remarkable catalytic influence of aluminium chloride on the rates of some Diels-Alder reactions. Later, Valenta et al[5,6,11] showed that not only do Lewis' acids such as $AlCl_3$ and $BF_3.Et_2O$ cause considerable rate enhancements, but also result in a reversal in the orientation of addition in the case of unsymmetrical reaction partners. Some examples of their results are shown in scheme 7.



Scheme 7

Diels-Alder reaction of 1-trimethylsilylbutadiene and unsymmetrical dienophiles, such as xyloquinone, were reported by Fleming and Percival[12]. 1-Trimethylsilylbutadiene proved not to be a very reactive diene giving poor yields, and in the reaction with xyloquinone there was no regioselectivity (scheme 8). In presence of borontrifluoride the reactions were mostly faster but the yields not always better. With xyloquinone the product was exclusively the adduct (10) of "reversed" orientation, formed in 43% yield.



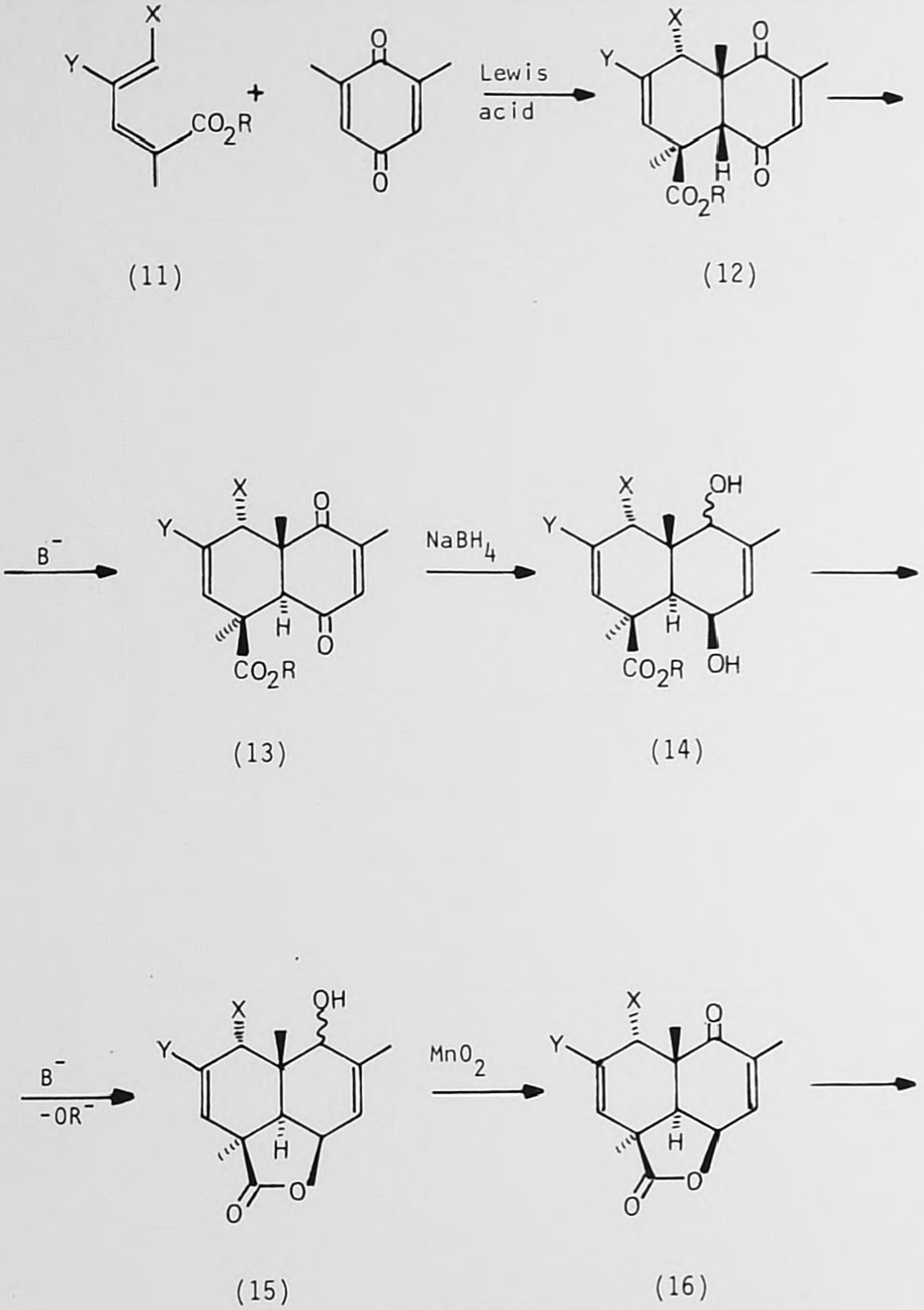
(10)

Scheme 8

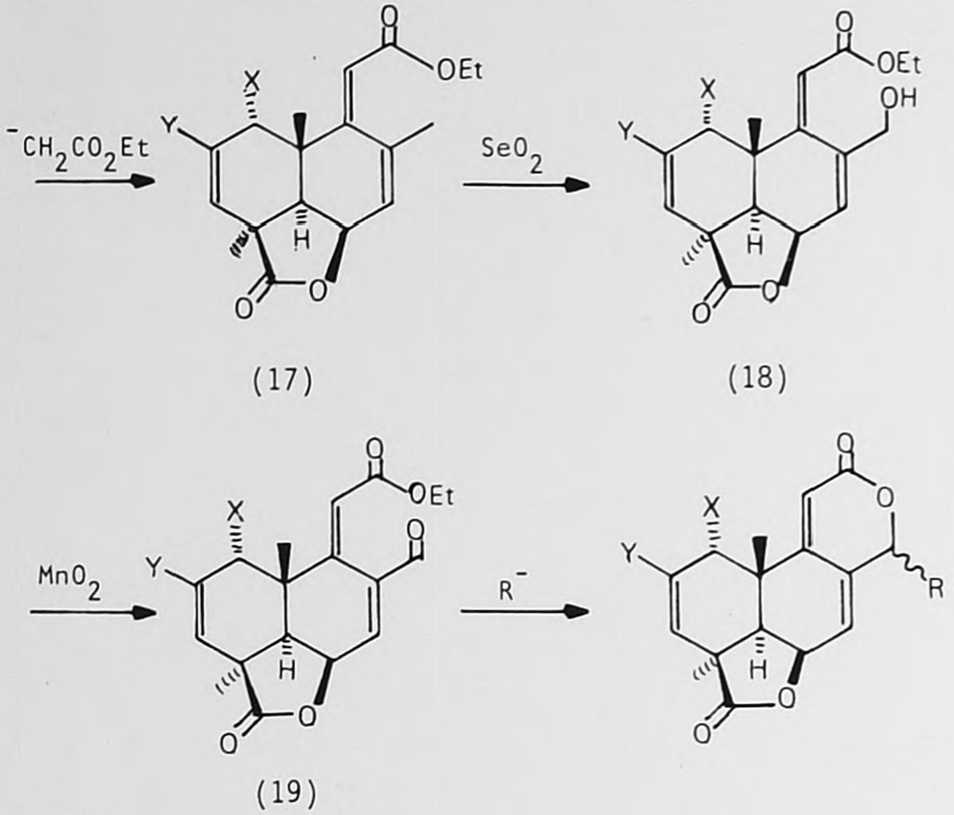
Particularly important conclusions from the work above are that the normal orientation of thermal Diels-Alder reactions can be reversed, with extremely high regioselectivity resulting, by the addition of Lewis acids, and that steric effects are much less pronounced in the catalysed reactions, so that even heavily substituted substrates will react efficiently under mild conditions.
Furthermore, the catalysed reactions still proceed highly stereoselectively, through the normal endo transition state.
Thus, the structure of the product of a Lewis-acid catalysed version of a Diels-Alder reaction can be predicted with great confidence.

6.3 Proposed synthetic approach to norditerpene lactones

In the light of the results described in Section 6.2, it is now possible to propose a route for the total synthesis of compounds of the diterpene dilactone type, as shown in scheme 9.



Scheme 9



Scheme 9 (cont.)

The cycloaddition of xyloquinone with a diene ester (11) having *Z*-stereochemistry about the 2,3-double bond should lead, under the control of a Lewis acid catalyst, to an adduct (12) by *non-ortho*, *endo* addition. The *cis* ring fusion of (12) will then be inverted to *trans* by the action of base, giving (13). The attack of borohydride ion on the C-6 carbonyl group of the latter should occur preferentially from the α-face (because of the β-methyl at C-10, as in steroid reactions); the direction of attack on the C-9 carbonyl and the subsequent orientation of the C-9 OH in (14) are unimportant, since after closure of the γ-lactone ring giving (15), the C-9 position will be reoxidised readily (allylic alcohol) to the ketone (16). The construction of the ring C skeleton requires the formal condensation of the C-9 carbonyl group with an acetic acid equivalent and this would be accomplished, in principle, by reaction with an ethyl acetate anion (Claisen condensation) with ethoxycarbonylmethylenetriphenylphosphorane (Wittig reaction) or with ethylbromoacetate and zinc (Reformatsky reaction), all of which will lead (after dehydration if necessary as a separate step) to the unsaturated ester (17). It only remains now to functionalize the allylic methyl groups (C-14) and this could be done in a variety of ways including, for example, as shown in scheme 13, by selenium dioxide oxidation (c.f. page 152). Attachment of further substituents at C-14 can then be achieved by oxidation to the aldehyde level and carbanion condensations.

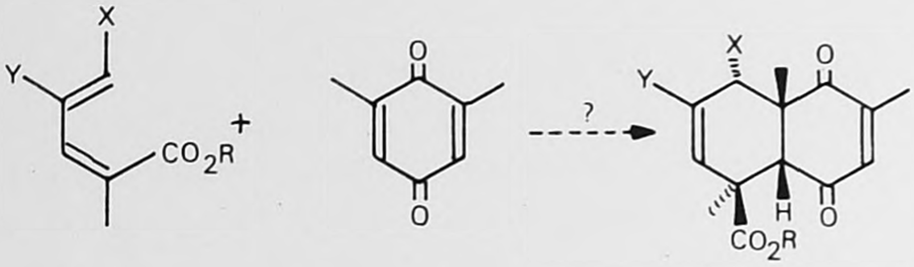
6.4 References

1. Ian Fleming, "Frontier Orbitals and Organic Chemical Reactions", 1976, John Wiley & Sons, Ltd., London, New York, Sydney, Toronto.
2. M.T.H. Liu and C. Schmidt, Tetrahedron, 1971, 27, 5289.
3. C. Schmidt, J. Org. Chem., 1970, 35, 1324.
4. P. Yates and P. Eaton, J. Am. Chem. Soc., 1960, 82, 4436.
5. Z. Stojanac, R.A. Dickinson, N. Stojanac, R.J. Woznow and Z. Valenta, Can. J. Chem., 1975, 53, 616.
6. N. Stojanac, A. Sood, Z. Stojanac, and Z. Valenta, Can. J. Chem., 1975, 53, 619.
7. B.B. Snider, J. Org. Chem., 1974, 39, 255.
8. C. Schmidt, S.D. Sabnis, E. Schmidt, and D.K. Taylor, Can. J. Chem., 1971, 49, 371.
9. M.F. Ansell and A.H. Clements, J. Chem. Soc. (C), 1971, 269.
10. Y.A. Titov, Russian Chem. Rev., 1962, 31, 267.
11. R.A. Dickinson, R. Kubela. G.A. MacAlpine, Z. Stojanac, and Z. Valenta, Can. J. Chem., 1972, 50, 2377.
12. I. Fleming, and A. Percival, J.C.S. Chem. Comm., 1976, 17, 681.

CHAPTER 7. SYNTHESIS OF XYLOQUINONE AND OF SOME PENTA-2,4-DIENOIC ESTERS

7.1 Introduction

In Chapter 6 an approach to the total synthesis of norditerpene dilactones was proposed, which called for a Lewis-acid controlled cycloaddition reaction between xyloquinone (1) and dienic esters of the general structure (2).



(2)   (1)

This Chapter will discuss synthetic approaches to these starting materials and preliminary attempts to effect the desired cycloaddition.

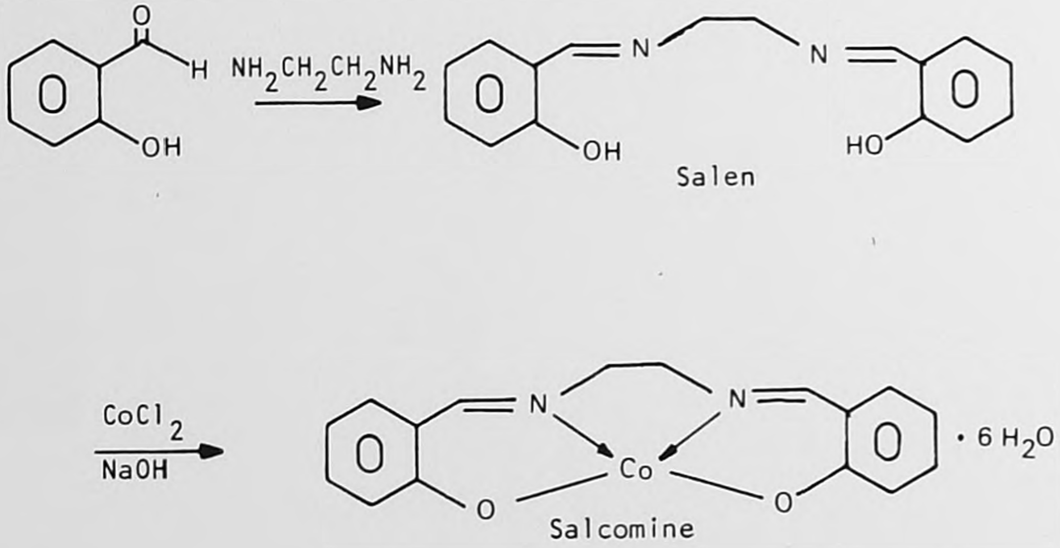
7.2 Synthesis of xyloquinone

Xyloquinone (1) is prepared by the oxidation of 2,6-dimethyl phenol, which is a readily available and cheap starting material. Numerous methods for carrying out the oxidation have been reported in the literature, including the use of copper^{II} nitrate[1], lead dioxide in formic acid[2], potassium nitrosodisulfonate[3], titanium salts in aqueous hydrogen peroxide[4], and peracetic acid[5].

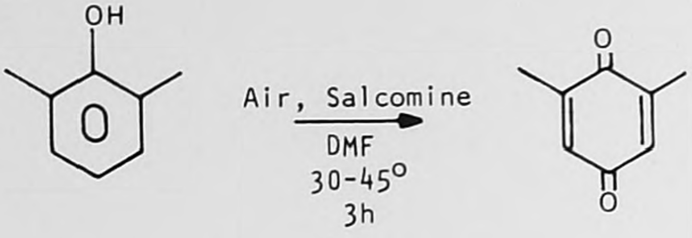
Of all the other methods reported, the one which appeared to be most convenient and to give the best yield (68%) was the use of peracetic acid, prepared *in situ* from acetic/sulphuric acid and 80% hydrogen peroxide. However, when this reaction was carried out on a tenth-molar scale, a violent explosion occurred after mixing the reagents. A second attempt on a twentieth-molar scale with increased cooling and careful precautions failed completely, no product being obtained.

A further literature search for related reactions then revealed an interesting method for the oxidation of 2,6-disubsituted phenols to the corresponding quinones, which had been applied to the di-*t*-butyl, diphenyl and dimethoxy compounds. This method[6] involved the air oxidation of the phenol, using as catalyst salcomine, which is the cobalt (II) complex of bis(salicylidene)ethylenediimine.

A sample of this complex (which is only moderately stable) was prepared freshly[7] (Scheme 1), and used for the aerial oxidation of 2,6-dimethyl phenol (Scheme 2). This gave xyloquinone in excellent yield (90%) and high purity and is undoubtedly the best method for the synthesis of this compound, being cheap, safe, rapid and amenable to large-scale operation.



Scheme 1

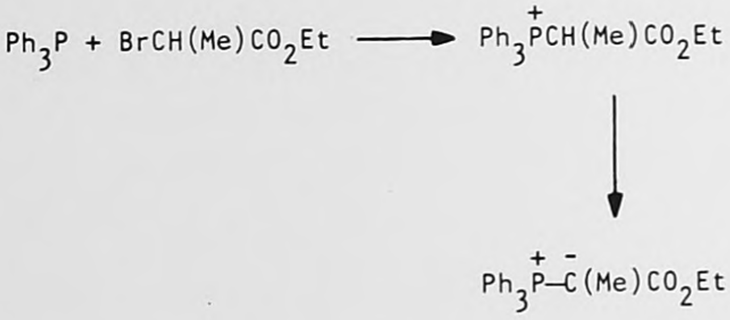


(1)

Scheme 2

7.3 Preparation of some model dienes

The Wittig reagent (3) was prepared by condensation of ethyl 2-bromopropionate with triphenylphosphine and subsequent treatment with base (Scheme 3).

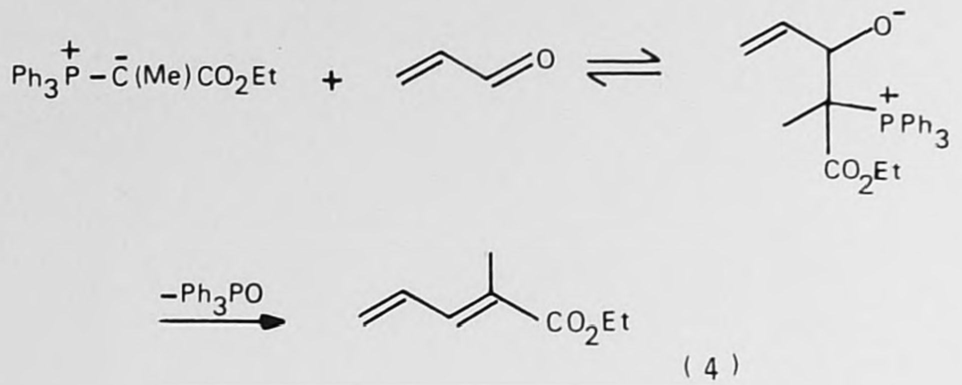


$$Ph_3P + BrCH(Me)CO_2Et \longrightarrow Ph_3\overset{+}{P}CH(Me)CO_2Et \longrightarrow Ph_3\overset{+}{P}-\overset{-}{C}(Me)CO_2Et$$

(3)

Scheme 3

Although Wittig reactions of simple alkylidene-phosphoranes can often be used for the production of cis-olefins, House and Rasmusson have shown that stabilized ylids such as (3) give exclusively products of E-stereochemistry. Their explanation[8] for this is that betaine formation is reversible for the stabilized ylid and therefore leads to the thermodynamic product (Scheme 4).



(4)

Scheme 4

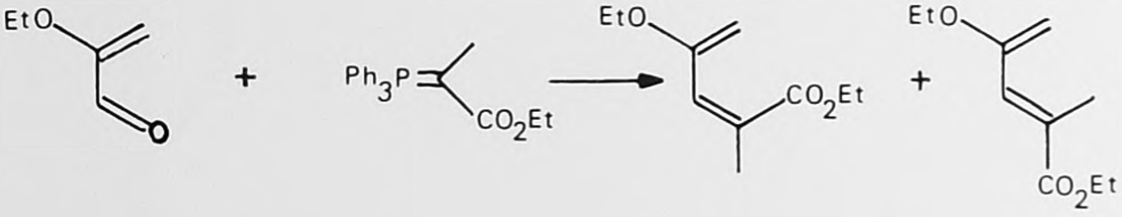
Despite the fact that the product has the "wrong" stereochemistry for the proposed approach to norditerpene lactones, the easy access to compound (4) via Scheme 4 makes this diene an attractive model compound for exploring the cycloaddition process.

The α-ethoxycarbonyltriphenylphosphorane (3) was prepared by the literature method[8,9] and condensed with acrolein. After removal of the precipitated triphenylphosphine oxide, vacuum distillation gave ethyl E-2-methylpenta-2,4-dienoate (4) as a colourless mobile liquid with the correct spectroscopic properties. The oil became more viscous on standing for a few weeks and eventually became transformed into a rigid glass, suggesting that slow polymerization was occurring. Consequently, samples were freshly prepared within a few days of any subsequent use.

It was also considered desirable to prepare an alkoxy-substituted diene ester, in the hope that the presence of the electron-releasing alkoxy group would facilitate the cycloaddition reaction and would subsequently serve as a useful handle for further modification of ring A. 2-Ethoxyacrolein, which is a very unstable and reactive aldehyde, was prepared in poor yield by the literature method[10-15] (Scheme 5), and was condensed at once with the Wittig reagent (3). After the usual work-up and vacuum distillation, a clear, mobile oil was obtained in two fractions. The ^{1}H NMR spectra of both fractions showed the presence of two methyl signals at δ1.83 and δ2.16, the

Scheme 5

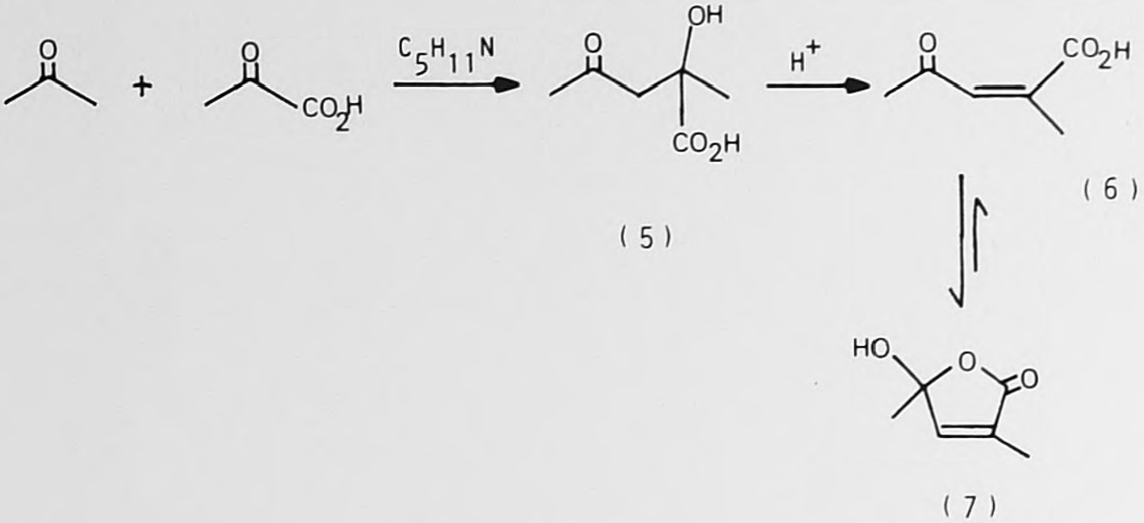
former being attributed to the methyl group in the Z-isomer (Z) and the latter to that in E-isomer (E). The lower boiling fraction was richer in the Z-isomer and the higher boiling fraction was richer in the E-isomer. Thus, the presence of the additional ethoxy-substituent in the aldehyde results in a loss of stereoselectivity in the Wittig reaction of the stabilized phosphorane (3).



(Z) (E)

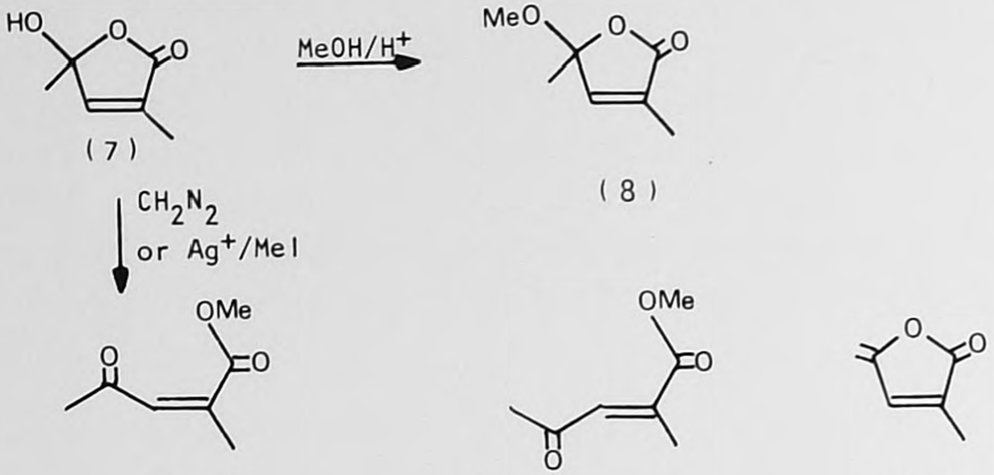
The above reactions confirmed our view that it would be necessary to develop a new method for the synthesis of dienoic esters of the required stereochemistry. After a search of the literature, it was decided to explore a reaction in which the desired Z-geometry about the 2,3-double bond in an unsaturated ester would be controlled by the intermediacy of a cyclic structure.

Buchta and Satzinger have described[16] the condensation of acetone with pyruvic acid, leading to the α-methyl-β-acetylacrylic acid (6) which is formed exclusively in the Z-form as a consequence of its preferred existence in the cyclic form[17] as the lactanol (7).



After condensation of acetone and pyruvic acid in the presence of piperidine, the reaction mixture is acidified with HOAc/conc.HCl and heated in order to effect the dehydration of the intermediate alcohol (5). In the literature procedure, the reaction mixture is then heavily diluted with water and re-evaporated to remove water and acid. This time-consuming procedure leads eventually to a 19% isolated yield of the lactanol. After carrying out this procedure in the first instance and obtaining the results described in the literature, the procedure was modified somewhat in subsequent runs, by saturating the diluted, aqueous product solution with sodium chloride and extracting thoroughly with ether. This procedure was found to be much more rapid and also gave an improved yield of 24%. The product isolated showed ν_{max} ($CHCl_3$) 1765 cm^{-1}, λ_{max} (MeOH) 215 nm (λ_{max} (MeOH/NaOH) 247 nm), indicating it to be present largely or exclusively in the cyclic form as the lactanol (7).

According to the literature the esterification of the lactanol (7) may take two different courses[18,19]. Treatment with methanol and acid was reported to lead to a derivative retaining the cyclic structure and described as a "pseudoester" (8). By contrast, reactions with diazomethane and with silver oxide and methyl iodide were claimed to give the "normal" ester (9).



(7) (8) (9) (11) (10)

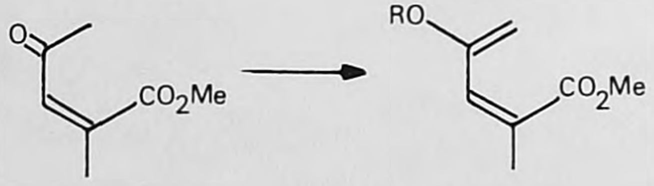
All three of these methods of esterification were re-investigated and the products carefully examined by analytical HPLC and by spectroscopy. Treatment of the lactone with methanol/HCl gave a product the ^{1}H NMR spectrum of which was largely in accord with that reported for the pseudoester (8). However, additional signals were also present which could be interpreted as being due to the ester (9) and also the γ-methylenelactone (10). Compound (10) was subsequently isolated from another reaction and its structure is discussed later in this chapter. The presence of all three of these compounds was confirmed by analytical HPLC comparison with authentic samples and the ratio of products (8):(9):(10) was determined to be about 70:10:20 by integration of signals in the ^{1}H NMR spectrum.

Formation of the silver salt of the lactanol/acid (7)/(6) and treatment with methyl iodide gave, in agreement with the literature, the ester (9), but in a poor yield (25%). In addition, the ^{1}H NMR spectrum indicated that the product was contaminated with traces of the E-isomer (11).The vinylic proton at C-3 in the E-structure appears more downfield (δ7.20) than in the Z-form (9)(δ6.14).

Treatment of the lactanol (7) with diazomethane solution gave the ester (9) rapidly and clearly as the only product, as judged by HPLC and ^{1}H NMR. This method was clearly superior to the silver salt-methyl iodide procedure in terms of speed, cost and yield. However, in large-scale runs with tens of grams of lactanol, the diazomethane reaction was found to be rather sluggish and incomplete. A report in the literature that silica gel aided the O-methylation of caprolactam and valerolactam[20] encouraged us to attempt a similar catalysis in our own reaction. Very pleasingly, treatment of the lactanol (7) with ethereal diazomethane in the presence of silica gel gave the ester (9) in an isolated yield of 95% and high purity.

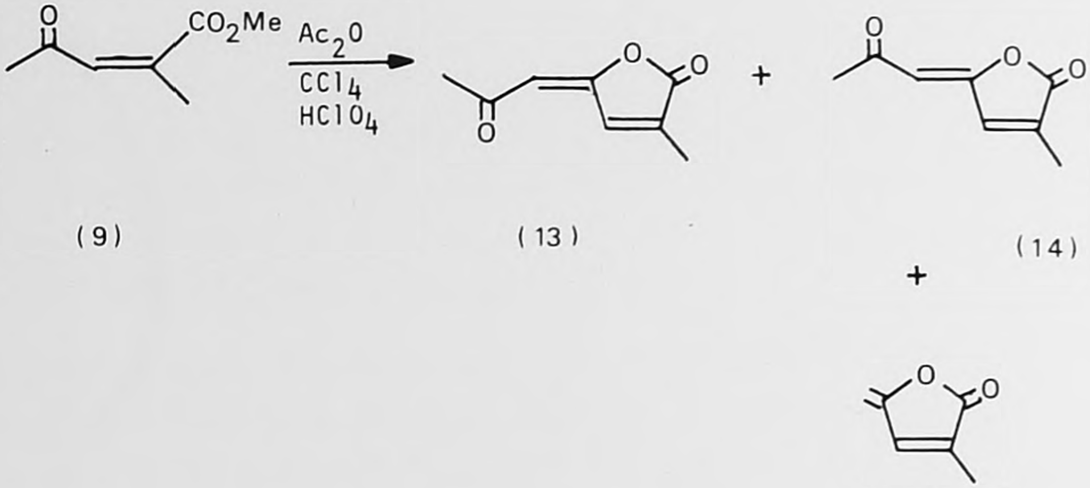
The ester (9) and pseudoester (8) are readily differentiated by IR. The methoxylactone shows only one strong peak at 1765 cm^{-1}, whereas the open chain keto ester has two carbonyl absorptions at 1735 cm^{-1} and 1690 cm^{-1}. The 'H NMR spectra of these two compounds show a difference in the position of the OCH_3 signal, which appears at δ3.19 in the methoxylactone and at δ3.79 in the methyl ester.

The methyl (Z)-4-keto-2-methylpent-2-enoate (9) which was thus obtained appeared to be a promising intermediate for the synthesis of dienoic esters of structural type (2). In principle, all that is required is to convert the methyl ketone into an enol derivative in order to generate a diene (12) suitable for the projected Diels-Alder studies.



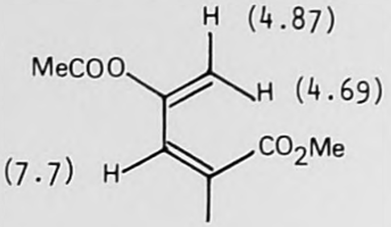
(9)   (12)

Several methods of forming enol derivatives of the ketone (9) were therefore investigated. Following the method of Barton et al[21,22] O-acetylation was attempted using acetic anhydride in carbon tetrachloride and $HClO_4$ as catalyst. This afforded a complex mixture (TLC and HPLC) which was separated by chromatography on silica, yielding three principal products, none of which was the desired enol acetate. The products were shown to be (13) and (14), by comparison with literature data[23,24], and the previously unreported γ-methylene lactone (10), which was identified by its spectroscopic data.



(9) (13) (14) (10)

Close examination of the crude reaction mixture showed the presence of very weak signals at δ4.47 and 4.56 (small coupling) and 7.10 (broad) which are similar to the values calculated by the Tobey-Simon Rules for the vinylic protons in the desired enol ester (15). However, if this compound was present in the reaction mixture, it must have been in small amount and was impossible to isolate.



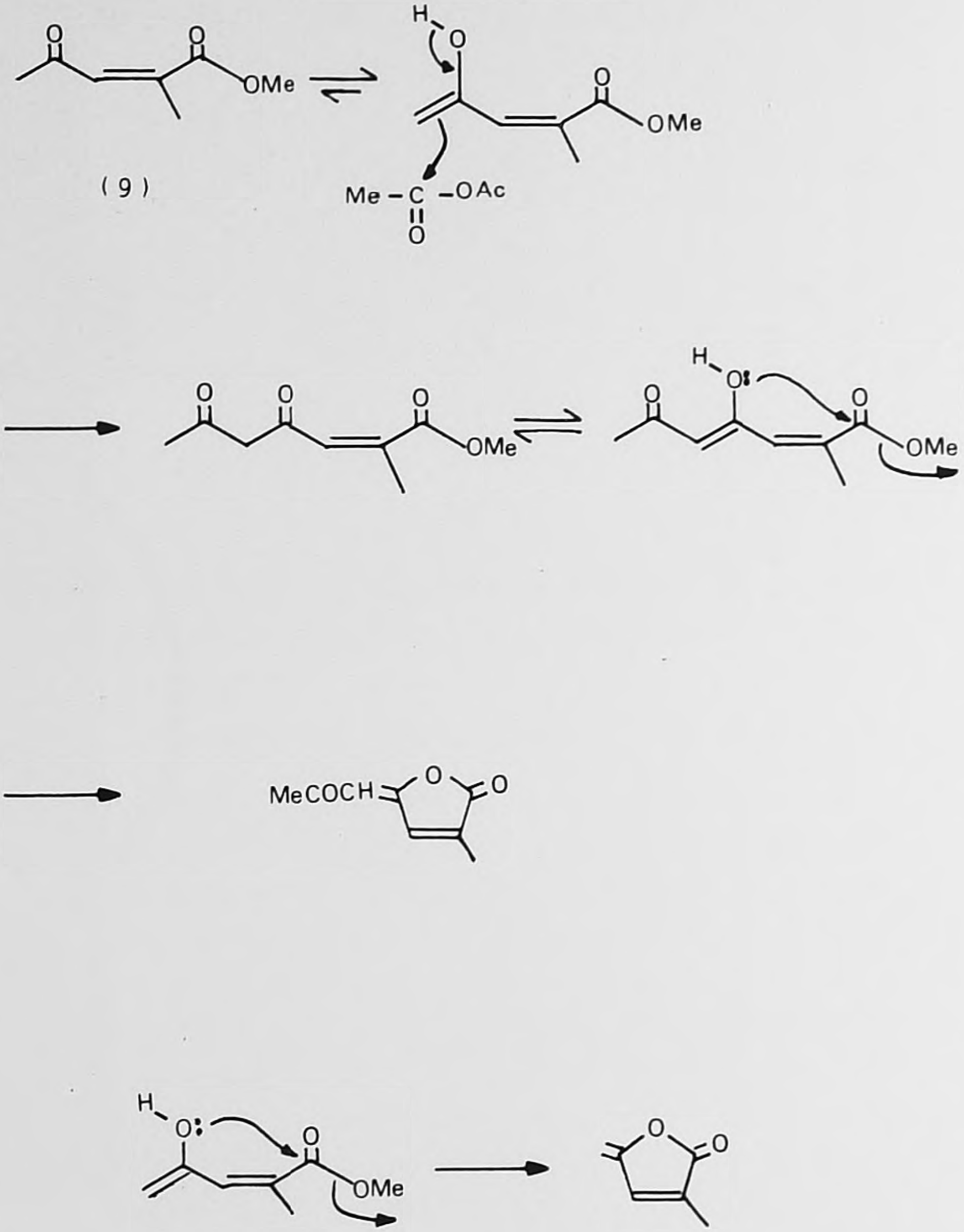
(15)

A survey of a wide range of other acetylating conditions was then made, using both acetic anhydride and acetyl chloride in a wide range of solvents and with a variety of different acid catalysts. All of these reactions gave either no products or mixtures containing mainly the C-acylated products (13) and (14). In no case could the desired O-acylated product (15) be obtained.

The results can be explained by assuming that the enolized form of the ketone (9) prefers to undergo C-acylation to O-acylation and that when O-acylation does take place, it does so internally by attack of the ketone oxygen on the ester carbonyl (Scheme 6).

These results find analogy in the study of protoanemonin derivatives by Eisner, Elvidge and Linstead[25].

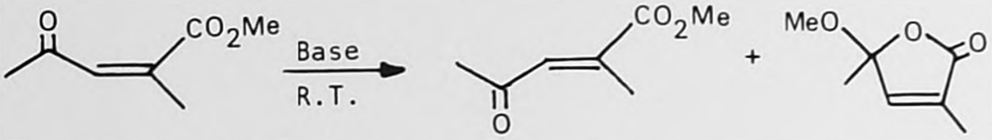
Attention was then turned to attempts to O-alkylate the ketone (9), by treatment with triethyloxonium fluoroborate (Meerwein's salt) in the presence of non-nucleophilic, hindered bases such as 1,8-bis(dimethylamino)naphthalene (proton sponge), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,4-diazabicyclo[2.2.2]octane (DBO). The results obtained can be summarized as follows:



Scheme 6

(i) No formation of C- or O-alkylated products.

(ii) The (Z)-ketoester (9) isomerizes in the presence of bases, giving as a major product the (E)-ketoester (11) and as a minor product the pseudoester (8)(Scheme 7).



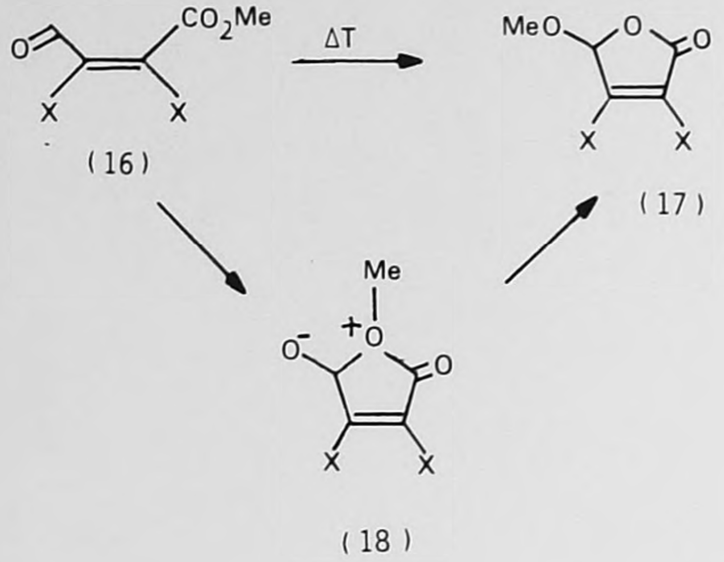
Scheme 7

(iii) By following the reactions by HPLC, it was established that the rate of isomerization is base-dependent, in the following order:

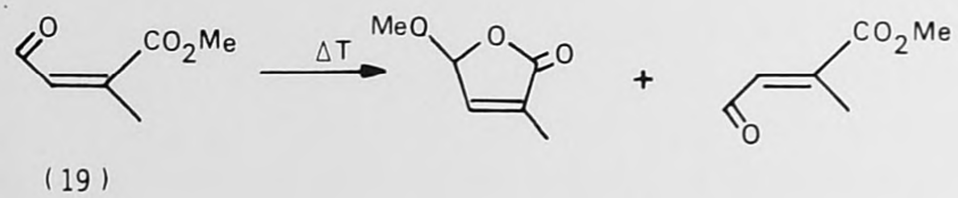
DBN > Proton sponge > DBU

DBO - no reaction.

(iv) The amount of pseudoester formed increased considerably with increasing temperature. This is in agreement with the results obtained by Beska et al[26], who showed that the methyl esters of 2,3-dihalogenated 3-formyl-acrylic acid (16; X = Cl or Br) are thermolabile and isomerize to give pseudoesters (17). They suggested that the mechanism involves ring closure by attack of the methoxyl oxygen on the formyl group to give an oxonium ion (18), followed possibly by the shift of a methyl cation.

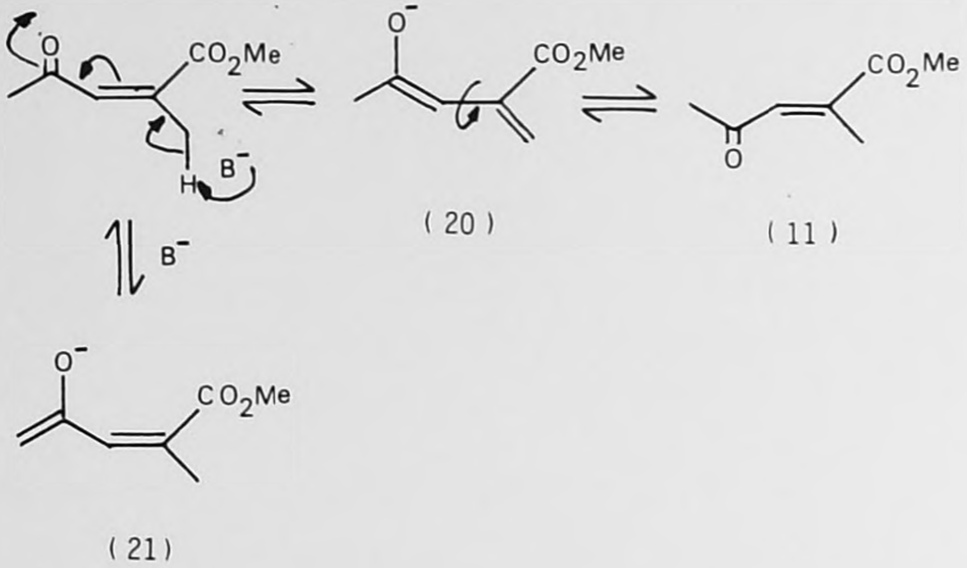


Fariña and Martin (27) have also reported similar results, in which the 3-formyl methacrylate ester (19) isomerized on heating in an inert solvent (Scheme 8).



Scheme 8

In the case of our own reactions at room temperature, the isomerization of (9) to (11) is clearly not thermal, but is a base-induced process. The mechanism is believed to be one in which the base abstracts a proton from the 2-methyl group, forming a dienolate anion (20) in which rotation can occur, so that re-protonation gives rise to the isomer (Scheme 9).



Scheme 9

At first sight, it seems surprising that the dienolate anion (20) should be forming, apparently in preference to the anion (21). It was reasoned that the anion (21) would be the product of kinetic deprotonation but that the alternative anion (20) would be the thermodynamically more stable one. The use of hindered amine bases at room temperature would be expected to give the thermodynamic enolate. According to the literature[28-31], lithium dialkylamides in THF or dimethoxyethane (DME) at low temperatures are bases which convert ketones into enolate anions under conditions of kinetic control. In particular, the strong, hindered base lithium diisopropylamide (LDA) offers a number of advantages including the fact that it is soluble in common solvents, it can be estimated by the use of indicators such as 2,2-bipyridyl, and the diisopropylamine is cheap, readily available and reasonably volatile.

A reaction was therefore carried out by addition of the ketoester (9) to LDA at -78^{0}, followed by addition of Meerwein's salt in an attempt to trap the kinetic enolate. However, no C- or O-alkylated products were obtained under these conditions. The reaction mixture was found to be complex by HPLC, and by means of preparative HPLC it was possible to isolate again the pseudoester (8) as a major product and the γ-methylene lactone (10) as a minor one.

Finally, two procedures for the preparation of trimethylsilyl enol ethers were investigated, involving reaction of the ketoester with
(i) trimethylsilyl chloride and triethylamine in DMF[31], and
(ii) trimethylsilyl chloride and a triethylamine-zinch chloride complex in benzene[32].
In both cases, no enol ether was formed.

7.4 Attempted cycloaddition reactions with the model dienes

Attempts were made to carry out cycloaddition reactions of the dienoic ester (4) and the mixture of ethoxy-substituted derivatives (Z/E) with xyloquinone. A variety of thermal conditions (refluxing toluene and O-dichlorobenzene; dichloromethane in a sealed tube at 150^{0}) and Lewis acid catalysis ($BF_3.Et_2O$ in dichloromethane) were investigated. In all cases, the dienes gradually disappeared (TLC), but no products recognisable as resulting from cycloaddition could be isolated.

Presumably, the lack of reactivity is a consequence of the large steric hindrance of the cis group at the 2-position of the 2,4-dienoic ester. This will have two effects, firstly tending to disfavour the S-cis conformation of the diene which is required for the Diels-Alder reaction, and secondly destabilizing the transition state for cycloaddition, particularly with xyloquinone which contributes its own steric effect from the methyl substituent.

7.5 Experimental

2,6-dimethylbenzo-1,4-quinone (1). In a 500 ml, three-necked flask equipped with a mechanical stirrer, a thermometer, and a gas-inlet tube were placed 42.5 g (0.35 mole) of 2,6-dimethylphenol in 300 ml of dimethylformamide and 2.5 g of salcomine. With stirring, oxygen was introduced at such a rate that the temperature did not exceed 45°C. This was continued for 3 hours. At the end of the reaction the temperature dropped to about 28°C. The reaction mixture was then poured onto 500 g of crushed ice and 15 ml of 4N hydrochloric acid. A yellow precipitate was formed. After filtration, the precipitate washed on the filter three times with 50-ml portions of 1N hydrochloric acid, three times with 100-ml portions of water, and twice with 25-ml portions of cold ethanol. Dried under reduced pressure at 50°C for 3h. Yield 90%, mp 70-71°, $\nu^{max}_{(nujol)}$-1655 cm^{-1}, NMR $\delta(CDCl_3)$ 2.08 (3H,s), 6.60 (1H,s) ppm.

Bis(salicylidene)ethylene diimino-cobalt(II)(salcomine). This compound was synthesized under procedure described by Diehl and Hack in Inorg. Synthesis[7]. The yield obtained was in agreement with the literature (5 g of disalicylalethylene diamine (SALEN) afforded 5.5 g of salcomine).

(α-Ethoxycarbonyl ethylidene)triphenylphosphorane (3). By the literature method[1,2] from ethyl 2-bromopropionate and tripenylphosphine. Mp 156-7° (Lit:[2] 156-7°).

Ethyl E-2-methylpenta-2,4-dienoate (4). Acrolein (4.2 g; 0.075 mole) was added dropwise with stirring to a solution of the phosphorane (3)(21.3 g; 0.061 mole) in redistilled dichloromethane (40 ml) at 20-25°C during 0.5 h. The mixture was then refluxed for 3.5 h and half the solvent distilled off through a 30 cm Vigreux column. To the remaining solution was added pet. ether (120 ml; 60-80° fraction) to precipitate triphenylphosphine oxide, which was filtered off and washed with a further 30 ml pet. ether. The combined organic layer was evaporated under reduced pressure and the residue vacuum distilled at 60°/9 mm to afford 3.4 g (44%; Lit: 60%) of a colourless, mobile, sweet-smelling liquid. λ_{max}^{EtOH}: 251 nm; ν_{max}^{film} 1710, 930 cm^{-1}; $\delta(CDCl_3)$: 1.36 (3H,t), 2.00 (3H,s), 4.26 (2H,q), 5.6 (2H,dd), 6.76 (1H,dt), 7.44 (1H,d) ppm.

Bromoacetaldehyde diethylacetal. By the literature method[3] from vinyl acetate (86.0 g; 1 mole) and bromine (160 g; 1 mole) in CCl_4 (150 ml) followed by addition to ethanol (500 ml), bp 52-4°/10 mm (Lit:[3] 64-5°/16 mm), yield 162 g, 83% (Lit:[3] 77%).

1,1,2-Triethoxyethane. By the literature method[4] from bromoacetaldehyde diethylacetal (162 g; 0.823 mole) and sodium ethoxide (1M) in absolute ethanol (650 ml). Yield 67 g (50%), bp 162-4°C (Lit:[4] 61% yield, bp 164-6°C).

2-Ethoxyacrolein[13-15]. 1,1,2-Triethoxyethane (22.0 g; 0.136 mole) was stirred with 3M HCl (200 ml) at 25°C for 45 min., then neutralized at 0°C with 6M NaOH using a pH meter. This solution was added in one portion to 37% formalin (11.6 ml), diethylamine hydrochloride (14.9 g) and hydroquinone (100 mg) in a 4-necked 500-ml flask fitted with a reflux condenser, mechanical stirrer, N_2 inlet and pH electrodes. The flask was maintained at 60°C during the addition, the pH then adjusted to 7.5 and the mixture stirred for 2.5 h at 60°C. After cooling to 0°C and extraction with ether, the organic layer was washed with water, brine and dried over anhydrous sodium sulphate. Evaporation of the solvent and distillation of the residue gave 2-ethoxyacrolein, bp 22°/13 mm, yield 0.7 g, 5.2% (Lit:[6] bp 41°/18 mm, yield 44%).

Ethyl 4-ethoxy-2-methylpenta-2,4-dienoate. A Wittig reaction[6] between 2-ethoxyacrolein (2.3 g; 0.023 mole) and (α-carbethoxyethylidene-triphenylphosphorane (8.1 g; 0.023 mole) in refluxing dichloromethane (15 ml) was followed by the usual work-up. Distillation gave two fractions:

(i) bp 52-62°/7-8 mm (0.2 g)
(ii) bp 62-69°/7-8 mm (0.5 g).

Both showed ν_{max}^{film} 1710, 1590 cm^{-1}.

(Z)4-Keto,2-methyl,pent-2-enoic acid (6) (Buchta and Satzinger procedure modified). Pyruvic acid (44 g, 0.69 mole) in dry acetone (180 ml) was cooled in an ice bath and piperidine (86 g) added in small portions carefully to control fuming. The reaction mixture was left at room temperature for two days, then evaporated to give a viscous oil. To this was added a mixture of glacial acetic acid (400 ml) and conc. hydrochloric acid (160 ml) in small portions with stirring. The mixture was heated for 1 h at 90°C and then poured into 1500 ml of distilled water. Instead of evaporating the mixture on a steam bath as described by Buchta and Satzinger the water was saturated with NaCl and extracted several times with ether. The combined ethereal extracts were dried over Na_2SO_4 and evaporated to dryness. Methanol was added to the residue and re-evaporated to remove traces of acetic acid. The crude, syrup product was crystallised from benzene to give 2-3 crops of crystal with mp 98-101°. Yield 24%, λ_{max}^{MeOH} 210 nm, $\lambda_{max}^{MeOH+NaOH}$ 247 nm, ν_{max}^{nujol} 3260, 1763 (CO lactone ring) cm^{-1}, $\delta(CDCl_3)$ 1.70 (3H,s), 1.86 (3H,d 1.8) 6.90 (1H,d 1.8) ppm.

Methyl,(Z)-4-keto,2-methyl,pent-2-enoate (9)

(i) Diazomethane procedure

A solution of the acid (6)(20 g, 0.16 mole) in 500 ml of ether was treated over a period of 3 h with amounts of ethereal solution of diazomethane in presence of 40 g of silica until a yellow colour persisted. The mixture was filtrated and the precipitate washed several times with ether. The ether was evaporated and replaced with hexane and separated from the unreacted (6).

One batch of the obtained ketoester (6) was purified by vacuum distillation bp 98-100°/20 mm and another batch by column chromatography in SiO_2GF_{254} (for TLC plates) and eluted with gradient elution CH_2Cl_2/CH_3CN. The purification of the ester by comlumn chromatography showed to be much more efficient. Yield 95%, ν_{max}^{film} 1730, 1690, 1620 cm^{-1}, λ_{max}^{MeOH} 225 mm (ϵ7100), $\delta(CDCl_3)$ 2.00 (3H,d 1.8), 2.20 (3H,s), 3.79 (3H,s) 6.14 (1H,q 1.8) ppm, MS no $M^{+\cdot}$, base peak 127, 110, 99, 82, Anal. calcd. for $C_7H_{10}O_3$ C 59.14; H 7.09%.Found C 58.89; H 7.09%.

Diazomethane

Nitrosomethylurea (60 g) finely powdered, was added slowly to a mixture of ether (700 ml) and 96 g of potassium hydroxide in H_2O (194 ml) cooled to 0^oC with stirring. The ether layer was decanted off onto KOH pellets, for 30 minutes, the pellets changed and left for another 30 minutes. The solution stored in cool, over calcium chloride with bunsen valve.

(ii) Silver salt-methyl iodide procedure

A solution of (6)(1 g, 0,0007 mole) in water (25 ml) was treated with 0.5 N sodium hydroxide (16 ml). The aqueous solution of the sodium salt of (6) was subsequently treated with silver nitrate (1.33 g) and evaporated to dryness in vacuum at room temperature. The residue was dried by azeotropic distillation with benzene. The residue of dry salts was suspended in benzene (50 ml), treated with an excess of methyl iodide (3.0 ml) and refluxed for 10-12 h. At the end of this period the benzene solution was filtered from the precipitated silver iodide, washed with aqueous sodium hydrogen carbonate, evaporate to dryness. The dark yellow oil obtained was distilled at 100-105°C/10 mm. Yield 25%. This material was spectroscopically identical with (6) prepared with diazomethane.

Methyl,(E)-4-keto,2-methyl,pent-2-enoate (11). A solution of (Z)-ketoester (9)(0.100 g, 0.0007 mole) in dry CH_2Cl_2 (3 ml) was stirred at room temperature for 5 h with 2 drops of 1,5-diazabicyclo [4.3.0] non-5-ene (DBN). The reaction mixture was poured onto a solution of aqueous sodium hydrogen carbonate and the organic layer extracted, washed with water and dried over magnesium sulphate.
The product was isolated by preparative thin-layer-chromatography (SiO_2GF_{254}) and twice eluted respectively with CH_2Cl_2 and CH_2Cl_2/CH_3CN (99:1), $\nu_{max}^{CHCl_3}$ 1720, 1690, 1620 cm^{-1}, λ_{max}^{MeOH} 235 nm (log ε=3.99), $\delta(CDCl_3)$ 2.24 (3H,d 1.5), 2.36 (3H,s), 3.88 (3H,s), 7.20 (1H,q 1.5) ppm, MS no $M^{+\cdot}$, base peak at 110, other peaks at 127, 111, 99, 82.

3,5-Dimethyl,5-methoxy-3-butenolactone (8). A solution of (6)(500 mg, 0.0039 mole) in methanol (70 ml) containing 7% (W/V) of dry HCl were allowed to stand for 16-18 h. The reaction mixture was concentrated in vacuum, flushed several times with benzene and the solvent evaporated under vacuum. The product purified by vacuum distillation, bp 110-115^{o}C/20 mm, yield 51%, ν_{max}^{film} 1765, 1620 cm^{-1}, λ_{max}^{MeOH} 220 nm, $\delta(CDCl_3)$ 1.60 (3H,s), 1.96 (3H,d 2.0), 3.19 (3H,s), 6.78 (1H,q 2.0), anal. calc. for $C_7H_{10}O_3$ C 59.14; H 7.09%, found C 59.18; H 6.96%.

3-Methyl-5-exo-methylene-3-butenolactone (methylprotoanemonin)(10). Ketoester (9)(5 g, 0.0350 mole) dissolved in carbon tetrachloride (150 ml) was refluxed for 6 h with acetic anhydride (25 ml) and $HClO_4$ (1 ml)(added last). After refluxing, the reaction mixture was neutralized with 8% aqueous solution of sodium hydroxide (20 ml) and washed several times with water. The organic layer was dried over magnesium sulphate and evaporated on buchi.
The reaction mixture (3.1 g) chromatographed on SiO_2 column (SiO_2GF_{254}) with gradient elution of CH_2Cl_2/CH_3CN, afforded one first fraction (trace amount) not identified, and the second fraction eluted with CH_2Cl_2/CH_3CN (88:2) was compound (10)(160 mg), $\nu_{max}^{CHCl_3}$ 1775 (s) cm^{-1}, λ_{max}^{MeOH} 260 nm (ε14,000), $\delta(CDCl_3)$ 2.0 (3H,s), 4.71 (1H,d 2.0), 5.01 (1H,d, 2.0), 6.96 (1H,m), MS no $M^{+\cdot}$, base peak at 83, other peaks 110, 96, 85, 68.

(2Z,4E)-2-methyl-6-oxohepta-2,4-dien-4-olide (13). The same procedure as for compound (10). Compound (11) was the third fraction to come out of the column chromatography eluted with CH_2Cl_2/CH_3CN (85:5)(100 mg) yellow crystals, mp 86-89^{o}, $\nu_{max}^{CHCl_3}$ 1790, 1690, 1620, 1610 cm^{-1}, λ_{max}^{MeOH} 285 nm (ε22,000), $\delta(CDCl_3)$ 2.10 (3H,d 2.0), 2.32 (3H,s), 6.20 (1H,s), 8.0 (1H,q 2.0), ppm. MS no $M^{+\cdot}$, base peak 137, other peaks 152, 69, 43, anal. calc. for $C_8H_8O_3$, C 63.20, H 5.26%, found C 63.10, H 5.21%.

(2Z,4Z)-2-methyl-6-oxohepta-2,4-dien-4-olide (14). The same procedure as for compound (10). Compound (14) was the fourth fraction to come out of the column chromatography eluted with CH_2Cl_2/CH_3CN (85:5)

(220 mg), mp 120-121°, $\nu_{max}^{CHCl_3}$ 1790, 1665, 1620, 870 cm^{-1}, λ_{max} 285 nm (ε22,000), δ($CDCl_3$) 2.16 (3H,d 2.0), 2.60 (3H,s), 5.60 (1H,s), 7.30 (1H,q 2.0), ppm, MS no $M^{+\cdot}$, base peak 137, other peaks 152, 127, 69, 43; anal. calc. for $C_8H_8O_3$, C 63.20; H 5.26%, found C 62.92; H 5.16%.

General procedure followed for the reactions of the ketoester (9) with $Et_3O^+BF_4^-$, lithium diisopropylamide and 2,2-bipyridil as indicator

In one 10-ml two-necked round bottom flask equipped with magnetic stirrer and inlet for nitrogen, were placed 5 ml of dry THF, 10 mg of indicator and diisopropylamine (130 mg, 0.0007 mole). N-butyl lithium was then added until change of colour of the solution to deep red orange. To this mixture was added the ketoester (9)(100 mg, 0.0007 mole) followed by freshly prepared $Et_2O^+BF_4^-$. The reaction mixture maintained at -78° in a cardice/acetone bath.

Triethyloxonium tetrafluoroborate. In a 500-ml 3-necked flask fitted with a stirrer dropping funel and condenser protected by a drying tube, was placed a solution of freshly distilled boron trifluoride etherate (31.04 ml, 0.314 mole), bp 115-116°, in 100 ml dry ether. Freshly distilled epichlorohydrin (17.8 ml, 0.276 mole), bp 112-113°C was added dropwise at such a rate that gentle reflux was maintained by the exothermic reaction. The mixture was then heated to reflux for a further 45 min. and left at room temperature. The ether layer extracted and the white precipitate washed with 400 ml of dry ether. The product was stored under dry ether.

7.6 References

1. I.B. Afanas'ev, L.F. Gur'yanova, N.G. Baranova, G.I. Samokhvalov, Zh. Org. Khim., 1978, 14, 569.
2. C.R.H.I. de Jonge, H.M. van Dort, and L. Vollbracht, Tetrahedron Lett., 1970, 1881.
3. H.J. Teuber and W. Rau, Ber., 1953, 86, 1036.
4. R.G.R. Bacon, A.R. Izzat, J. Chem. Soc. (C), 1966, 791.

5. D. Bryce-Smith, and A. Gilbert, *J. Chem. Soc. (C)*, 1964, 873.
6. C.R.H.I. de Jonge, H.J. Hageman, G. Hoentjen, and W.J. Mijs, *Org. Synt.*, 1977, **57**, 78.
7. H. Diehl, and C.C. Hack, *Inorg. Syn.*, 1950, **3**, 196.
8. H.O. House and G.H. Rasmusson, *J. Org. Chem.*, 1961, **26**, 4278.
9. O. Isler, H. Gutmann, M. Montavon, R. Ruegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, 1957, **40**, 1242.
10. P.Z. Bedoukian, *J. Amer. Chem. Soc.*, 1944, **66**, 651.
11. S.M. McElvain and C.H. Stammer, *J. Amer. Chem. Soc.*, 1951, **73**, 915.
12. E.N. Marvell and M.J. Joncich, *J. Amer. Chem. Soc.*, 1951, **73**, 973.
13. W.C. Agosta and W.W. Lowrance, *J. Org. Chem.*, 1970, **35**, 3851.
14. G.A. Russel and M. Ballenegger, *Synthesis*, 1973, 104.
15. M.F. Shostakovskii and N.A. Keiko, *Doklody Akad. Nauk SSSR*, 1965, **162**, 362.
16. Buchta and Satzinger, *Chem. Ber.*, 1959, **92**, 449.
17. K. Bowdem, and M.P. Henry, *J.C.S.P.II*, 1972, 206.
18. N. El Ghandour, O.H. Rousseau and J. Soulier, *Bull. Soc. Chim. France*, 1972, No. 7, 2817.
19. N.L. Wendler, and H.L. Slates, *J. Org. Chem.*, 1967, **32**, 849.
20. H. Nishiyama, H. Nagase, and K. Ohno, *Tetrahedron Lett.*, 1979, 4671.
21. D.H.R. Barton, R.M. Evans, J.C. Hamlet, P.G. Jones and T. Walker, *J. Chem. Soc.*, 1954, 747.
22. D.H.R. Barton, D.A.J. Ives, and B.R. Thomas, *J. Chem. Soc.*, 1954, 903.
23. C.F. Ingham, R.A. Massy-Westropp, and G.D. Reynolds, *Aust. J. Chem.*, 1974, **27**, 1477.
24. C.F. Ingham, R.A. Massy-Westropp, G.D. Reynolds, and W.D. Thorpe, *Aust. J. Chem.*, 1975, **28**, 2499.
25. U. Eisner, J.A. Elvidge, and R.P. Linstead, *J. Chem. Soc.*, 1951, 1501.
26. E. Beška, P. Rapoš, and P. Winternitz, *J. Chem. Soc. (C)*, 1969, 728.

27. F. Fariña and M.V. Martin, *An. R. Soc. Esp. Fis. y Qui., Quimica*, 1971, **67**, 315.

28. H.O. House, M. Gall, and H.D. Olmstead, *J. Org. Chem.*, 1971, **36**, 2361.

29. G.H. Posner and G.L. Loomis, *J.C.S. Chem. Comm.*, 1972, 892.

30. M.W. Rathke, and A. Lindert, *J. Amer. Chem. Soc.*, 1971, **93**, 2318.

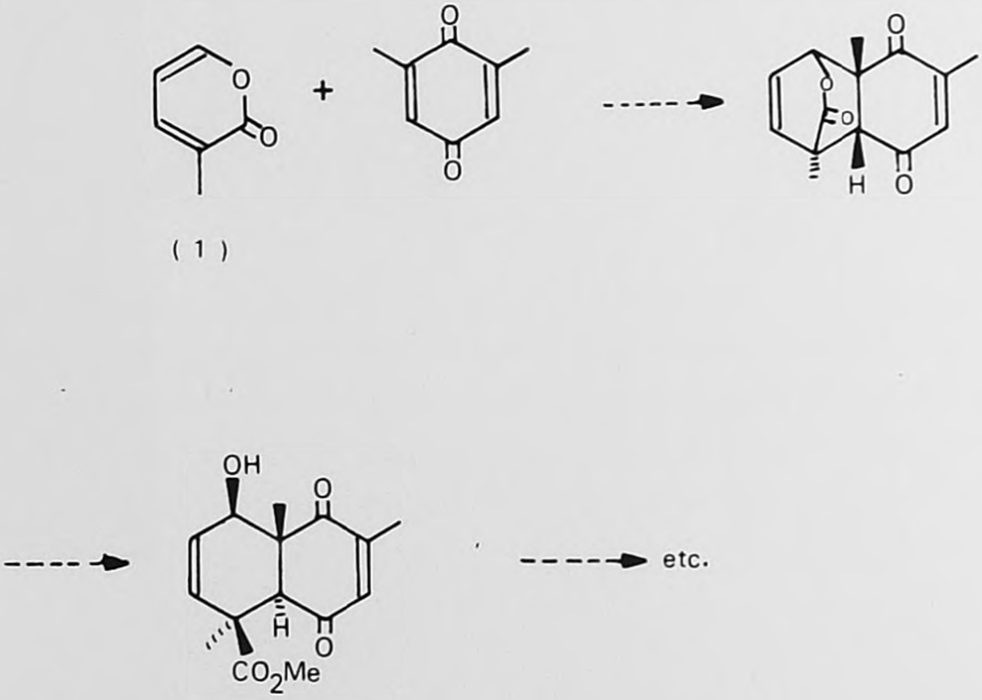
31. H.O. House, L.J. Czuba, M. Gall, and H.D. Olmstead, *J. Org. Chem.*, 1969, **34**, 2324.

32. S. Danishefsky, and T. Kitahara, *J. Am. Chem. Soc.*, 1974, **96**, 7807.

CHAPTER 8. SYNTHESIS AND DIELS-ALDER REACTIONS OF 2-PYRONES

8.1 Introduction

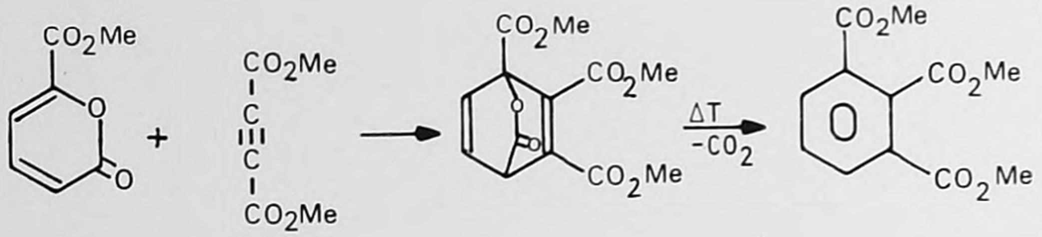
In view of the failure of the open chain dienes to take part in cycloaddition reactions (Chapter 7), attention was turned to the possibility of using cyclic dienes[1] in which the steric and conformational problems would be greatly reduced (e.g. the considerable reactivity of cyclopentadiene compared with the hexa-2, 4-dienes). A re-analysis of the structural requirements for the proposed synthetic scheme (Chapter 7) then suggested that 2-pyrones, and in particular 3-methyl-2-pyrone (1), would be suitable candidates (Scheme 1).



Scheme 1

A search of the literature revealed that Diels-Alder reactions of 2-pyrones have been investigated in some detail, giving confidence in the viability of the proposed route (Scheme 1). These reactions were reviewed in depth in 1974 by Shusherina[2] and only a brief outline of relevant details will therefore be presented here.

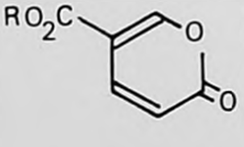
The pyrone ester (2) has been used in the synthesis of aromatic compounds[1,3] by cycloaddition followed by thermal elimination of CO_2, as in Scheme 2.

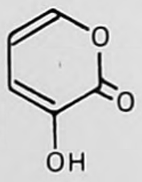


(2)

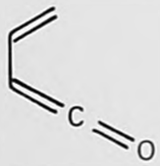
Scheme 2

Similarly, alkyl coumalates (3; R=Me or Et) undergo cycloaddition with various dienophiles, including benzyne, maleic anhydride and benzoquinone[4]. In all of these cases, thermal elimination of the CO_2 bridge at high temperatures occurred, and this elimination reaction has been studied in some detail by Goldstein and Thayer[5].



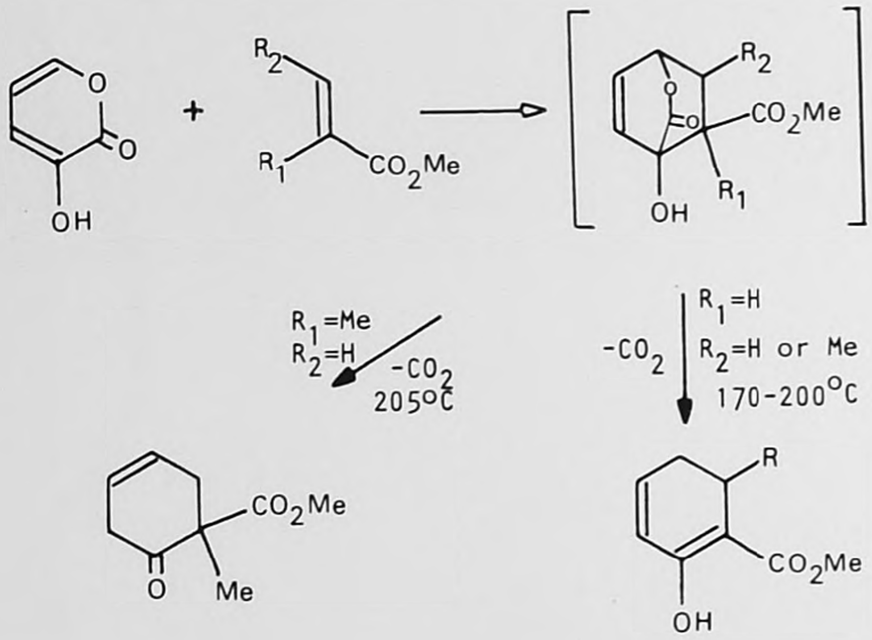


≡



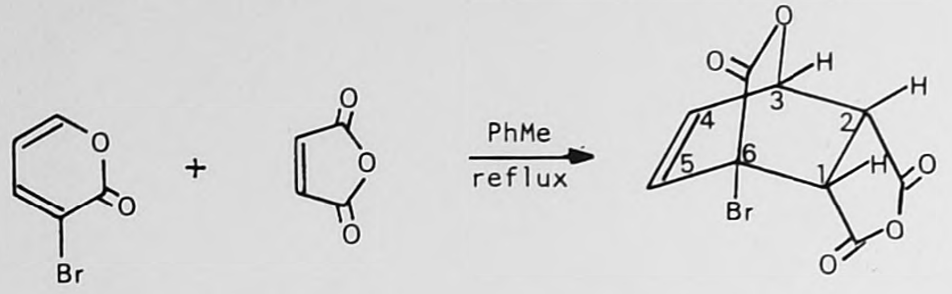
(3) (4) (5)

There are relatively few examples of cycloaddition with 3-substituted-2-pyrones. 3-Hydroxy-2-pyrone (4) has received some attention, notably by Corey[6], since it can be regarded as a vinylketene (5) equivalent. In thermal reactions with unsymmetrical dienophiles, it appears to follow the normal ortho rule (Scheme 3).



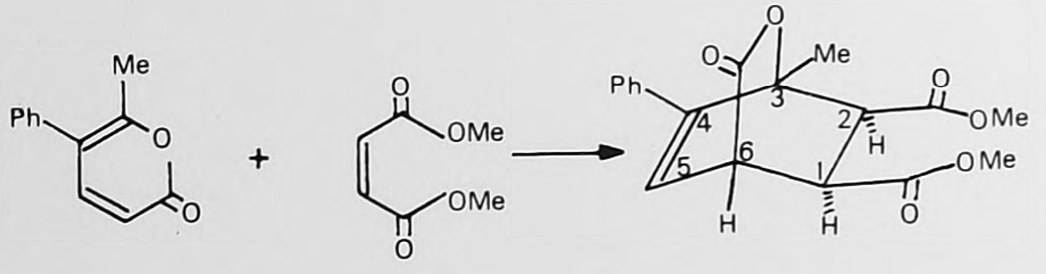
Scheme 3

3-Bromo-2-pyrone (6) forms an adduct with maleic anhydride on refluxing in toluene[7]. At this temperature, the CO_2 bridge is retained and it was shown by NMR that the cycloaddition takes place with endo selectivity, giving the adduct (7). In particular, the H_2-H_3 coupling constant was found to be 4.59 Hz.



(6) (7)

Interestingly, Shusherina *et al*[7,8] also showed that 6-methyl-5-phenyl-2-pyrone gave the *exo*-adduct with maleic ester (Scheme 4), and other 5,6-dialkyl-2-pyrones behaved similarly. The H_1-H_6 coupling constant in (8) was 1.90 Hz.

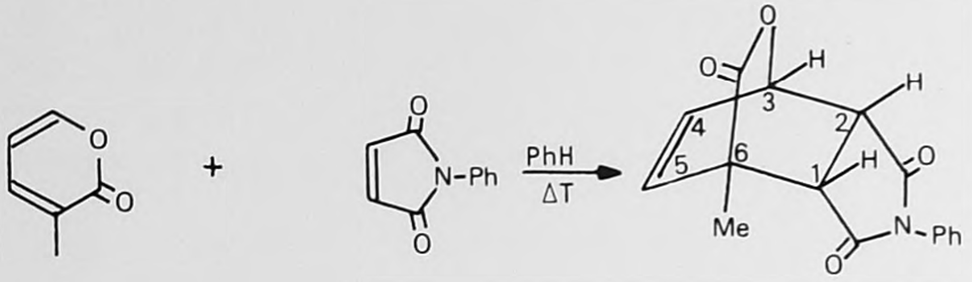


(8)

Scheme 4

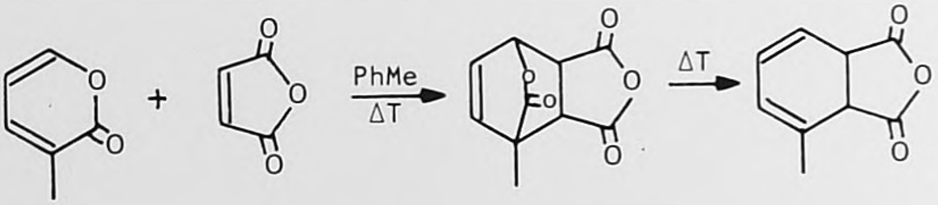
It was later shown[9] that the production of the *exo*-adduct is a specific property of the 6-methyl substituent.

There has been very little work reported on the cycloaddition reactions of 3-methyl-2-pyrone. Its thermal Diels-Alder reaction with N-phenylmaleimide in refluxing benzene[10] gave the adduct (9) in 60% yield. This adduct was shown to have <u>endo</u> stereochemistry by the characteristic $J_{2,3}$ coupling of 4.8 Hz.



(9)

A reaction with maleic anhydride in refluxing toluene was reported[11] to give an adduct (10) of undefined stereochemistry, which underwent decarboxylation to (11) on heating at 160-230°C.



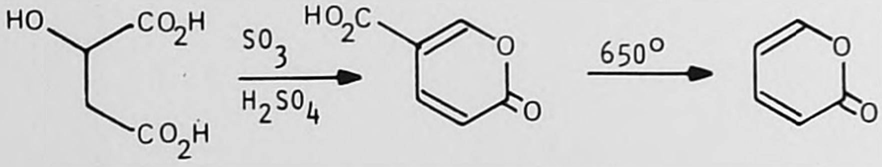
(10) (11)

8.2 Synthesis of 3-methyl-2-pyrone

Only two procedures are described in the literature for the synthesis of 3-methyl-2-pyrone. One of these, which has been used by the Russians[12], involves the selective chloromethylation of 2-pyrone at the 3-position, followed by reduction. The second procedure, reported by Julia and Jassonneix[11] involved a 6-stage synthesis proceeding in very poor yield, involving unstable products in the last two stages and giving a final material which was difficult to purify.

Initially, it was decided to examine the Russian procedure. Although 2-pyrone is commercially available, its great expense precludes its purchase in bulk and it was therefore necessary to synthesize this compound.

Both of the major literature procedures for making 2-pyrone were investigated. A method given in Organic Syntheses[13] involves the decarboxylation of coumalic acid (12) which is readily prepared[14] from malic acid and fuming sulphuric acid (Scheme 5).



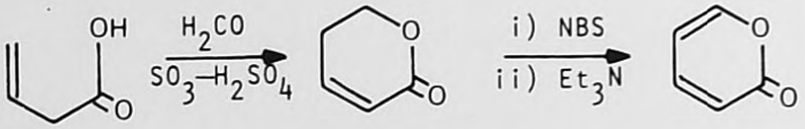
(12)   (13)

Scheme 5

Coumalic acid was prepared in good yield according to the literature procedure[14]. However, its decarboxylation by the vapour phase pyrolysis method[13] proved to be extremely difficult to carry out in practice with the apparatus available and only very small samples of 2-pyrone (13) could be obtained in this way. The further refinements of the apparatus which would have been necessary in order to make this procedure suitable for large-scale work would have been extremely costly and therefore alternative procedures were examined.

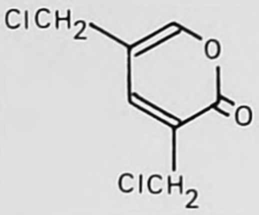
Attempts to decarboxylate coumalic acid using the classical copper-quinoline method[15] gave only a black, intractable tar.

A more recent procedure[16] for the synthesis of 2-pyrone was then examined, involving the acid-catalysed hydroxymethylation of but-3-enoic acid and subsequent bromination-dehydrobromination (Scheme 6). This method was found to work without difficulty, although both the literature yield (23%) and our own yield (20%) were modest.

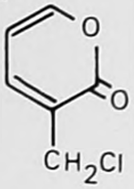


Scheme 6

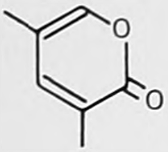
The Russian procedure[12] for chloromethylation of 2-pyrone was then examined. The pyrone was treated with chloromethyl methyl ether in acidic medium maintaining the temperature below 48°. However, the product obtained on work-up proved to be the 3,5-bis(chloromethyl)-2-pyrone (14) rather than the expected 3-chloromethyl-2-pyrone (15).



(14)



(15)

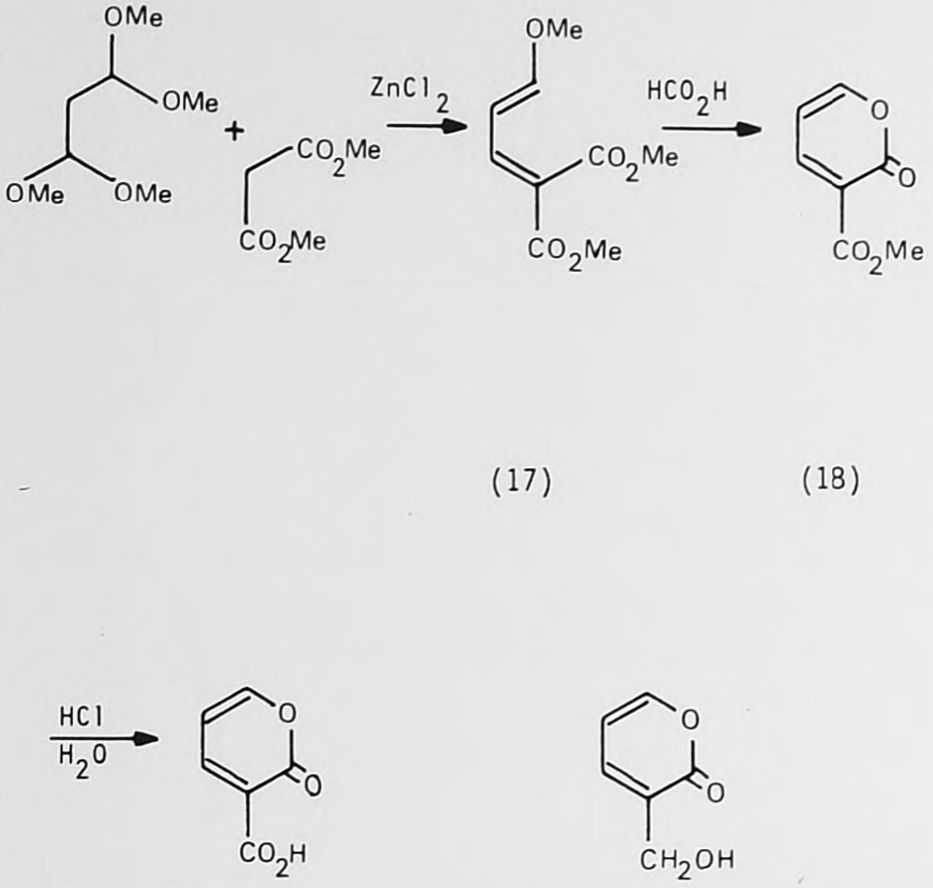


(16)

Attempts to control the chloromethylation by use of lower temperatures led to a product the ^{1}H NMR spectrum of which indicated it to be a mixture of 3-chloromethyl and 5-chloromethyl isomers.

Zinc reduction of the bis(chloromethyl)-2-pyrone proceeded as described in the literature[12], furnishing 3,5-dimethyl-2-pyrone (16). However, in view of the difficulty of obtaining selective 3-chloromethylation of the 2-pyrone, it was felt that alternative routes should be examined.

Using the method of Windholz et al[17] the methyl ester[1] of 2-pyrone-3-carboxylic acid (18) was synthesised and hydrolysed to the free acid (19), as shown in Scheme 7.



(17) (18)

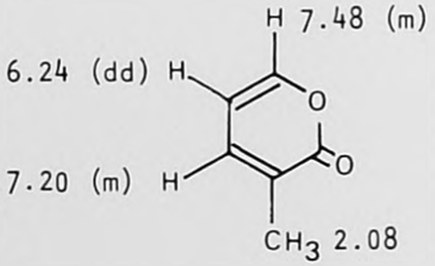
(19) (20)

Scheme 7

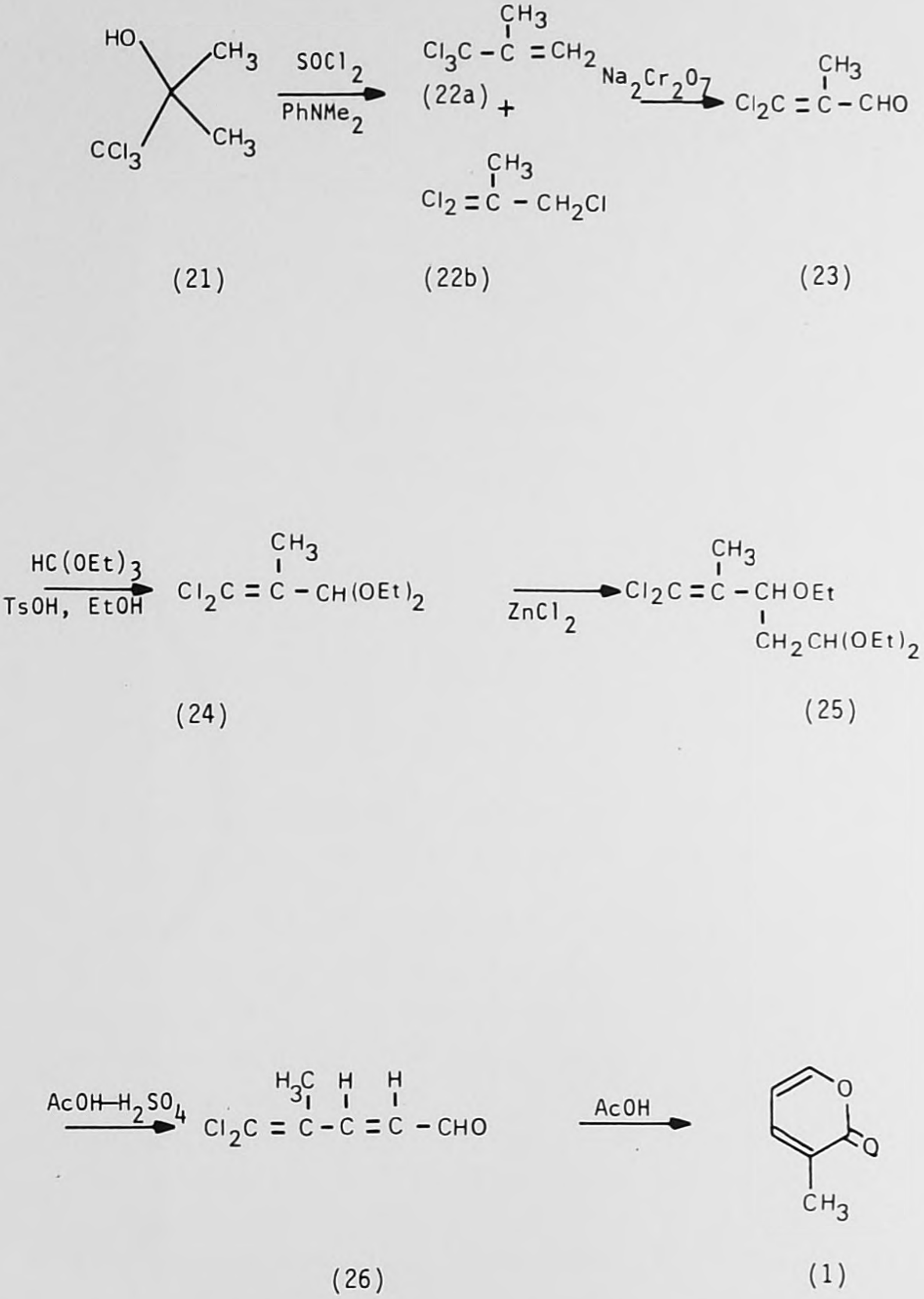
It was hoped that the acid (19) could be reduced to the alcohol (20), which would then be chlorinated to give 3-chloromethyl-2-pyrone. The most attractive method for carrying out the selective reduction of the side-chain in (19) appeared to be the use of diborane[18]. However, this procedure gave only a complex reaction mixture from which no identifiable products could be isolated.

Finally, therefore, attention was turned to the method of Julia and Jassonneix[11] for the synthesis of 3-methyl-2-pyrone (Scheme 8). Chlorination of 250 g of acetone-chloroform (21) led to a mixture of trichloromethyl propenes (22) in 60% yield (lit.[11] 75% yield) which was oxidized to the dichloropropenal (23) in 35% yield (lit.[11] 50% yield). The aldehyde was converted into its ketal (24), in 50% yield (lit.[11] 79% yield). The ketal (24) was condensed with ethyl vinyl ether using Isler's catalyst (10% $ZnCl_2$ in EtOAc) to give the tri-ethoxy compound (25) in 98% yield (lit.[11] 79% yield), and this was hydrolysed to the aldehyde (26). This was obtained quantitatively in the crude form, but after distillation the yield of pure material was 55%. The French workers report a 96% yield of material which is unstable and readily decomposes. The diene aldehyde (26) was cyclized by refluxing in glacial acetic acid and giving 60% of crude product which was purified by thin-layer-chromatography, giving the pure 3-methyl-2-pyrone (1) in a yield of 10%, together with about 10% of recovered starting material. In the literature procedure, the crude reaction product (91%) was found to be too unstable for distillation.

The product (1) obtained showed ν_{max} (film) 1710 (s) and 1640 (m) cm^{-1} and had an NMR spectrum (1a) entirely in accord with the required structure[19].



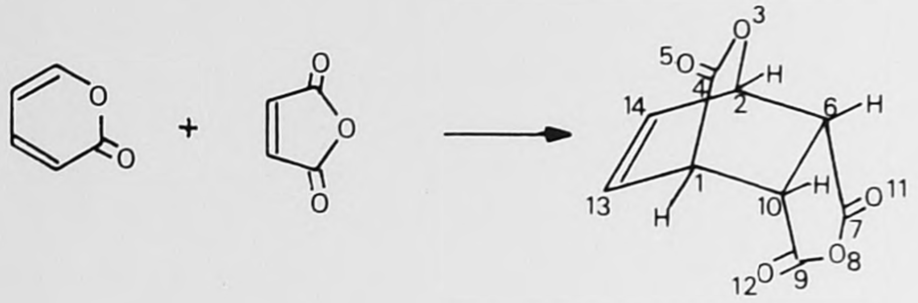
(1a)


HO
CH3
CCl3
CH3
SOCl2
PhNMe2
Cl3C − C = CH2
CH3
(22a) +
Cl2 = C − CH2Cl
CH3
Na2Cr2O7
Cl2C = C − CHO
CH3
(21)
(22b)
(23)
HC(OEt)3
TsOH, EtOH
Cl2C = C − CH(OEt)2
CH3
ZnCl2
Cl2C = C − CHOEt
CH3
CH2CH(OEt)2
(24)
(25)
AcOH−H2SO4
Cl2C = C − C = C − CHO
H3C
H
H
AcOH
O
O
CH3
(26)
(1)

Scheme 8

8.3 Diels-Alder reactions of 2-pyrones

As a model reaction, the thermal cycloaddition of 2-pyrone with malic anhydride[5] was repeated, affording the adduct (27).



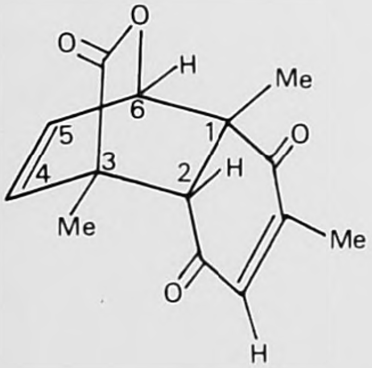
(27)

A thermal reaction between 2-pyrone and xyloquinone was also carried out, but failed to give any identifiable products.

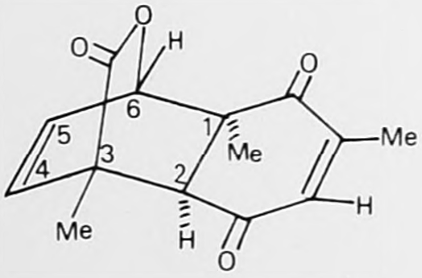
Since only a limited amount of 3-methyl-2-pyrone was available it was only possible to carry out one reaction with this compound. The pyrone (1) and xyloquinone were stirred together in equimolar amounts in dichloromethane in the presence of 1.3 equivalents of aluminium chloride, initially at -78^0 for 2 h and then at room temperature overnight. A further 0.5 equivalents of aluminium chloride was then added and stirring continued for an additional 24 hours. Preparative TLC of the reaction mixture then yielded, in addition to recovered xyloquinone and 3-methyl-2-pyrone, a fraction the NMR spectrum of which suggested the presence of the desired adduct, in an impure form. Further purification by preparative HPLC yielded a homogeneous material in very small quantity, which was characterised only by 400 MHz ^{1}H NMR. This revealed the presence of three methyl singlet signals, in accordance with the formation of a 1:1 cycloadduct (28)-(31). That the adduct was not of an "ortho" type

(30) or (31) was revealed by the presence of a 1-H singlet at δ3.48. This must be due to the H-2 in an adduct of type (28) or (29), since the H-1 in an adduct (30) or (31) would have shown[7] a coupling of either 4-5 Hz for the endo case (30) or 1.9 Hz for the exo case (31). The allylic methyl group in the adduct appears at δ3.10, rather low field because of the de-shielding effect of the in-plane carbonyl group at C-7. The other two methyl groups also appear at surprisingly low field, δ2.18 and 2.26 and this allows the product to be assigned as the endo adduct (28) rather than the exo isomer (29), since in the former the two methyl groups also find themselves in positions which are strongly de-shielded by the C-10 carbonyl and the lactone bridge respectively (as seen in molecular models). These de-shielding influences are absent in the exo isomer.

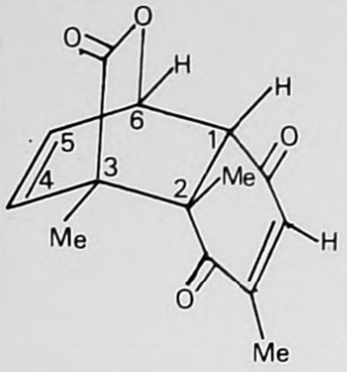
Hence, it is tentatively concluded that the product isolated is the desired non-ortho, endo isomer (28). The rest of the NMR spectrum was unfortunately obscured by residual solvent peaks.



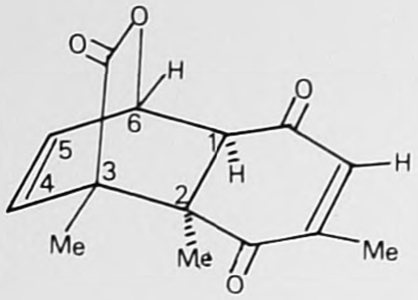
(28)



(29)



(30)



(31)

Clearly, considerably more work needs to be done, to isolate further quantities of the cycloadduct and confirm its structure. This work must unfortunately await the development of an improved synthesis of 3-methyl-2-pyrone, since the French route is clearly impractical for large-scale work.

If the assignment of the structure of the adduct as (28) is correct and this compound can be produced in a reasonable yield, then it will become the key synthetic intermediate for the preparation of a very wide range of terpenoid compounds.

8.4 Experimental

Coumalic acid(12). By the procedure described in Organic Syntheses[14], from malic acic and conc. and fuming sulphuric acid. Yield 66%, mp 207^{0}(Lit:[14] mp 206-209^{0}), $\nu^{nujol}_{max.}$ 3200-2700, 1720, 1680 cm^{-1}, N.m.r. δ $(CD_3)_2CO$) 6.36(H_3,dd 1.5,10), 7.84 (H_4dd 10,3), 8.40 (H_6 dd 3,1.5) ppm.

2-Pyrone(13) a) Via decarboxylation of coumalic acid The procedure followed is described in Organic Syntheses[13]. The Vycor tube used by the authors for the vapour phase pyrolysis was replaced by a copper tube and the connections to the glassware made by the teflon tube. The pressure was held all the time below 5mm Hz. The oven used for heating the copper tube at 650^{0} was calibrated with a thermocouple of platinum, rhodium (13%). Owing to blockage of the connection tubing by the subliming coumalic acid, the yield of 2-pyrone obtained was very poor. ν^{film}_{max} 1720, 1620 cm^{-1}, N.m.r. δ ($CDCl_3$) (in agreement with lit.[20]) 6.24 (H_3,m), 7.34 (H_4,m), 6.38 (H_5,m), 7.50 (H_6,m) ppm; Ms: (also in agreement with lit.[21]) $M^{+\cdot}$ 96(41.4), 95(M-1)(9), base peak 68 $(M-CO)^{+\cdot}$, 50(11.3).

b) Via 5,6 dihydro 2-pyrone A mixture of 5,6 dihydro 2-pyrone (31.2g, 0.32 mol), N-bromosuccinimide (56.7g, 0.32 mol) and benzoyl peroxide (1.6g, 0.007 mol) in carbon tetrachloride (1 ℓ) was refluxed for 45 min. The reaction mixture was filtered and the filtrate evaporated. To the crude product obtained was added triethylamine (distilled and dried over sodium-lead alloy)(400 ml) and the reaction mixture refluxed for 25 min. The residue obtained after evaporation of excess of triethylamine was distilled to give 2-pyrone in 56% yield (bp 115^{0}/33mm) (Lit[16] bp 109^{0}/30 mm). The spectroscopic data was similar to those described in a).

5,6, Dihydro 2-pyrone. A mixture of but-3-enoic acid (86g, 1 mol), paraformaldehyde (30g, 1 mol) and conc. sulphuric acid (4 ml) in 250 ml of acetic acid, was refluxed for 6.5 hr and the solvent was removed after addition of anhydrous sodium acetate (20g,0.24 mol). The residue neutralized with sodium hydroxide (2N) and extracted with ether. The ether extract after washing with water was dried and evaporated in vacuo to give 48.5 g of a crude mixture. Distillation afforded the required product in 35.5% yield, bp 102-104/10 mm (Lit.[16] 98-100°/10 mm), $\nu_{max.}^{film}$ 1730 cm^{-1}, N.m.r. δ ($CDCl_3$) 2.40-2.60 ($2H_5$,m), 4.48 ($2H_6$,m), 6.04(H_3,m), 7.02 (H_4,m) ppm.

<u>3,5 Bis(chloromethyl)2-pyrone(14)</u>. To a solution of 2-pyrone (11g, 0.11 mol) in 40 ml of 96% (W/V) sulphuric acid was added dropwise 20 ml of mono-chloromethyl methyl ether. After 6-8 hr heating at 45-48°, cooling and pouring into 100g of iced water, the resulting oil was extracted with ether. The aqueous phase was neutralized with sodium hydroxide (2N) and the product was extracted several times with ether. The combined ethereal extracts were washed with sodium hydrogen carbonate and water, dried over magnesium sulphate and the solvent distilled. The product was purified by preparative T.L.C. (SiO_2GF254 Type 60), eluent CH_2Cl_2 (two elutions), yield 38% (Lit.[12] 36%), ν_{max}^{film} 1735, 1650, 1580 cm^{-1}, N.m.r. δ ($CDCl_3$) 4.36(2H,s), 4.48 (2H,s), 7.60 (2H, broad peak) ppm, in good agreement with the literature[12].

<u>3,5 Dimethyl 2-pyrone(16)</u>. To a solution of the bis-chloromethyl pyrone(14) (8.5g;0.044 mol) 200 ml of ethanol was added 60g of activated zinc and dropwise 80 ml of conc. hydrochloric acid. A violent reaction occurred and the reaction mixture refluxed for 1.5 h without any external heating. After cooling, the mixture was filtered and the ethanol and hydrochloric acid evaporated in vacuo. The residue was dissolved in 75 ml of ether and subsequently washed with water (5 x 100ml) and the aqueous phase back-extracted several times with ether. The combined ether extracts were dried over magnesium sulphate, evaporated and the residue submitted to preparative column chromatography (SiO_2 for T.L.C., GF 254 Type 60) eluent $CHCl_3$/MeOH, which yielded 1.2g of product (22%)(Lit.[12] 26%), N.m.r. δ($CDCl_3$) 1.93 (3H,s), 2.08(3H,s), 7.04(1H, broad s), 7.16 (1H, broad s) ppm.

(<u>Note:</u> The zinc was activated by washing successively with dichloromethane, ether, a solution of hydrochloric acid/water (1:1), followed by further washing with water and ether, and dried in vacuo at 50^{0} for 3h).

Dimethyl 3-methoxyallylidenemalonate(17). 1,1,3,3-Tetramethoxypropane (82g, 0.5 mol) was heated to reflux with dimethyl malonate (66g, 0.5 mol) in acetic anhydride (200 ml) for 2 h in the presence of zinc chloride (0.7g, 0.005 mol), giving the product(17); yield 55%, bp. 130^{0}/0.2 mm, $\nu_{max.}^{film}$ 1720, 1620 cm^{-1}, N.m.r. δ ($CDCl_3$) 6.20 (1H, dd 11.5, 13.0), 7.12 (1H,d 13.0), 7.48 (1H,d 11.5) ppm.

Methyl 2-pyrone-3-carboxylate(18). 0.6g of compound(17) was stirred and heated (80^{0}C) for 1 h in the presence of 1.5 ml of formic acid. The solvent was evaporated under reduced pressure and the crude mixture purified by preparative T.L.C. (SiO_2GF 254 Type 60) eluent CH_2Cl_2 (twice eluted), affording an oil, $\nu_{max.}^{film}$ 1760, 1730, 1620 cm^{-1}, N.m.r. δ ($CDCl_3$) 3.82 (3H,s), 6.48 (H_5dd 5.7,4.2), 7.86(H_6dd 4.2, not possible to determine $J_{6,4}$), 8.30 (H_4,dd,5.7-) ppm.

2-Pyrone-3 carboxylic acid(19). The ester(18) (3.5 g, 0.022 mol) was hydrolized in the presence of 20ml of hydrochloric acid/water (1:1) affording a yellow solid, yield 50%, mp 128-130^{0} (Lit.[17] 127-128^{0}), $\nu_{max.}^{nujol}$ 3270, 1725, 1690, 1615 cm^{-1}, N.m.r.δ ($CDCl_3$) 6.36 (H_5,m), 7.60 (H_6,m), 8.24(H_4,m) ppm.

3,3,3-Trichloro 2-methyl prop-1-ene(22a). By the procedure described in the literature[11] from acetone-chloroform (250g, 1.34 mol) and distilled thionyl chloride (500ml) in the presence of dimethyl/aniline (1.3ml). The product(22a) was isolated in 60% yield, bp 124-134^{0} (Lit.[11] 131-137^{0}), $\nu_{max.}^{film}$ 790 cm^{-1} v. strong (polichlorinated compounds), δ ($CDCl_3$) 2.20 (3H,s), 5.20 (1H,s), 5.81 (1H,s), ppm.

3,3-Dichloro 2-methyl propenal(23). By the literature procedure[11] from (22a)(115g 0.63 mol), using sodium dichromate dihydrated (78.11g, 0.26 mol) and sodium carbonate decahydrate (13.7g) in 335 ml of water as oxidant. The product was obtained as a pale yellow liquid, yield 35%, bp 55-58/16mm (Lit.[11] 45-46/12 mm), $\nu_{max.}^{film}$

1685, 1625, 1590 cm^{-1}, N.m.r.δ($CDCl_3$) 1.96(3H,s),10.14(1H,s).As minor fraction of this reaction was obtained in approximately 7% yield. 3,3-dichloro-2-methyl prop- 2 en-ol bp 78-80%/16mm $\nu^{\text{film}}_{\text{max.}}$ 3430, 1625 cm^{-1}, N.m.r. δ ($CDCl_3$) 2.00 (3H,s), 4.28 (2H,s) ppm.

1,1-Dichloro-3,3-diethoxy-2-methylprop-1-ene(24). By the literature procedure[11] from compound(23) (30g, 0.214 mol) and triethylorthoformate (66.4g, 0.449 mol), yield 50%, bp 83-86^{o}/16mm (Lit.[11] 92-93^{o}/20mm),$\nu^{\text{film}}_{\text{max.}}$ 1630, 1240, 1110, 1070, 890, 690cm^{-1}, N.m.r. δ ($CDCl_3$) 1.24 (3H,t,d), 1.84(6H,s) 3.58 (4H,m), 5.30 (1H,s), ppm.

1,1-Dichloro-2-methyl-3,5,5-triethoxypent-1-ene(25). By the literature procedure[11] from compound(24) (19g, 0.089 mol), $ZnCl_2$ (~~0.28~~)(0.2g) in acetic acid (2ml) and ethyl vinyl ether (6.3g, 0.089 mol). Yield 98%, $\nu^{\text{film}}_{\text{max.}}$ 1615, 1270, 1065, 930, 750 cm^{-1}, N.m.r. δ ($CDCl_3$) 1.30 (9H,m), 1.80 (3H,s,1.84(2H,s), 3.50 (6H,m), 4.60 (2H,m) ppm.

5,5-Dichloro 4-methylpenta 2,4-dienal(26) By the procedure described in the literature[11] from compound(25) (25g, 0.088 mol) refluxed under N_2 with acetic acid (70ml), conc. sulphuric acid (0.18ml) and a pinch of hydroquinone. The crude product distilled under nitrogen bp. 73/1.5mm (Lit.[11] 73/1.5mm) $\nu^{\text{film}}_{\text{max.}}$ 1695, 1610 cm^{-1}, N.m.r. δ ($CDCl_3$) 2.09 (3H,s), 6.30 (1H,dd,9.0, 15.0) 7.70 (1H, d 15.0), 9.65(1H,d 9.0)ppm.

3-Methyl-2-pyrone(1) By the literature method[11] from compound(26) (2.07, 0.012 mol) refluxed for 15 h with acetic acid (20ml) yield 60%. The crude(1) was purified by preparative T.L.C. (SiO_2GF 254 Type 60), eluent CH_2Cl_2/CH_3CN (93:7), $\nu^{\text{film}}_{\text{max.}}$ 1715, 1640 cm^{-1}, N.m.r.δ($CDCl_3$) 2.08 (3H,s), 6.24 (H_5dd) 7.20 (H_4,m), 7.48 (H_6,m) ppm.

Maleic anhydride adduct of α-pyrone(27) A mixture of 2-pyrone (0.5g, 0.0052 mol), maleic anhydride (0.5, 0.0052 mol) and 10ml of toluene was refluxed for 2 h. Yield 82%, mp difficult to observe because at 175^{o} the product starts decomposing. At 190^{o} all the adduct is melted. $\nu^{\text{film}}_{\text{max.}}$ 1785, 1750, 1620 cm^{-1}, δ (CD_4CN)

4.05 ($3H_{1,10,6}$,m), 5.72 (H_2,m), 6.72 ($2H_{13,17}$,m) ppm.

2-6-Dimethylbenzoquinone adduct of 3-methyl-2-pyrone(28). An equimolar mixture of 3-methyl-2-pyrone (1) (0.149g, 0.0013 mol) and xyloquinone (0.184g, 0.0013 mol) in 15 ml of dry dichloromethane was stirred at room temperature for 38 h in the presence of aluminium chloride (0.340g, 0.0025 mol). The crude mixture obtained was fractionated by preparative T.L.C. (SiO_2GF 254 Type 60), eluent CH_2Cl_2/CH_3CN (98:2), affording 14mg of adduct. In addition, there was recovered some xyloquinone and 3-methyl-2-pyrone. The adduct was further purified by semi-preparative H.P.L.C. on a silica column, eluent Hexane/Isopropanol (99:1). δ ($CDCl_3$) 2.18 (3H,s), 2.26(3H,s), 3.10 (3H,s), 3.48 (1H,s), ppm.

8.5 References

1. T.A. Bryson and D.M. Donelson, *J. Org. Chem.*, 1977, **42**, 2930.
2. N.P. Shusherina, *Russ. Chem. Rev.*, 1974, **43** (10), 851.
3. E. Wenkert, D.B.R. Johnston, and K.G. Dave, *J. Org. Chem.*, 1964, **29**, 2534.
4. A.K. Bahl, and W. Kemp, *J. Chem. Soc. (C)*, 1971, 2268.
5. M.J. Goldstein and G.L. Thayer, Jr., *J. Amer. Chem. Soc.*, 1965, **87**, 1925.
6. E.J. Corey and A.P. Kozikowski, *Tetrahedron Lett.*, 1975, 2389.
7. N.P. Shusherina, M.V. Gapeeva, A.U. Stepanyants, D.M. Mondishka, and R.Y. Levina, *Zh. Org. Khim.*, 1971, **7** (7), 1383.
8. E.Y. Vashakidze, N.P. Shusherina, A.U. Stepanyants, D. Mondishka, and R.Y. Levina, *Zh. Org. Khim.*, 1970, **6** (9), 1918.
9. N.P. Shusherina, M.G. Hakimi, V.S. Deeva, L.V. Betaneli, *Zh. Org. Khim.*, 1975, **11** (9), 1966.
10. N.P. Shusherina, M.G. Hakimi, A.U. Stepanyants, I.V. Zlokazova, *Zh. Org. Khim.*, 1977, **13** (4), 854.
11. M. Julia and C.B. Jassonneix, *Bull. Soc. Chim. France*, 1975, 743.
12. N.P. Shusherina, M.H. Hakimi, A.U. Stepanyants, and I.V. Zlokazova, *J. Org. Chem. URSS*, 1977, **13**, 779.
13. H.E. Zimmerman, G.L. Grunewald, and R.M. Paufler, *Org. Synt.*, 1966, **46**, 101.
14. R.H. Wiley and N.R. Smith, *Org. Synt.*, 1963, **CV4**, 201.
15. D.M. Burness, *Org. Synt.*, 1963, **CV4**, 628.
16. M. Nakagawa, M. Tonozuka, M. Obi, M. Kiuchi, T. Hino, *Synthesis*, 1974, 510.
17. T.B. Windholz, L.H. Peterson, G.J. Kent, *J. Org. Chem.*, 1963, **28**, 1443.
18. N.M. Yoon, C.S. Pak, H.C. Brown, S. Krishnamurthy, and T.P. Stocky, *J. Org. Chem.*, 1973, **38**, 2786.
19. W.H. Pirkle and W.V. Turner, *J. Org. Chem.*, 1975, **40**, 1617.

20. Pirkle and Dines, J. Heterocycl. Chem., 1969, 6, 1.

21. W.H. Pirkle and M. Dines, J. Amer. Chem Soc., 1968, 90, 2318.

SECTION 3

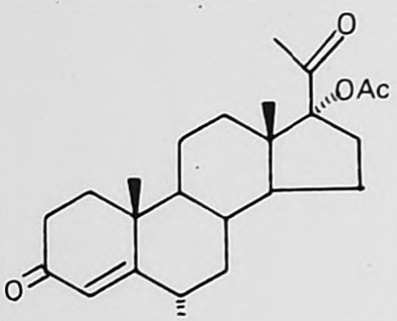
CHAPTER 9. HPLC ANALYSIS OF STEROID ESTERS

9.1 Introduction

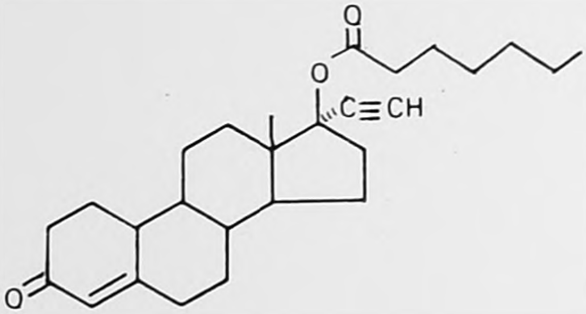
During the entire period of the 3-year research programme described in this thesis, a second, part-time project was undertaken in addition to the main work on Podocarpus constituents. This second project concerned the HPLC analysis of steroid esters and provided valuable experience in the development and refinement of HPLC systems for the separation of closely similar compounds. Application of this expertise in the work with norditerpene lactones was of considerable benefit, as described in Section 1 of this thesis.

Between the early 1960's and the mid-1970's, two compounds became available commercially for use as long-acting injectable contraceptive agents in women. These were medroxyprogesterone acetate (1) (DMPA; Upjohn) and norethisterone enanthate (2)(NET-EN; Schering), with the former having a roughly 3-month duration of activity at a dose of 150 mg injected (aqueous microcrystalline suspension) and the latter a duration of activity of ca. 2 months at a dose of 200 mg injected (oily solution). However, these durations of activity are somewhat shorter than the optimum desirable (6 months). Moreover, the two compounds, although widely used around the world, failed to gain acceptance in a number of countries as a result of both medical and political consideration. Most notably, the Federal Drug Administration in the USA has repeatedly refused to license DMPA for use there. These circumstances led to a decline in interest on the part of the major pharmaceutical companies in the development of further long-acting agents during the early 1970's, despite the continuing demand for such agents in many Third World Countries.

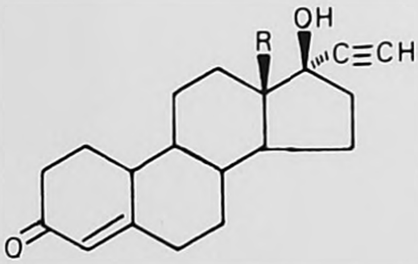
It was against this background that in 1975, the World Health Organization (WHO) instituted a new research effort through the special Programme of Research, Development and Research Training in Human Reproduction. The objectives of the new research programme were to synthesize a large number of new steroid derivatives and to screen them for use as long-acting agents. The chemical design was based on the principle of taking known, active oral progestogens such as norethisterone (3) and levonorgestrel (4) and converting them into esters, which would be themselves relatively inactive "prodrugs". On administration by injection, the ester would be slowly released from the depot site and hydrolyzed by enzymes to the active, free steroid alcohol. Thus, the final activity might be a function of chemical characteristics (e.g. ease of hydrolysis) and/or physicochemical characteristics (solubility, partition coefficient, transport factors, etc.).



(1)

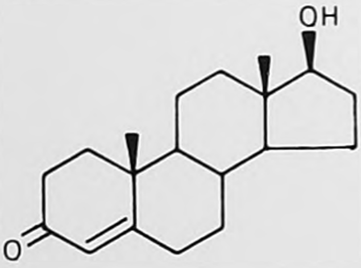


(2)

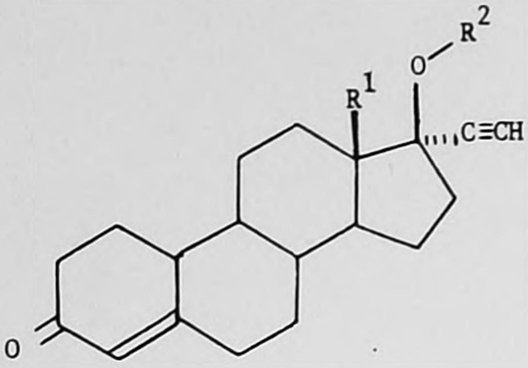


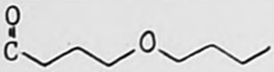
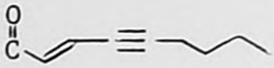
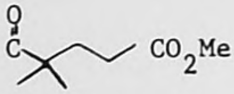
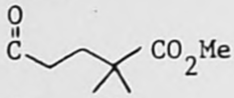
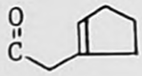
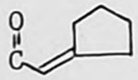
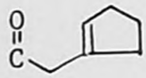
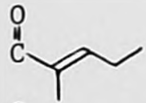
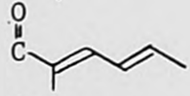
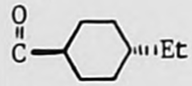
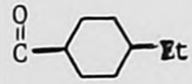
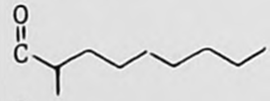
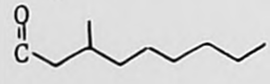
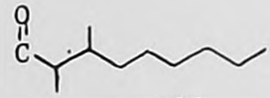
(3) R = Me

(4) R = Et



(5)



Compound	R^1	R^2
6	Me	
7	Me	
8	Me	
9	Me	
10	Me	
11	Me	
12	Me	
13	Et	
14	Me	
15	Me	
16	Me	
17	Me	
18	Me	
19	Me	
20	Me	

A preliminary list of esters was drawn up by the Steering Committee of the Programme, which included eminent steroid chemists such as Carl Djerassi, Pierre Crabbé and Jozef Fried, and was later added to on the basis of progress reports and ad hoc submissions. Pierre Crabbé acted as Chemical Coordinator for the WHO and maintained close links with all of the separate participating groups throughout the period of work.

During a period of 5 years, more than 200 derivatives of norethisterone and levonorgestrel were prepared in the laboratories of universities based in about a dozen Third World countries. After proper registration and coding at the WHO Headquarters in Geneva, these compounds were delivered to our laboratory in London for Quality Control, after which the pure, or purified, samples were forwarded for formulation (School of Pharmacy, London) [redacted]

As a result of this unique endeavour in international cooperation, the Programme has succeeded in the identification of several new esters of levonorgestrel which have durations of activity more than four times greater than that of norethisterone enanthate. These compounds have been patented by WHO and are undergoing further development. They show considerable promise, at this stage, of providing the next generation of long-acting, injectable contraceptive agents for women.

As part of the same overall Programme, efforts have also been made to identify new, long-acting, injectable androgenic agents based on esters of testosterone (5). More than 100 newly synthesized esters of testosterone have been submitted to screening during the same 5-year period.

9.2 Principles of the Quality Control Procedure

It was recognized at the outset of the Programme that the physical form of the ester might be of critical importance in determining its rate of release from the depot site and hence its overall duration of activity (it is interesting to note that investigations by Enever and Lewis at the School of Pharmacy have subsequently established that this is indeed the case for many of the esters). Since the physical form might be influenced strongly by the presence of impurities which could affect crystallinity, rate of dissolution, etc., it was decided at the outset that a consistent, high degree of purity ($\geq$99.5%) would be required in order to obtain significant data and make meaningful comparison between esters. Consequently, the decision was taken to establish an independent, rigorous and uniform quality control procedure for all the samples submitted and this task was allocated to us.

The compounds received for quality control had all been prepared by the esterification of the 17β-hydroxyl group of norethisterone, levonorgestrel or testosterone (which were supplied in high purity to the synthetic chemists), generally with a carboxylic acid (which frequently had to be synthesized), its acid chloride or mixed anhydride, using a variety of coupling procedures. A number of possible sources and types of contamination could therefore be anticipated, including unreacted 17-hydroxysteroid, free acid, coupling agent, decomposition products, solvent, solvent impurities and plasticizers, etc. The unusually high purity required for bioassay meant that the most rigorous efforts had to be made to search for impurities.

On receipt, each sample (typically 1-5 g synthesized) was subjected to a general, physical inspection (state, colour, melting point, solubility in several solvents and search for insoluble residues, etc.) as a preliminary to quantitative analysis. The latter was devided into two main phases, involving firstly a search for volatile components (weight loss on vacuum drying at 40°C for 24 h) and secondly an examination of the involatile material by chromatography.

Qualitative chromatographic analysis by TLC on both silica and alumina, with UV inspection and iodine staining, was considered important as a preliminary screen, particularly to detect the presence of any non-UV absorbing involatile impurities (e.g. certain aliphatic alicyclic acids) which might be missed elsewhere in the analysis.

The most crucial part of the quantitative analysis was the application of HPLC. The major objective with this technique was to determine the presence of steroidal impurities such as the alcohol, isomers, and decomposition products. Detection was carried out at 243 nm, on the assumption that no steroidal materials present would be likely to lack a 4-en-3-one chromophore.

Samples failing quality control analysis were purified by preparative HPLC, using either a Waters Prep 500, Jobin-Yvon Prep 100 or laboratory-assembled equipment. Only the results of the analytical work will be discussed in detail in the present chapter.

9.3 Experimental

Solvents were either purchased as HPLC Grade (acetonitrile, methanol) or were distilled before use (cyclohexane, dichloroethane, dichloromethane, ethyl acetate).

The HPLC equipment consisted of a Waters 6000M pump, a Cecil 2012 detector fitted with an 8μl flow cell, and a Linseis recorder. Injections were made either via a septum injector based on a modified Swagelok fitting, using a high pressure SGE Type B syringe, or with a Rheodyne 7125 loop injector. All columns were 1/4" o.d., 4.5 mm i.d. 316SS, 10-25 cm in length, except for the Corasil II column which was 1/8" o.d., 2 mm i.d., and were fitted with zero dead volume unions. Columns were packed by using high pressure slurry techniques with downward or upward delivery.

Samples were injected as 10% solutions (w/v) in dichloromethane.

9.4 Results and discussion

For adsorption chromatography, binary solvent combinations which were sufficiently transparent for use at 243 nm in the HPLC system and which gave the required control of retention times included 1,2-dichloroethane-cyclohexane, ethyl acetate-cyclohexane and acetonitrile-dichloromethane. Eventually, the last of these was adopted as the standard binary mixture for analytical work. Nearly all the steroids could be eluted with reasonable k' values with mixtures containing 2-10% acetonitrile and this combination was found to be convenient for scale-up to preparative HPLC.

The selection of stationary phases was dictated by two main factors: the need to resolve closely similar components such as structural and stereo-isomers and the requirement to be able to elute both very polar and very non-polar contaminants. Initially, a 10μm, irregular microparticulate silica (Lichrosorb SI60) was selected for adsorption chromatography. An example of its use in the analysis of the norethisterone ester (6) is shown in Figure 1, revealing the presence of several impurities, one of which corresponds to unreacted norethisterone.

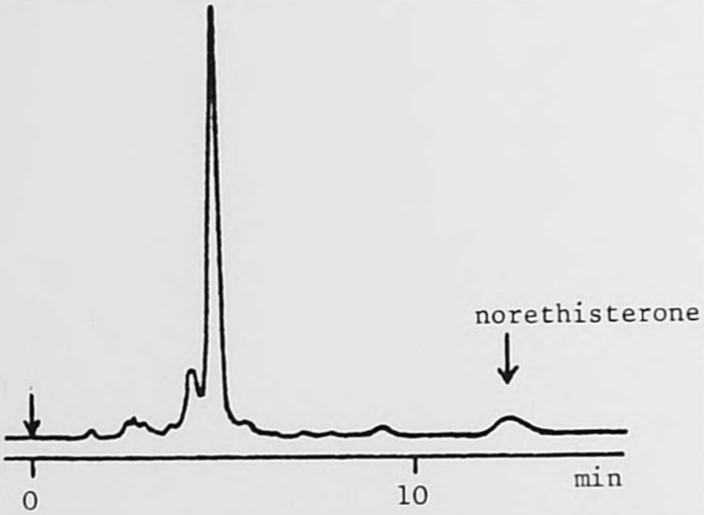


Figure 1. HPLC analysis of norethisterone 3,3-dimethylbutanoate (6). Column: 25 cm Lichrosorb SI60; mobile phase: MeCN:CH_2Cl_2, 10:90.

At an early stage of the work, we examined the use of Corasil II pellicular silica as a second analytical column. This type of packing is of rather low resolution, but also produces low k' values, making it possible to rapidly screen for fairly polar contaminants by a fast column-switching technique with a single set of chromatographic equipment.

The introduction of 5μm spherical silicas has made possible much higher resolution than could be obtained previously and we therefore replaced the Lichrosorb used at the outset with Hypersil 5μm SiO_2. With the availability of high resolution, reverse phase packings based on 5μm supports, which offered scope not only for screening for very polar contaminants but also for separating close-running, isomeric compounds, the use of the Corasil II column was phased out. Both short and long chain alkyl silicas have been used. Although SAS-Hypersil gave good results, as illustrated (Figure 2) by the analysis of the norethisterone ester (7), the longer chain ODS-Hypersil was eventually adopted as the standard phase, primarily because it was considered to have better potential for scale-up in the event that preparative reverse-phase HPLC would be required for the purification of samples.

Figure 3a shows a sample of steroid (8) contaminated with many impurities including a number of very polar ones. The isocratic elution on silica is unable to cover the complete polarity range of the sample, but is nicely complemented by the analysis on ODS-Hypersil (Figure 3b). It was not usual to find such gross contamination of the samples analysed in this work: most had purities greater than 95%. In the case of the ester (8) the high level of impurities probably reflects the instability of the aliphatic 2-en-4-ynoate side chain.

Besides allowing efficient screening of a very broad polarity range, the adoption of a dual analysis procedure involving both normal and reverse phase modes permitted a wide variety of isomeric mixtures to be separated. Figure 4 shows the results of analysing a sample reported to have the structure 9. The observation of two components in a 1:1 ratio suggested the presence of a positional isomer. This was confirmed subsequently when the two

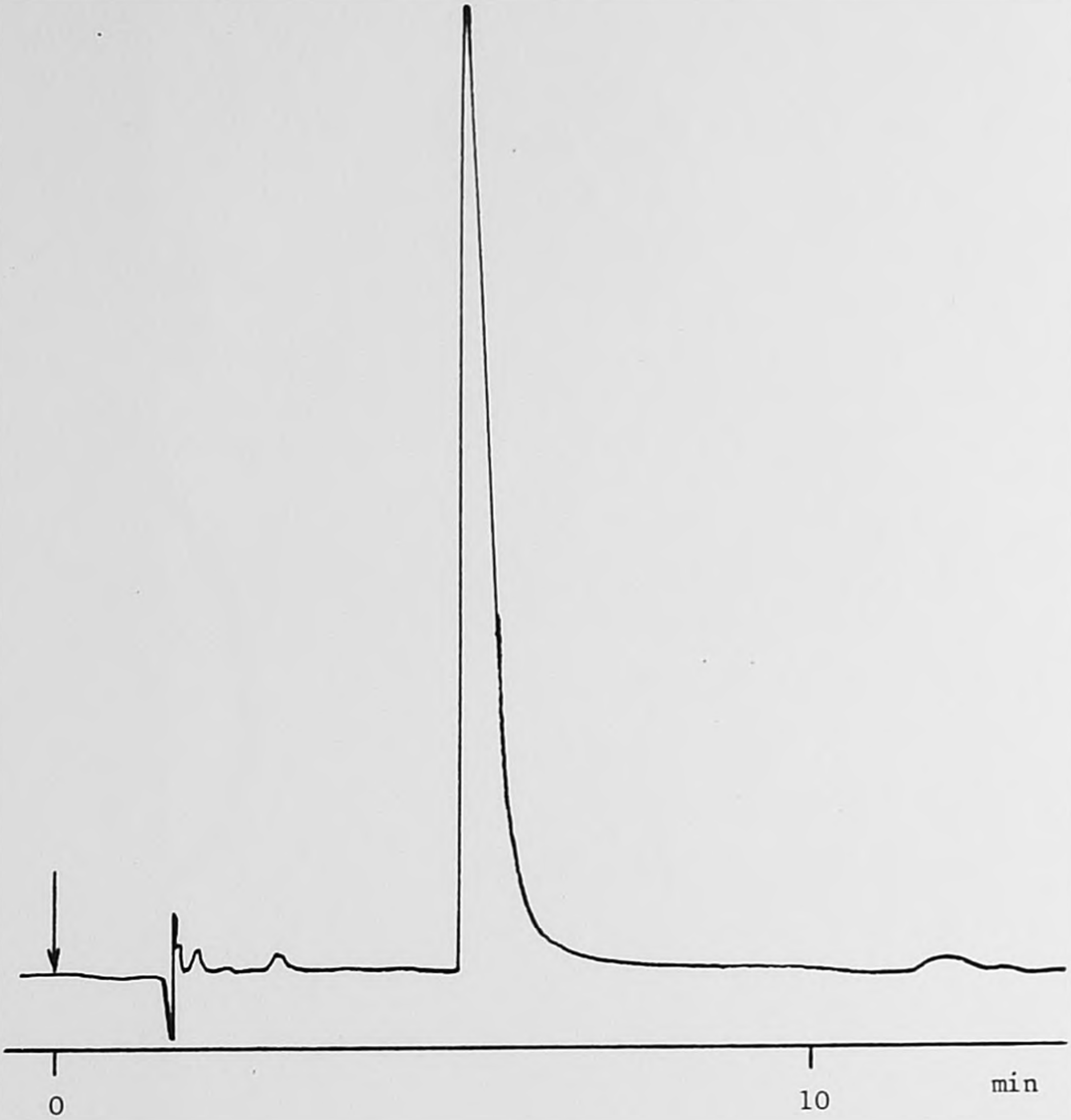
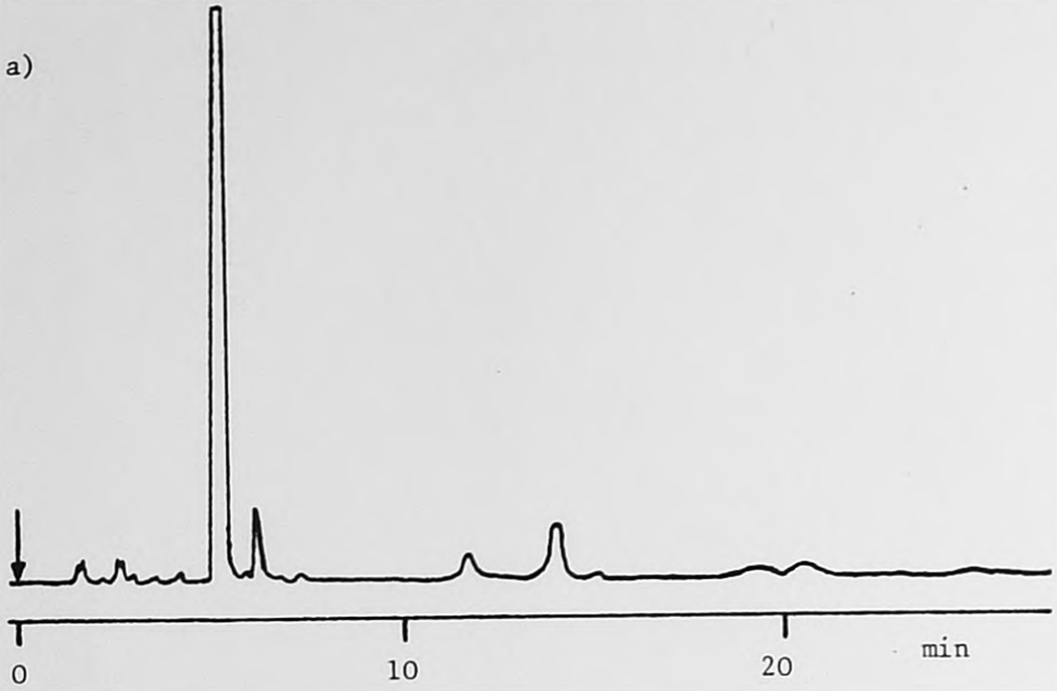


Figure 2. HPLC analysis of norethisterone 4-butoxybuanoate (7). Column: 12.5 cm SAS-Hypersil; mobile phase: MeOH:H_2O, 80:20.



a) Column: 25 cm Hypersil 5 µm.

Mobile phase: MeCN:CH_2Cl_2, 5:95.

b) Column: 12.5 cm ODS-Hypersil.

Mobile phase: MeOH:H_2O, 90:10.

Figure 3. HPLC analysis of norethisterone E-non-2-en-4-ynoate (8).

components were separated by preparative HPLC and were shown to have almost identical NMR spectra. The second component is believed to have the structure (10).

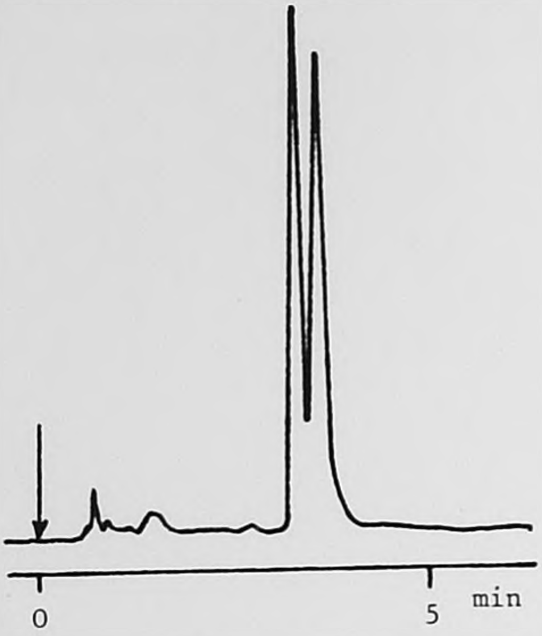


Figure 4. HPLC analysis of norethisterone methyl 2,2-dimethylglutarate (9/10).
Column: 25 cm Hypersil 5μm; mobile phase: MeCN:CH_2Cl_2, 10:90.

A second example of positional isomerism is furnished by the case of compound (11). The ODS-Hypersil column showed the presence of ca. 15% of a component which was less well resolved on the silica column (Figure 5). In view of the known instability of such β,γ-unsaturated esters with respect to the conjugated α,β-isomers, it was suspected that the contaminant had the structure (12). A preliminary inspection of the ^1H NMR spectrum of the steroid sample revealed the presence of only the two required olefinic signals at 4.15τ and 4.45τ. However, the signal for the side chain olefinic proton in (12) would be expected to coincide with that of the C_4-H of the steroid nucleus and, indeed, the signal for the peak at 4.15τ was slightly large. Confirmation came from the addition of $Eu(fod)_3$, which selectively complexed to the C_3 oxygen and rapidly shifted the C_4-H downfield, leaving behind the previously hidden signal of the vinylic α-proton in the side chain of the contaminant (Figure 6). A similar observation of positional isomerism was made with the corresponding levonorgestrel ester (13), the presence of the α,β-isomer again being revealed by reverse phase HPLC, with considerably worse resolution on silica.

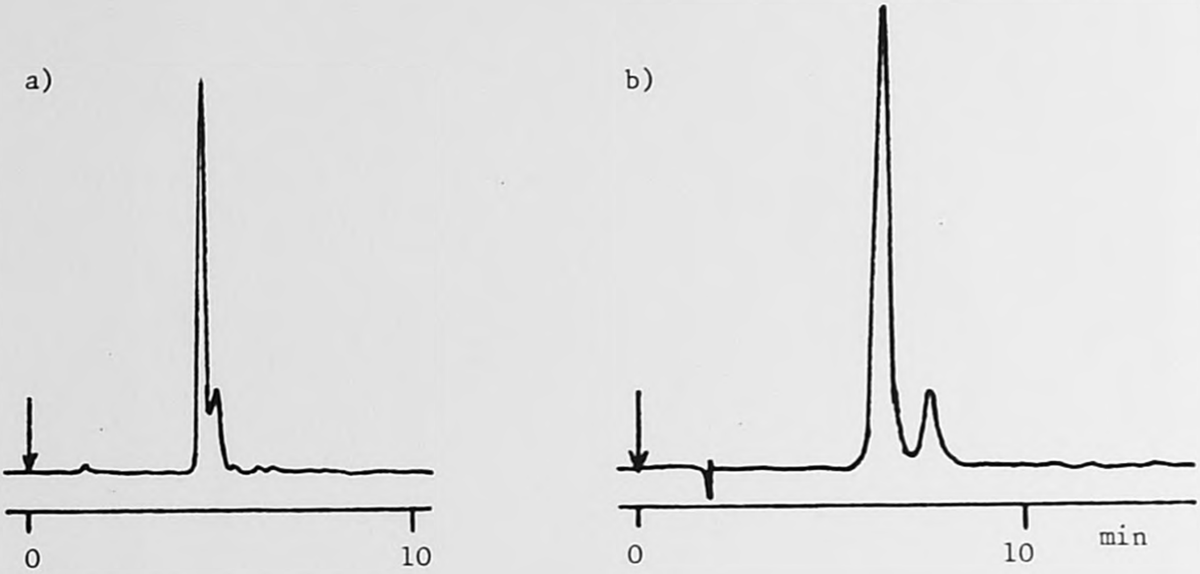


Figure 5. HPLC analysis of norethisterone 1-cyclopentenylacetate (11).

a) Column: 25 cm Hypersil 5 μm; mobile phase: MeCN:CH_2Cl_2, 5:95.

b) Column: 25 cm ODS-Hypersil; mobile phase: MeOH:H_2O, 80:20.

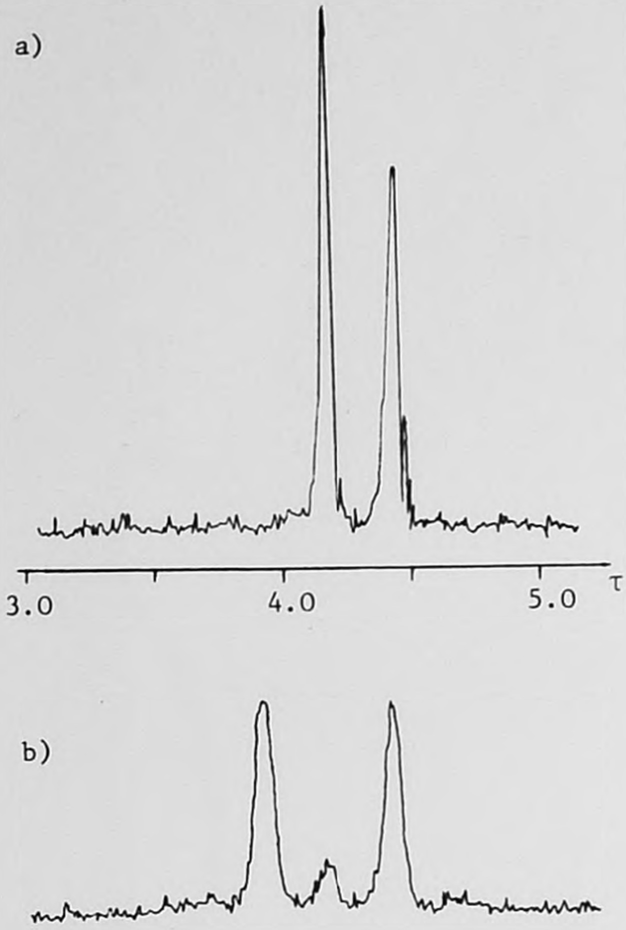


Figure 6. Olefinic region of ^1H NMR spectrum of norethisterone 1-cyclopentenylacetate (11).

a) Normal spectrum in $CDCl_3$.
b) Spectrum with addition of $Eu(fod)_3$.

In contrast to these results for positional isomers, the geometric isomerism of olefinic side chains was always visible on silica and usually poorly, if at all, revealed on ODS-Hypersil. For example, analysis of the norethisterone ester of E-2-methylpent-2-enoic acid (14) showed the presence of the Z-isomer (Figure 7). For the 2-methyl-E, E-hexa-2, 4-dienoate of levonorgestrel (15), only one component was visible on ODS-Hypersil but a very poorly resolved shoulder was apparent on the 5μm Hypersil column using 5:95 MeCN-CH_2Cl_2 as mobile phase. Slightly better results were obtained with 2:98

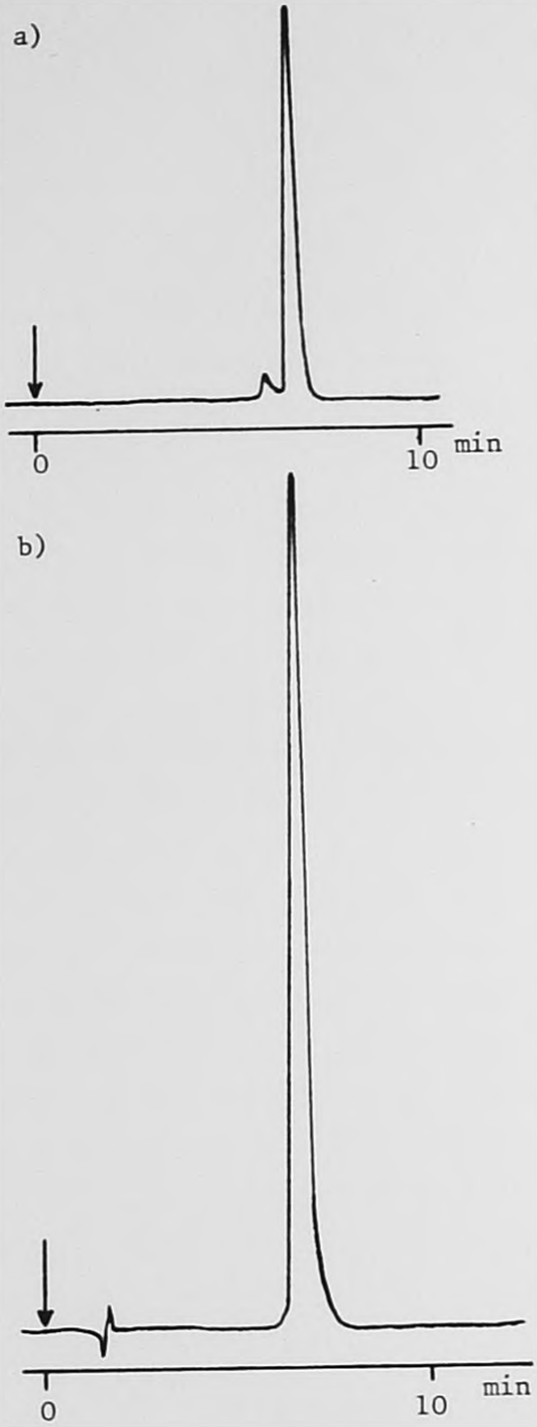


Figure 7. HPLC analysis of norethisterone E-2-methylpent-2-enoate (14).

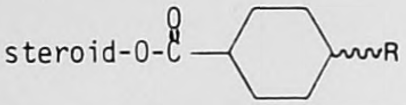
a) Column: 25 cm Hypersil 5 μm; mobile phase: MeCN:CH_2Cl_2, 5:95.
b) Column: 25 cm ODS-Hypersil; mobile phase: MeOH:H_2O, 80:20.

$MeCN-CH_2Cl_2$, but the difficulty of this separation led us to examine the use of the 3μm Hypersil which has recently become available. The new material gave a much more satisfactory separation of the impurity in (15), which is believed to be a geometric isomer, the two main components having α=1.04.

Further types of isomerisation which were detected during analysis included structures in which the esterifying acid is based on a disubstituted, saturated ring. The esters (16) and (17) are diastereoisomers, differing only in relative ring geometry in the side chain (note that the acid itself has a plane of symmetry and is achiral). This class of diastereoisomers, resulting from geometric isomerism in a 4-alkylcyclohexylcarboxylate side chain, is readily resolvable on both Hypersil and ODS-Hypersil, with α values generally around 1.1 - 1.2. We observed such separations in every one of seven pairs of isomeric 4-substituted cyclohexylcarboxylates examined. The results are presented in Table 1. It is interesting to note that in the case of these stereoisomers, the usual rule of reversal of retention order on changing from normal to reverse phase was not followed. The cis isomer always eluted before the trans isomer on both Hypersil and ODS-Hypersil, for all seven pairs of compounds. The results suggest that for diastereoisomers of this type, the separation is not primarily determined by the polarity of the molecules but by their sterically controlled approach to the support surface, which is independent of the chemical character of the surface. The consistency observed in relative retention order indicates that HPLC has a valuable role to play in the assignment of stereochemistries to isomers of this class.

The diastereoisomeric esters resulting from the combination of a steroidal alcohol with a racemic, aliphatic acid containing a single asymmetric centre behaved differently. Ten such cases were examined, typical examples including the norethisterone esters of the branched acids (18) and (19) and in no case was there any evidence for resolution of the diastereoisomeric pairs on either Hypersil or ODS-

Table 1. Comparison of retention data for steroidal 4-substituted-cyclohexylcarboxylates.



Structure		Retention Data[a]					
Steroid	R	Hypersil SiO_2			ODS-Hypersil[d]		
		k'_C	k'_T	α	k'_C	k'_T	α
testosterone	cyclohexyl[b]	2.14	2.29	1.07	7.71	9.50	1.23
testosterone	methyl[c]	1.57	1.86	1.18	2.36	2.50	1.06
testosterone	propyl[c]	1.50	1.64	1.09	4.14	4.64	1.12
testosterone	pentyl[c]	1.43	1.64	1.15	7.51	8.79	1.16
norethisterone	methyl[b]	1.71	1.86	1.09	2.17	2.29	1.06
norethisterone	propyl[b]	1.57	1.71	1.09	2.21	2.50	1.13
norethisterone	pentyl[b]	1.57	1.71	1.09	3.86	4.50	1.17

Notes: a. Both columns 25 cm.

$$k' = \frac{R_t - R_o}{R_o}$$, where R_t is retention time of compound
R_o is elution time for zero retention (1.40 min.).

k'_C and k'_T refer to cis and trans isomers, respectively.

$\alpha = k'_T/k'_C$.

b. Mobile phase: $MeCN:CH_2Cl_2$, 5:95 on Hypersil.

c. Mobile phase: $MeCN:CH_2Cl_2$, 7:93 on Hypersil.

d. Mobile phase: $MeOH:H_2O$, 90:10 on ODS-Hypersil.

Hypersil. The norethisterone ester of the branched acid (20), containing two chiral centres in the side chain, also showed no sign of resolving into the four diastereomeric components present.

Out of more than 300 samples of steroid esters analysed in the present work, about 50% passed quality control inspection without requiring purification. A further 25% were between 95.0 and 99.5% pure. About 15% of all the samples had purities below 90%. Thus, the decision by the WHO to institute quality control procedures is justified by this outcome and by the demonstration by other participants in the programme of the crucial role played by the physicochemical properties of the esters in determining duration of activity.

The WHO has made arrangements for a complete volume of the journal Steroids to contain publications on all aspects of the programme, [redacted] will appear in print during 1982.