

THE CHEMISTRY OF SOME PLANT EXTRACTIVES

A thesis submitted in
partial fulfilment of the requirements
for admission to the degree of

DOCTOR OF PHILOSOPHY

by

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PREFACE

Unless otherwise indicated, the results recorded in this thesis are those of the author.

The work in this thesis was carried out under the supervision of Dr. W.C. Taylor and the author is grateful for his helpful guidance. Thanks are also due to Dr. E. Ritchie, F.A.A., and Dr. S. Sternhell for many valuable discussions, Professor L.H. Briggs, Department of Chemistry, University of Auckland, N.Z., for samples of meliternatin and melisimplexin and to Mr. W.T. Jones, C.S.I.R.O., Brisbane, for collecting the plant material.

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ROBUSTA

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PART I

THE CHEMICAL CONSTITUENTS OF

MELICOPE BROADBENTIANA

INTRODUCTION

Chemically, the family Rutaceae presents a striking picture of synthetic versatility. The structural diversity of the constituents together with their abundance throughout the genera distinguishes the family from all others. The leaves in many species have been found to be relatively rich in essential oils and terpenes. Karrer¹ notes the wide occurrence of coumarins and chromenes throughout the family whilst Geissman² draws attention to the variety of flavonoid compounds found in the group. Other classes of compounds that are recorded in the family are the lignans^{3,4} and triterpenes^{1,5,6}. Price⁷ comments on one aspect of the chemistry of the Rutaceae stating that its capacity to add a prenyl unit to a wide variety of molecular types appears to be biochemically characteristic of it. But perhaps the most striking example of this synthetic versatility is furnished by the number of alkaloid types found in members of the family; the acridine, the furoquinoline, the quina-zoline, the indoloquinazoline, the canthinone, the imidazole, the oxazole, the benzyloquinoline groups and a number of simple aromatic amines and amides have been found⁸ as constituents.

Members of the rutaceous genus Melicope are small trees or shrubs growing in sub-tropical habitats in Australia and

New Zealand. All the species that have been chemically examined show this diversity of plant constituents not only within the genera but in the individual species. The New Zealand species Melicope ternata⁹ (J.R. and G. Forst.) and M. simplex¹⁰ are quite remarkable in that they produce a series of six flavones; five of these possess a methylenedioxy group which is otherwise quite rare in flavonoids. Three of these flavones meliternatin (1), meliternin and ternatin (from M. ternata) also occur in the Australian species M. mantelli⁴ (Buch). M. ternata contains besides flavonoids the complex coumarin, xanthoxyletin⁹. Accompanying the flavones in M. simplex are two chromenes, evodionol and alloevodionol which were first isolated from the leaves of Evodia littoralis¹¹ (Endl.) and E. elleryana (F. Muell.)¹², Queensland trees of the closely related genus Evodia. M. fareana (Engl.) was found by Price¹³ to contain three acridine alkaloids, melicopicine, melicopine and melicopidine. They were accompanied by small amounts of furoquinoline bases, skimmianine and acronycidine. Members of the acridine group of alkaloids have been isolated from two other rutaceous genera indigenous to the rain forests of Northern Australia, from one Brazilian and one Indian genus; the furoquinoline alkaloids, on the other hand, occur in twenty nine rutaceous genera of the thirty six from which alkaloids have been isolated⁸. M. sarcococca (Laut.) has been shown by Geissman¹⁴

to contain two new flavanones, 4',5-dihydroxy-3',7-dimethoxy-flavanone and its 4'-isopentenyl ether.

As M. broadbentiana (Bail.)¹⁵ is a member of such an interesting genus, an examination of its constituents promised to be rewarding. The species is endemic to Australia, growing only in rain forest areas on the higher mountain slopes of the Atherton Tableland, North Queensland. It is a slender erect shrub which reaches a height of 12 feet and has a sparse covering of relatively large leaves. The wood of the tree is extremely hard and is covered with a thin sheet of yellowish bark.

THE CONSTITUENTS OF MELICOPE BROADBENTIANA

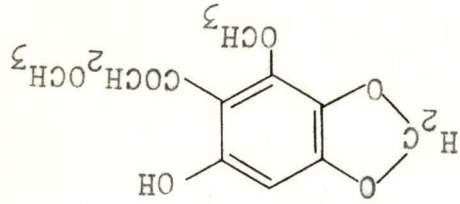
The bark, wood and leaves were examined separately. Each was extracted in turn with light petroleum, ether and methanol. The extracts were concentrated to small volumes and refrigerated for several weeks. Any solid material that precipitated was collected and purified by chromatography on silica gel. A test for alkaloids on each of the extracts with Meyer's Reagent, carried out in the usual way, was negative. Further fractionation was achieved by shaking an ethereal solution of each concentrate with 5% sodium hydrogen carbonate, 5% sodium carbonate and 2% sodium hydroxide solutions.

Four batches of leaves were extracted in order to obtain sufficient amounts of the constituents. The relative amounts of extractives varied quite markedly from batch to batch, presumably the result of collecting from different areas, since each collection was made at the same time of the year. One batch each of wood and bark was extracted.

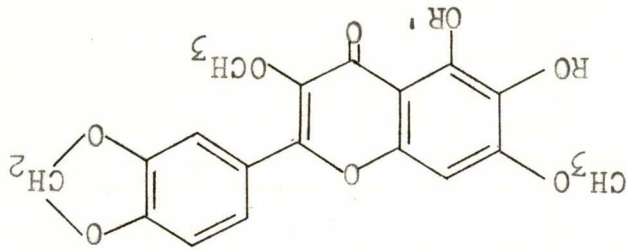
The Constituents of the Bark

A new flavone, "melibentin" (0.5%) was isolated from the light petroleum and ether extracts; its structure is discussed below. Two known flavones (meliternatin(1) (0.06%) and melisimplexin (0.04%) (2)) were also found in the ether

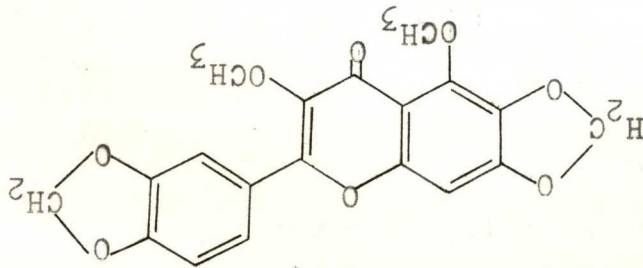
(4)



- (2) $R=CH_3, R'=CH_3$
(3) $R=CH_3, R'=H$
(9) $R=H, R'=CH_3$



(1)



extracts.

The Constituents of the Wood

The wood contained the same flavones as the bark but in greater amounts viz. melibentin (1.1%), meliternatin (0.3%) and melisimplexin (0.02%).

The Constituents of the Leaves

A new phenol "melicopin", (0.2%) was isolated from the phenolic fraction of the ether extract. A related phenol, (0.14%) "methylnelicopin", was found in the fraction which separated from the light petroleum extract. The structures of these two substances are discussed below. Two flavones, meliternatin (0.7%) and melisimplin (0.002%) (3), were present in both the light petroleum and ether extracts. The aliphatic alcohol, triacontanol, was found in 0.5% yield in the light petroleum soluble fraction.

Identification of Known Compounds

Meliternatin (1)

This flavone was identified as meliternatin by m.p., mixed m.p. and comparison of IR spectra with an authentic specimen from M. ternata. The structure of meliternatin has been shown by degradation and synthesis⁹ by Briggs and Locker to be (1). Hydrolysis of the flavone in the usual way gave the products isolated by Briggs and Locker, namely,

piperonylic acid and 2-hydroxy- ω ,6-dimethoxy-4,5-methylene-dioxyacetophenone (4).

Examination of the NMR spectrum of (1) confirmed the assigned structure. Two signals, each of three-proton intensity, at 3.87 and 4.08 ppm were attributed to the methoxyl protons, whilst a sharp four-proton singlet at 5.86 ppm was due to the methylenedioxy protons. A signal at 6.64 ppm was consistent with the expected¹⁶ chemical shift of the 8-H. The signals from the aromatic protons of the substituted B ring formed a well-resolved ABX pattern. The 5'-H, shielded by the methylenedioxy group, appeared as a one proton doublet centred at 6.90 ppm; the value of the coupling constant (J 8c/s) indicated ortho coupling between the 5'-H and 6'-H. The 2',6'-protons resonated as anticipated¹⁶ at lower field; the singlet at 7.54 ppm, partially superimposed on the signal from the 6'-H, was attributed to the 2'-H, whilst the broad doublet (J 8c/s), centred at 7.62 ppm, was clearly due to the remaining proton, 6'-H.

Melisimplexin (2)

Identification of this compound was also made by m.p., mixed m.p. and comparison of the IR spectra with an authentic specimen of melisimplexin from M. simplex.

The NMR spectrum confirmed the assignment of structure made by Briggs and Locker¹⁰. A series of three-proton

singlets at 3.94, 3.98, 4.04 and 4.06 ppm confirmed the presence of four methoxyl groups whilst a two-proton singlet at 6.12 ppm indicated a methylenedioxy group. The aromatic portion of the spectrum was identical with the corresponding portion of the spectrum of (1) except for the chemical shift of the 8-H which now appeared at 6.81 ppm.

Melisimplin (3)

As insufficient material was isolated to allow identification to be made by the usual methods, it was decided to synthesise simple derivatives of the isolated flavones in the hope of achieving an identification. Melibentin (see later) was smoothly demethylated to yield a compound (5) which was not identical with the natural product. The formation of quinones by oxidative demethylation of phenolic ethers has been known for many years and several examples are cited by Ansell et al.¹⁷. Treatment of an ethereal solution of (5) with concentrated nitric acid at 0° gave the desired quinone (6). Comparison of IR spectra and m.p.s showed clearly that the natural product was not identical with it.

The pigment was finally recognised as melisimplin (3) by direct comparison with an authentic sample of this substance, prepared by demethylating melisimplexin with aluminium chloride in refluxing nitrobenzene¹⁸. The co-occurrence of these two

flavones might have been expected as they are also both present in M. simplex¹⁰.

Triacontanol

The waxy solid which was present in the leaf light petroleum extract analysed for $C_{30}H_{62}O$ and had characteristic hydroxyl absorption in the IR at 3350 and 1030 cm^{-1} . It was probably "triacontanol", a very common constituent of leaf extracts¹. No further examination of it was made.

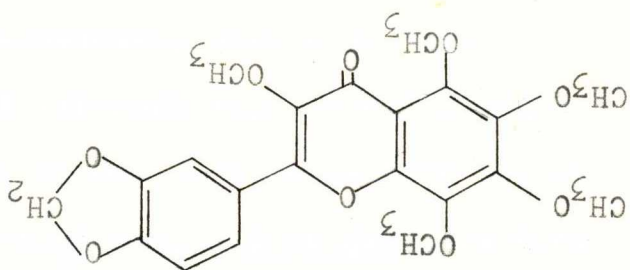
THE STRUCTURE OF MELIBENTIN

Melibentin crystallised as yellow prisms from methanol, m.p. 134-135°. Its structure has been firmly established as (7).

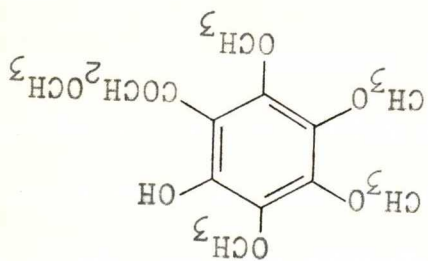
By analysis the molecular formula was established as $C_{21}H_{20}O_9$, with five methoxyl groups. It was recognised as a flavonoid compound by its solubility in concentrated hydrochloric acid to give a bright yellow solution from which it was reprecipitated on dilution. Reduction with magnesium in hydrochloric acid gave a bright red colour, indicative of a flavone with a methoxyl at C3¹⁹. The presence of a methylenedioxy group was strongly suggested by positive Labat²⁰ and Hanson²¹ tests. Briggs and Colebrook²² have shown from a survey of the IR absorption of a series of methoxy- and methylenedioxyflavones that peaks at 2780, 926 (strong) and 719 cm^{-1} are diagnostic for the methylenedioxy group. These peaks were present in the spectrum of melibentin. A negative ferric chloride test and no hydroxyl absorption in the IR suggested a fully alkylated flavone. IR absorption at 1640 cm^{-1} and characteristic UV maxima at λ 240, 270sh, 340m μ (log ϵ 4.38, 4.20, 4.35) were quite compatible with this assignment (7).

Melibentin was then degraded by treatment with 5% alcoholic potassium hydroxide in the usual manner. Two

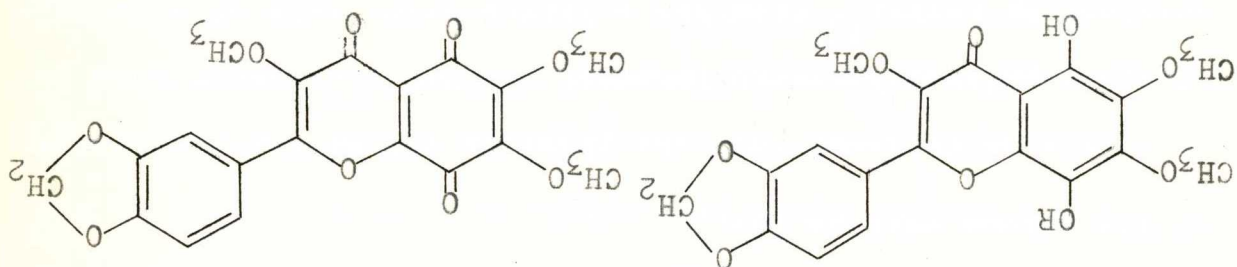
(7)



(8)



(5) R=CH₃.
(10) R=H.



products were isolated from this hydrolysis: a phenolic ketone (8), $C_{13}H_{18}O_7$ and an acid, $C_8H_6O_4$. The acid was identified as piperonylic acid by comparison with an authentic sample (m.p., mixed m.p. and identical IR spectra). Since melibentin contained five methoxyl groups, it followed that the structure of the ketone must be (8).

It also followed that the phenolic ketone was 2-hydroxy- ω ,3,4,5,6-pentamethoxyacetophenone. It had previously been obtained by Karrer²³ from the alkaline hydrolysis of dimethylcalycopterin and its reported m.p. agreed with that of the ketone from melibentin. The structure of calycopterin has been established by degradation²⁴ and synthesis²⁵ as 5,4-dihydroxy-3,6,7,8-tetramethoxyflavone. The structure of the ketone was confirmed by its NMR spectrum. The series of three-proton singlets at 3.52, 3.80, 3.85, 4.00 and 4.09 ppm were assigned to the five methoxyl groups and the sharp two-proton singlet at 4.65 ppm to the methylene protons α to the carbonyl group. The hydroxyl group appeared as a singlet at 11.17 ppm; this peak disappeared on exchange with deuterium.

This evidence established the structure of melibentin as 3'4'-methylenedioxy-3,5,6,7,8-pentamethoxyflavone (7).

Support for this structure was obtained from its NMR spectrum. Five-three-proton singlets at 3.90, 3.97, 4.00, 4.02, and 4.10 ppm were assigned to the methoxyl protons. The

methylenedioxy group gave rise to a low field singlet of two-proton intensity at 6.05 ppm. The three aromatic hydrogens of ring B formed an ABX pattern identical with that from the ring B hydrogens of meliternatin. This clearly confirmed the substitution pattern in ring B.

After this work had been completed, further proof of the structure of melibentin was supplied by its synthesis by Briggs and Cebalo¹⁸. Earlier work by Briggs¹⁰ had shown that meliternatin reacts with sodium methoxide to give 6-hydroxy-3,5,7-trimethoxy-3',4'-methylenedioxyflavone (9). Methylation of (9) with dimethyl sulphate and potassium carbonate afforded melisimplexin (2) which can be selectively demethylated to give melisimplin (3). Persulphate oxidation of melisimplin gave 5,8-dihydroxy-3,6,7-trimethoxy-3',4'-methylenedioxyflavone (10) which was smoothly methylated to give a compound identical with melibentin.

THE STRUCTURES OF MELICOPIN AND METHYLMELICOPIN

Melicopin crystallised from benzene or from methanol as cream needles, m.p. 133-134° and was optically inactive. Methylmelicopin crystallised from ether as yellow plates, m.p. 103-104°. The structures of melicopin and its methyl ether have been firmly established as (11) and (12) respectively and, for the sake of simplicity, the degradative evidence is discussed in terms of these structures. It may be pointed out here that both substances are rather unstable to acidic and to alkaline conditions.

The Functional Groups

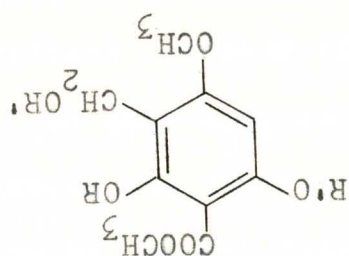
Analysis established the molecular formula of melicopin as $C_{19}H_{26}O_6$ and showed that it contained one methoxyl group. An X-ray analysis by Mathieson confirmed that the molecular weight was 351²⁶. Analysis ($C_{20}H_{28}O_6$) and the presence of one additional methoxyl group suggested that methylmelicopin was the methyl ether of melicopin. This was subsequently proved by methylation of melicopin with methyl iodide under strictly defined conditions to yield methylmelicopin.

Hydroxyl Groups

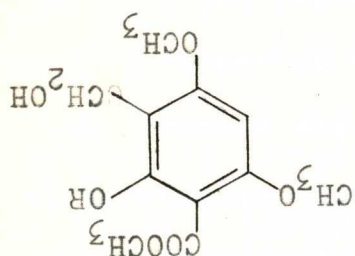
Melicopin was soluble in dilute sodium hydroxide, slightly soluble in hot sodium carbonate but insoluble in 5% sodium hydrogen carbonate. It gave a reddish-purple

$R = \text{geranyl}$
 $R' = \text{CH}_2\text{CO}$

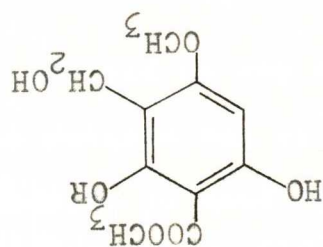
(14)



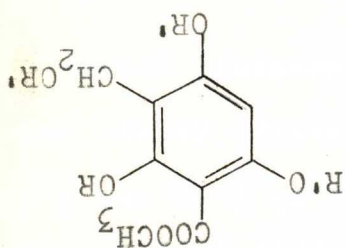
(15)



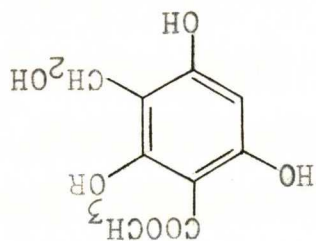
(12)



(13)



(11)



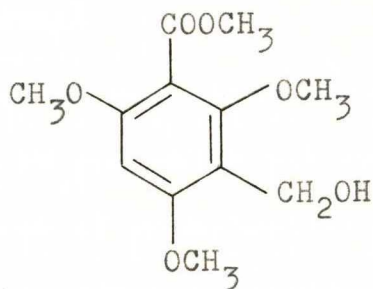
colour with ferric chloride. The UV spectrum in ethanol showed $\lambda_{\text{max.}}$ 232sh, 292, 325 m μ ($\log \epsilon$ 4.04, 4.26, 3.61); in alkaline solution a shift to $\lambda_{\text{max.}}$ 255, 326 m μ ($\log \epsilon$ 3.9, 4.6) was observed. This bathochromic shift is characteristic of phenolic groups in alkaline solution²⁷. The IR spectrum showed hydroxyl absorption at 3150-3450 cm⁻¹. On mild acetylation melicopin afforded the triacetate (13), whose IR spectrum showed maxima at 1770, and 1745 cm⁻¹ but no hydroxyl absorption. The frequencies of these maxima indicated that the acetates were derived from both phenolic and aliphatic hydroxyl groups. The NMR spectrum of (13) contained three singlets, each of intensity three protons, at 2.31 and 2.27 ppm due to the protons of the aromatic acetoxyl groups and at 2.13 ppm due to protons of the aliphatic acetoxyl group.

Thus melicopin was shown to contain three hydroxyl groups, two being phenolic, and one alcoholic.

Methylmelicopin (12) also behaved as a weak acid and gave a purple colour with ferric chloride. Its UV spectrum was identical with that of melicopin; a small shift to $\lambda_{\text{max.}}$ 292, 348 m μ in alkaline solution indicated that a phenolic group was present. Characteristic absorption in the IR at 3200-3500 cm⁻¹ also confirmed the presence of hydroxyl groups. Acetylation afforded a diacetate (14) which had IR absorption at 1775 (phenolic acetate) and 1755 cm⁻¹

(aliphatic acetate), but no hydroxyl absorption. The NMR spectrum of (14) confirmed this assignment. A three-proton singlet at 2.30 ppm corresponded to the phenolic acetoxyl and a higher field three-proton singlet at 2.13 ppm to the aliphatic acetoxyl group.

Methylation of methylmelicopin with dimethyl sulphate in refluxing acetone gave the neutral dimethyl ether (15). Strong absorption at 3540 cm^{-1} in its spectrum showed the presence of a free hydroxyl group. The UV spectrum of (15) showed no change in alkaline solution. Conclusive proof of the presence of a benzylic hydroxyl group came from the NMR spectrum of a degradation product (16). (see below). This



(16)

spectrum possessed a narrow two-proton doublet at 4.52 ppm; this signal was consistent with the presence of two benzylic protons split by interaction with the hydroxyl proton. On exchange with deuterium oxide this peak collapsed to a sharp two-proton singlet.

Methylmelicopin was further characterised as its tosyl derivative. Treatment of (12) with p-toluene sulphonyl chloride gave a monotosylate (17). The presence of only one tosylate group was shown by the analytical figures and its attachment to the benzylic oxygen followed from spectral data. A reversible bathochromic shift of the UV absorption maxima in alkaline solution was typical of a phenolic hydroxyl whilst the NMR spectrum possessed a one-proton singlet downfield at 12.84 ppm which corresponded to the bonded phenolic proton. Thus the product had the structure expected from the unreactivity of the bonded phenolic hydroxyl group.

Aromatic Methoxycarbonyl

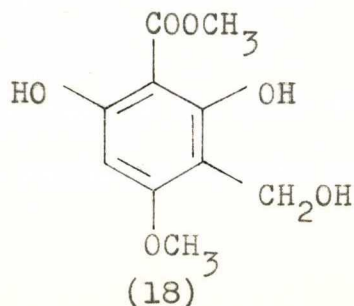
The NMR spectrum of melicopin contained a sharp three-proton singlet at 3.90 ppm which was assigned to methoxyl protons. The spectrum of methylmelicopin had, accordingly, two singlets of three-proton intensity, in this region of the spectrum (3.90 and 3.80 ppm). The IR spectra of both melicopin and methylmelicopin showed absorption at 1640 cm^{-1} which suggested a hydrogen-bonded carbonyl function; a DNP derivative could not be prepared. From a consideration of this spectral evidence, it seemed likely that the remaining three oxygens were present in ether and ester functions, one

of which involved a methoxyl group; i.e. as



To distinguish between these possibilities several mild alkaline hydrolyses of methylmelicopin were attempted. However, the reaction mixtures rapidly discoloured and only intractable oils could be isolated from them. It is not surprising that methylmelicopin with its phenolic and benzylic hydroxyl groups is so sensitive to both acidic and basic reagents. Mild acid hydrolysis yielded a black acidic fraction from which some crystalline material was isolated with some difficulty.

This compound analysed for $\text{C}_{10}\text{H}_{12}\text{O}_6$ and it also possessed IR absorption at 1630 cm^{-1} again assignable to a hydrogen-bonded carbonyl function. The NMR spectrum (in acetone solution) exhibited two singlets at 3.89 and 3.70 ppm, confirming that the two methoxyl groups were intact. The spectrum also indicated the removal of an unsaturated side chain (see later). It was therefore deduced that the product of acid hydrolysis was the dealkylated phenol (18).

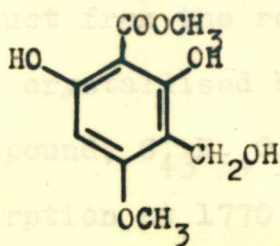


The IR spectrum of the phenol (18) had hydroxyl absorption at $3150-3520\text{ cm}^{-1}$ but in the NMR spectrum hydroxyl proton absorption was masked by the solvent. However, the presence of three hydroxyl groups was confirmed by formation of a tritosylate of (17) by reaction with p-toluene sulphonyl chloride. The IR spectrum of this derivative (19) showed carbonyl absorption at 1725 cm^{-1} , confirming the presence of a non-bonded aromatic methoxycarbonyl function.

The product from acetylation of this phenol (18) could not be crystallised but treatment of the gummy triacetate (20) with 5% sodium carbonate solution afforded an acidic compound, $\text{C}_{12}\text{H}_{14}\text{O}_9$, (21). The identity of this acetate followed from its spectral properties. Absorption in the IR at 1750 cm^{-1} and a three proton singlet in the NMR at 2.19 ppm indicated a benzylic acetoxyl group. The UV absorption maxima of phenolic acetates shift to higher wave lengths on adding alkali; on reacidification the UV spectrum of the parent phenol is observed. The UV absorption of (21), $\lambda_{\text{max.}}$ 233sh 285, 387 m μ ($\log \epsilon$ 4.03, 4.27, 3.51), shifted to $\lambda_{\text{max.}}$ 241 sh, 295 m μ ($\log \epsilon$ 4.06, 4.2) in alkaline solution. This shift was reversed on adding acid, showing that (21) was a benzylic acetate.

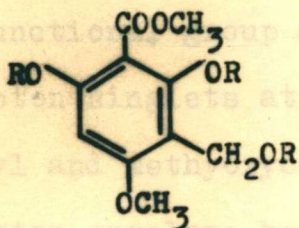
In order to characterise further the ester function it was decided to reduce methylmelicopin with lithium aluminium

hydrolysis. This was expected to afford the bis-benzylic alcohol (20). The product from this reaction obtained in low yield, could not be crystallized but on acetylation it furnished a neutral compound (21). The IR spectrum possessed carbonyl absorption at 1770 and 1720 cm^{-1} , assigned to phenolic acetate and non-bonded aromatic ester functions respectively. The NMR spectrum of the compound was consistent with structure (23).



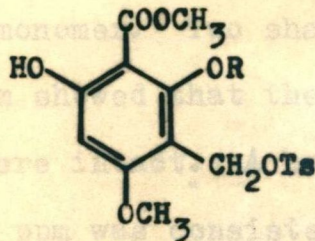
(18)

As this dimeric structure is symmetrical, the peaks for each functional group appeared as in a monomer. Sharp six-proton signals at 3.83 and 3.92 ppm showed that the methoxyl and hydroxymethyl protons were in equivalent environments. A six-proton envelope between 3.1 and 3.5 ppm was consistent with the six protons expected for the four olefinic protons of the two side chain (see later) and the two doubly benzylic protons.



(19) R = Ts

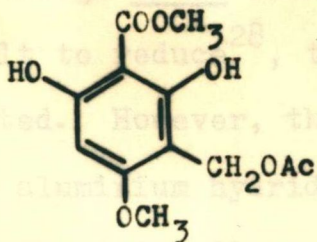
(20) R = CH_3CO



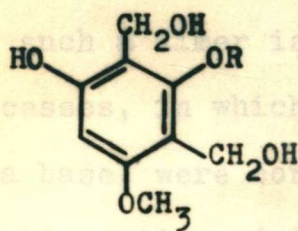
R = geranyl

(17)

Although ortho-disubstituted aromatic esters are frequently difficult to reduce, the formation of such a compound is still unexpected. However, the following processes, in which lithium aluminum hydride functions as a base, were considered to be mechanistically acceptable. The elimination of the benzylic hydroxyl group occurs essentially by a reversal of the esterification reaction and subsequent attack of the



(21)



R = geranyl

(22)

hydride. This was expected to afford the bis-benzylic alcohol (22). The product from the reaction obtained in low yield, could not be crystallised but on acetylation it furnished a neutral compound, $C_{43}H_{54}O_{12}$. The IR spectrum possessed carbonyl absorption at 1770 and 1720 cm^{-1} , assigned to phenolic acetate and non-bonded aromatic ester functions respectively. The NMR spectrum of the compound was consistent with structure (23).

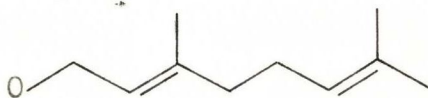
As this dimeric structure is symmetrical, the peaks for each functional group appeared as in a monomer. Two sharp six-proton singlets at 3.83 and 3.92 ppm showed that the methoxyl and methoxycarbonyl protons were intact. A broad six-proton envelope between 5.1 and 5.6 ppm was consistent with the signals to be expected for the four olefinic protons of the two side chain (see later) and the two doubly benzylic protons.

Although ortho-disubstituted aromatic esters are frequently difficult to reduce²⁸, the formation of such a dimer is still unexpected. However, the following processes, in which lithium aluminium hydride functions as a base, were considered to be mechanistically acceptable. The elimination of the formaldehyde residue occurs essentially by a reversal of the phenol-formaldehyde condensation and subsequent attack of the

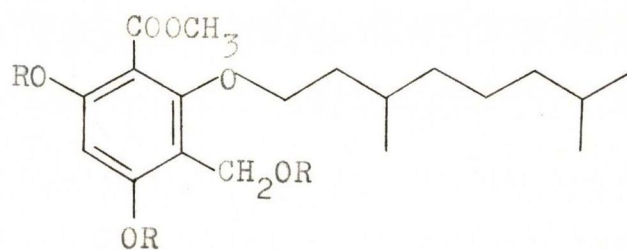
generated anion on a benzyl alcohol function or related zwitterionic species yields the dimeric product. As this product was of no diagnostic value, its chemistry was pursued no further.

Geranyl Side Chain

The molecular formula of the phenol (18), $C_{10}H_{12}O_6$, derived by acid hydrolysis of methylmelicopin indicated that a $C_{10}H_{18}$ fragment, containing two units of unsaturation, had been lost from the molecule. To determine its nature melicopin was hydrogenated with 5% palladium-charcoal catalyst. A tetrahydro-product (24) was isolated, and to check that only olefinic bonds had been reduced and that hydrogenolysis had not occurred, the triacetate (25) was prepared. Further confirmation of the presence of two double bonds was supplied by the formation of a tetrabromide of methylmelicopin (26). It seemed most likely that the C_{10} fragment was a geranyl residue, and evidence for this postulate was obtained from the NMR spectrum of melicopin.

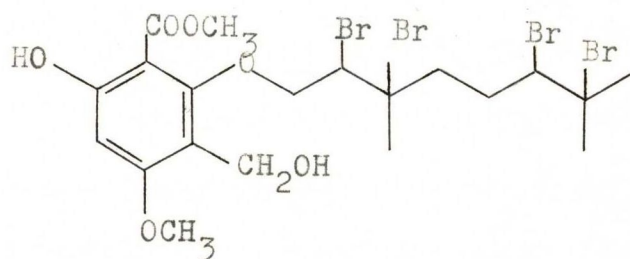


A series of three essentially unsplit singlets, each of three protons, at 1.62, 1.67 and 1.77 ppm, were assignable to the allylic methyl groups. A multiplet of four proton intensity centred at 2.12 ppm was attributed to the allylic

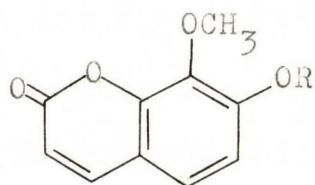


(24) $\text{R} = \text{H}$

(25) $\text{R} = \text{CH}_3\text{CO}$



(26)



$\text{R} = \text{geranyl}$

(27)

methylene protons. The resonance of the methylene protons next to oxygen appeared as a doublet at 4.53 ppm; the coupling constant (6.9 c/s) was consistent with interaction with an olefinic proton. The olefinic protons appeared as a triplet (J 6.9 c/s) at 5.5 ppm and as a multiplet at 5.1 ppm.

The NMR spectrum of the tetrabromide supported these assignments. All the signals suffered a down-field shift due to the deshielding effect of the bromine atoms. Two singlets of six- and three-proton intensity at 1.80 and 1.94 ppm respectively corresponded to the three methyl groups. The signals due to the four central methylene protons appeared as a multiplet centred at 2.43 ppm. The doublet at 4.67 ppm was due to the $O-CH_2$ protons whilst the methine protons now absorbed in the region from 5.5 to 5.8 ppm.

The structure of collinin has been established ²⁹ as a coumarin with an O-geranyl side chain. The NMR spectrum of collinin (27) was measured and the relevant peaks in the spectrum were found to be superimposable on those in the spectrum of melicopin. Chemical proof of the identity of the side chain was obtained by acid hydrolysis of methyl-melicopin with constant boiling hydrochloric acid. Geranyl chloride was isolated and characterised as its S-geranyliothiuronium chloride. This salt was identical (m.p., mixed

m.p. and IR spectra) with an authentic specimen prepared from geranyl chloride³⁰. The presence of the geranyl side chain explains the pleasant odour observed during almost any chemical manipulation of melicopin and methylmelicopin.

Melicopin had thus been shown to contain two phenolic hydroxyl groups, a benzyl alcoholic group, an aromatic methoxycarbonyl group and an O-geranyl side chain.

Degradation of Methylmelicopin

Most degradation experiments in this work were carried out with methylmelicopin as more of it was available. In an attempt to prepare the tetrahydro-derivative of methylmelicopin, it was hydrogenated with 5% palladium-charcoal as for melicopin. Surprisingly, hydrogenolysis occurred and the product of the reaction was the phenol (18) obtained from acid treatment of methylmelicopin. Of the two reactions, the allylic hydrogenolysis was the more efficient for the removal of the geranyl side chain.

To establish the nature of the nucleus of methyl-desgeranylmelicopin (18) it was desirable to degrade it to a known substance. The first step in this scheme involved methylation of (18) to give trimethyl-desgeranylmelicopin (16). Methylation with diazomethane was unsatisfactory but treatment with dimethyl sulphate in refluxing acetone gave a

40% yield of the neutral trimethyl ether (16). This substance had $\nu_{\text{max.}}$ 3500, 1698 cm^{-1} which suggested a benzylic alcohol and a free aromatic ester function. The UV absorption spectrum showed $\lambda_{\text{max.}}$ 280 $\text{m}\mu$ ($\log \epsilon$ 3.80), and was not altered on the addition of alkali; this confirmed the nature of the alcoholic group. The NMR spectrum of (16) (measured in benzene solution) exhibited four three-proton singlets at 3.30, 3.43, 3.68 and 3.77 ppm corresponding to the protons of the three methoxyl and the methoxycarbonyl groups. The remaining signals of the spectrum were also in accord with the assigned structure. The benzylic protons gave rise to a two-proton doublet at 4.52 (see later), the aromatic proton to a singlet at 6.30 and the hydroxyl proton to an envelope at 3.32 ppm.

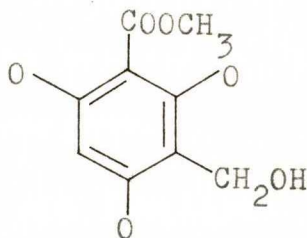
It was hoped that oxidation of the benzyl alcohol function in (16) would yield the corresponding aromatic acid which could then be methylated to give the known dimethyl 2,4,6-trimethoxyisophthalate (28).

Birkinshaw et al.³¹ have converted cyclopolic acid (29) to the corresponding tricarboxylic acid with dilute alkaline potassium permanganate. Oxidation of trimethyldesgeranylmelicopin under these conditions gave intractable oils.

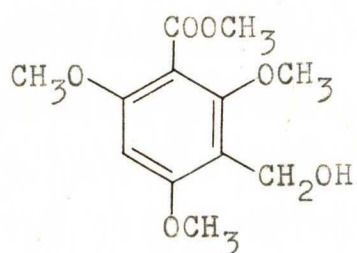
'Active' manganese dioxide, prepared from manganese sulphate and potassium permanganate, has been found by several workers^{32,33,}

to oxidise benzyl alcohols efficiently to the corresponding aldehydes. No crystalline material could be obtained by oxidizing (16) with this reagent. The reactivity of the substance (16) to oxidation was probably due in part to the activating effect of the methoxyl groups. Chromium (VI) oxide-pyridine has been used by Holm³⁴ to oxidize a number of methoxylated benzyl alcohols. This reagent (in small excess) successfully oxidized trimethyldesgeranylmelicopin in 10% yield to the corresponding acid (30). Methylation of the crude product gave a crystalline solid, m.p. 107-108°, whose IR spectrum contained a band at 1715 cm⁻¹, but showed no absorption due to hydroxyl; this indicated that the benzylic alcohol group had been converted to a methoxycarbonyl group. A mixed m.p. of this ester with a synthetic sample of dimethyl 2,4,6-trimethoxyisophthalate (28) showed no depression and both were spectroscopically identical.

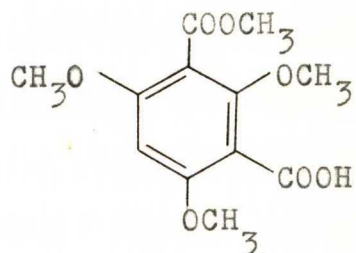
Thus, the skeleton of melicopin was established as (31)



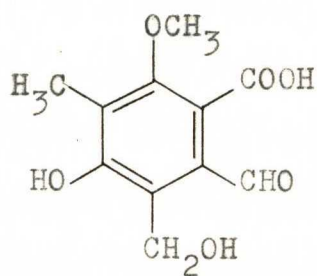
(31)



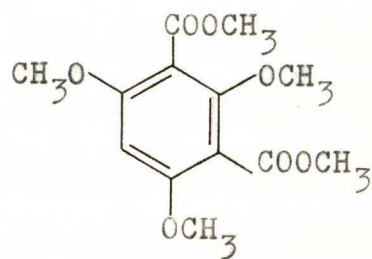
(16)



(30)



(29)



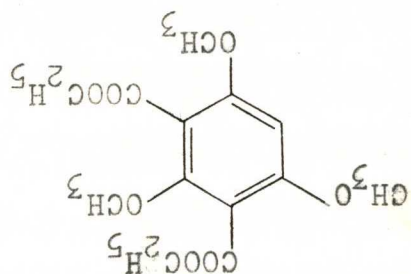
(28)

Dimethyl 2,4,6-trimethoxyisophthalic ester had been synthesised in the following way. Diethyl 2,4,6-trihydroxyisophthalate (32) was prepared in 25% yield by a Claisen condensation of diethyl malonate, according to the method of Leuchs and Geserick³⁵. Leuchs and Dzieng³⁶ had converted this phenol (32) to the trimethyl ether (33) with diazomethane. It was found, however, that this method was very slow and afforded a mixture of products. Methylation of (32) was finally effected by using diazomethane followed by treatment with dimethyl sulphate. The product, diethyl 2,4,6-trimethoxyisophthalate (33), had IR absorption at 1705 cm^{-1} and no hydroxyl absorption. The diester (33) was hydrolysed to the corresponding diacid (34), by the method of Leuchs³⁷, with minor modifications. Finally, methylation of the diacid with diazomethane afforded dimethyl 2,4,6-trimethoxyisophthalate (28), m.p. $107-108^{\circ}$. The identity of this ester (28) was confirmed by its NMR spectrum. A series of six-proton singlets at 3.88 and 3.83 ppm and a three-proton singlet at 3.81 ppm accounted for the protons of the three methoxyl and the two carbomethoxyl functions.

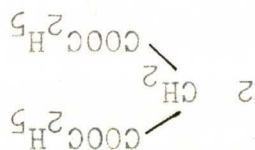
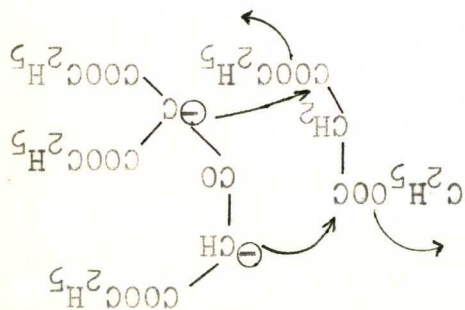
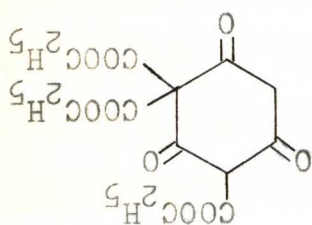
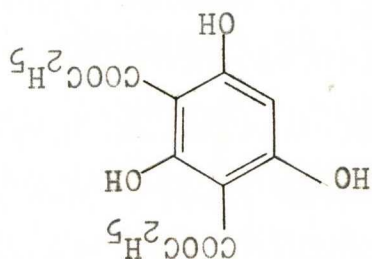
The Interrelationship of the Functional Groups

Having established the identity of the functional groups and the nature of the skeleton of melicopin and methylmelicopin, the next step was to place the substituents in relation to

(33)



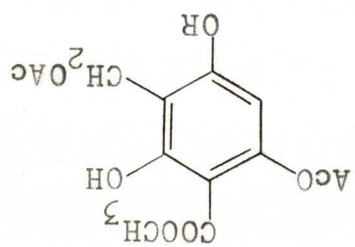
(32)



each other. This involved firstly showing that the methoxyl group was para to the methoxycarbonyl in methylmelicopin and secondly that the O-geranyl chain was ortho to both the methoxycarbonyl and benzyl alcohol functions.

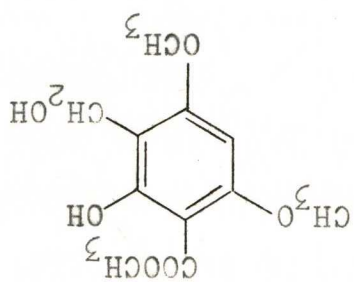
The Position of the Methoxyl Group

Before assigning the position of the methoxyl group in methylmelicopin it was necessary to show that the O-geranyl side chain was ortho to the methoxycarbonyl group. This was determined by considering the carbonyl stretching frequencies in the relevant IR spectra. Triacetylmelicopin (13) and diacetylmethylmelicopin (14) had peaks at $\nu_{\max}$ 1705 and 1700 cm^{-1} respectively, which were attributed to the free aromatic ester function. Treatment of triacetylmelicopin (13) with hydrogen in the presence of palladium-charcoal catalyst afforded the hydrogenolysed phenol, $\text{C}_{15}\text{H}_{16}\text{O}_9$. The NMR of this compound still possessed three singlets of three-proton intensity at 2.18, 2.31 and 2.40 ppm confirming that the three acetoxyl groups were intact whilst a one-proton singlet at 11.70 ppm corresponded to the bonded hydroxyl proton. This phenol was thus identified as triacetyldesgeranylmelicopin (35). In the same way, hydrogenolysis of diacetylmethylmelicopin yielded diacetylmethyldesgeranylmelicopin (36). The carbonyl absorption in these two phenols, (35) and (36), now appeared at lower frequencies with peaks at $\nu_{\max}$ 1635

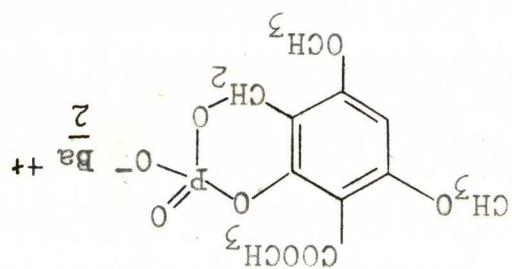


(35) R = Ac

(36) R = H



(37)



(39)

and 1640 cm^{-1} respectively, indicating hydrogen bonding in these phenols. This placed the geranyl ether group in melicopin and its derivatives ortho to the methoxycarbonyl group.

Confirmation of this assignment came from the IR spectra of the dimethyldesgeranylmelicopin (37). This compound was prepared by treating dimethylmelicopin (15) with hydrogen and palladium charcoal catalyst as above. Its identification followed from its analysis ($\text{C}_{11}\text{H}_{14}\text{O}_6$) and the fact that its NMR spectrum possessed three singlets each of three-proton intensity at 3.77, 3.91 and 4.04 ppm corresponding to the protons of the two methoxyl groups and of the methoxycarbonyl group respectively, and that it no longer had peaks arising from the protons of the side chain. The protons of the two hydroxyl groups absorbed at 3.64 (CH_2OH) and at 12.72 ppm (Ar-O-H). The characteristic shift of the carbonyl absorption frequency from $\nu_{\text{max.}} 1695\text{ cm}^{-1}$ in the IR spectra of (15) to $\nu_{\text{max.}} 1640\text{ cm}^{-1}$ in (37) again showed the presence of a hydroxyl group ortho to the aromatic ester in the desgeranylated compounds.

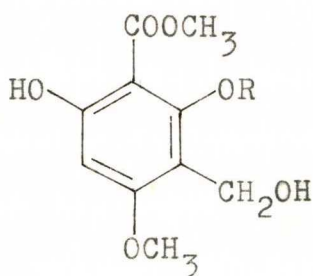
The methoxyl group in methylmelicopin was then shown to be para to the methoxycarbonyl function. In their IR spectra melicopin and methylmelicopin had a peak at $\nu_{\text{max.}} 1640\text{ cm}^{-1}$ which was attributed to the hydrogen-bonded aromatic ester

function. Dimethylmelicopin (15) had a strong absorption maximum at $\nu_{\text{max.}} 1695 \text{ cm}^{-1}$, characteristic of a free ester function. This indicated that the hydroxyl group in methylmelicopin was ortho to the methoxycarbonyl group and the methoxyl group para to it.

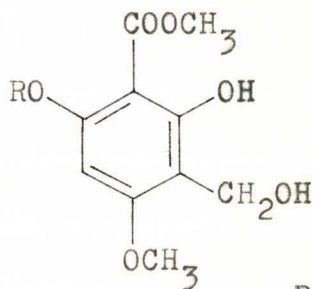
Additional support for this assignment came from the IR spectra of the acetates of these phenols (11) and (12). The ester absorption in triacetylmelicopin (13) and diacetylmethylmelicopin (14) had been raised to 1705 and 1700 cm^{-1} respectively. This change indicated that in methylmelicopin the ester group is hydrogen-bonded to an ortho hydroxyl substituent.

Environment of the Side Chain

Chemical and spectroscopic evidence at this stage allowed two possible structures for methylmelicopin.



(12)



R = geranyl

(38)

A decision in favour of (12) was reached from a consideration of the results of a series of Gibb's tests and from certain features in the NMR spectra of phenolic and related substances (see Part II).

A positive Gibbs test is generally recognised as diagnostic of the presence of a hydrogen para to a phenolic hydroxyl group. Triacetyldesgeranylmelicopin (35) and diacetylmethyldesgeranylmelicopin both gave a positive result in the spectroscopic modification of the test, absorbing at $\lambda_{\text{max.}}$ 670 m μ . In harmony with this result triacetylmelicopin (13) and triacetylmethyldesgeranylmelicopin (20) gave negative tests. However it was not safe on these grounds alone to conclude that methyilmelicopin was (12) since trimethyldesgeranylmelicopin (16) and dimethyldesgeranylmelicopin (37), which should then have given a negative and positive result respectively, both gave similar broad absorption between 400-700 m μ .

To settle the point chemically, attempts were made to prepare cyclic derivatives of dimethyldesgeranylmelicopin (37) which was expected to behave like saligenin towards phosgene, thionyl chloride and benzaldehyde. Reaction of (37) with phosgene and thionyl chloride both gave intractable tars whilst treatment with benzaldehyde³⁸ gave only unchanged

starting material. Reaction of (37) with phosphoryl chloride in pyridine, according to the method of Mosher et al.³⁹ was examined in the hope of isolating the cyclic phosphate. The product of the reaction was an acidic, crystalline solid (39) but it was so hygroscopic that it was impossible to characterise it.

Mellicopin is a rather unusual natural product. No other phluroglucinol derivative containing a benzylic alcohol function has been recorded. In fact natural benzyl alcohols themselves are quite rare and the function has been found only in certain anthraquinones, vanillyl alcohol and cyclopolic acid.

THE NUCLEAR MAGNETIC SPECTRA OF MELICOPIN
AND ITS DERIVATIVES

The signals in the nuclear magnetic resonance (NMR) spectra of melicopin and its derivatives, apart from those due to the protons of geranyl residue, were very well resolved and could be readily assigned to the respective protons. This section is concerned with the interpretation of the spectra of these molecules, with particular reference to that of methylmelicopin (Fig.1'). The structure (12) has been proposed for methylmelicopin and for the sake of simplicity the spectra will be interpreted in terms of this structure.

The chemical shifts of the aromatic proton and the two benzylic protons throughout the series of melicopin derivatives are shown in Table 1.

Aromatic Proton

The results in a series of model phenolic compounds have indicated that methylating a phenolic group will result in a

NMR spectra were recorded on a Varian A-60 spectrometer for solutions in deuteriochloroform unless otherwise stated. Chemical shifts (δ values) are expressed in ppm from tetramethylsilane as internal reference. Values for these shifts are derived from first order considerations only.

NMR Spectrum of Methylmellicopin

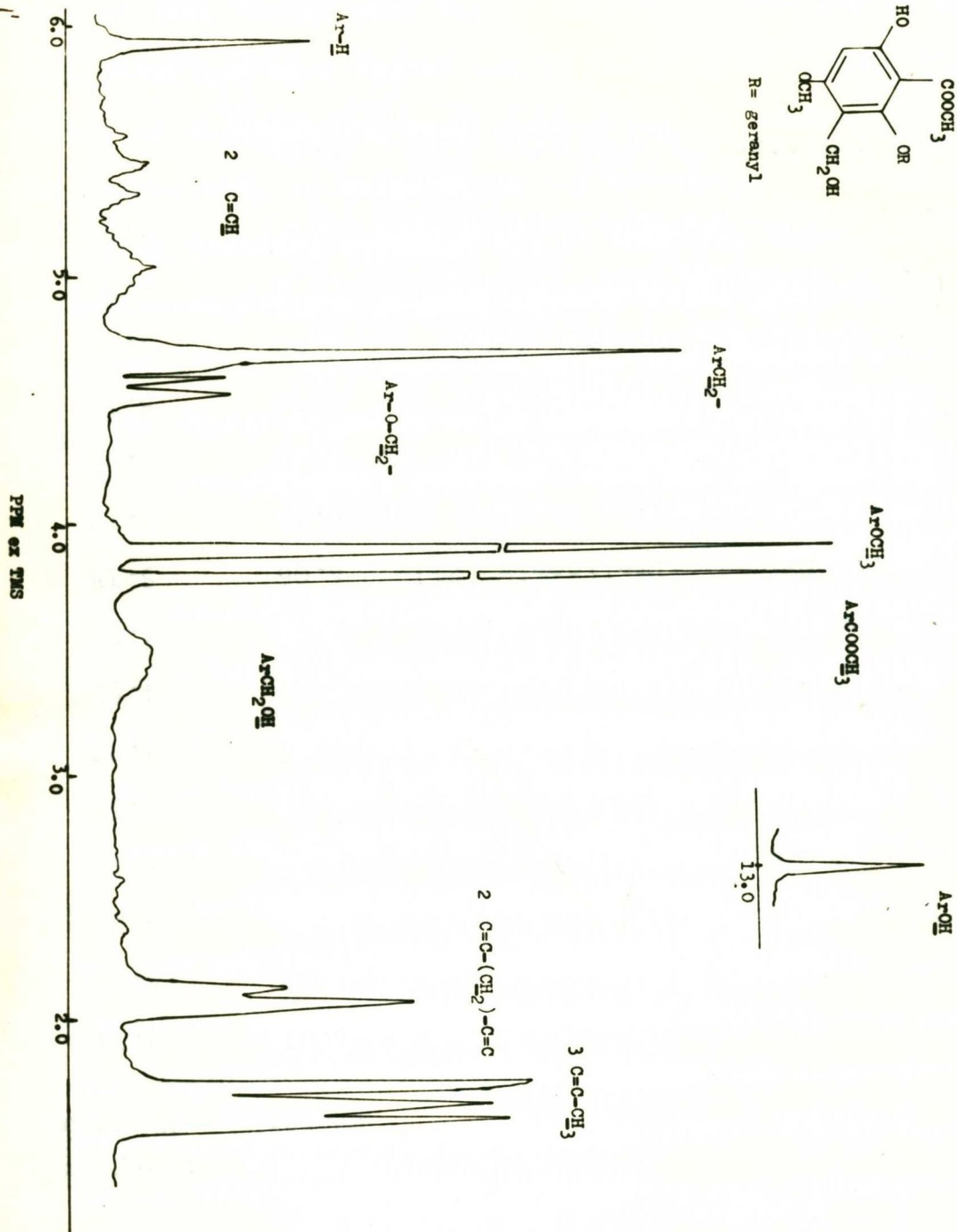
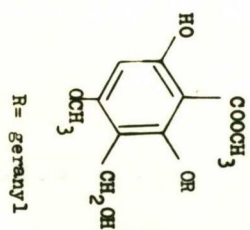


Fig. 1'

TABLE 1

Chemical Shifts (in ppm from TMS)

Compound	Aromatic Proton	Benzylic Protons
Mellicopin (11)	6.01	4.71
Triacetylmellicopin (13)	6.62	5.03
Methylmellicopin (12)	5.98	4.70
Diacetylmethylmellicopin (14)	6.36	5.02
Dimethylmellicopin (15)	6.30	4.53
Methyldesgeranylmellicopin (18)(acetone)	6.08	4.77
Trimethyldesgeranylmellicopin (16)	6.30	4.52
Dimethyldesgeranylmellicopin (37)	6.29	4.76
Acetylmethyldesgeranylmellicopin (21)	6.06	5.18
Diacetylmethyldesgeranylmellicopin (36)	6.42	5.12
Triacetylmethyldesgeranylmellicopin (20)	6.65	4.95
Triacetyldesgeranylmellicopin (35)	6.72	5.12
Methylmellicopin tetrabromide (26)	6.00	4.73
Tosylmethylmellicopin (17)	5.98	5.20
Tritosylmethyldesgeranylmellicopin (19)	6.74	4.76
Dimethyl trimethoxyisophthalate (28)	6.30	

paramagnetic shift of the signal due to the ortho hydrogen only. Both meta and para hydrogens will be unaffected by such a change. The application of this result to the shifts observed in the methyl derivatives of melicopin supported the assignment of structure (12) to methylmelicopin. The signal from the ring proton in methylmelicopin appeared at 5.98 ppm whilst in dimethylmelicopin (15) it had shifted downfield to 6.30 ppm. Removing the O-geranyl side chain from (15) produced the phenol (37) in which the aromatic proton also showed absorption at 6.30 ppm; methylating this phenol (37) gave trimethyldesgeranylmelicopin (16) in which the position of the signal was unchanged. These chemical shifts could be interpreted satisfactorily in terms of the assigned structures.

The study of the model phenolic compounds had also indicated that the resonance of an aromatic proton will undergo a paramagnetic shift on acetylation of the hydroxyl group, the shift being greater (and of similar magnitude) at positions ortho and para to the phenolic hydroxyl than at the meta position. Although the application of this result to the displacement of the signals from the aromatic protons of the acetyl derivatives in the series does not throw any light on the exact location of the side chain, they provide good support for the phloroglucinol pattern of the nucleus.

In the spectrum of mellicopin (11) the aromatic proton signal appeared as a singlet at 6.01 ppm; acetylation of (11) afforded the triacetate (13) in which the aromatic proton signal had undergone a downfield shift of 0.61 ± 0.03 ppm. As there were two acetoxyl groups in the molecule, both ortho to the aromatic proton, each could be considered to contribute equally to its chemical shift. This was seen to be correct from the chemical shift of the aromatic proton in diacetylmethylmellicopin (14), in which a paramagnetic displacement of 0.38 ± 0.03 ppm was consistent with the presence of one ortho acetoxyl group. The position of resonance of the aromatic proton in the corresponding desgeranyl compounds, triacetyldesgeranylmellicopin (35) and diacetylmethyldesgeranylmellicopin (36), was even further downfield than in (13) and (14), the displacements being 0.71 and 0.44 ± 0.03 ppm respectively. It is suggested that some steric inhibition of the ring current may be responsible for the slightly high value of the proton resonance in (13) and (14).

Benzylic Methylene Protons

Considerable data had been collected to show that the signals from the protons of the benzylic methyl group ortho to a phenolic hydroxyl group undergo an upfield shift on

acetylation. If this conclusion is applied to the chemical shift of the benzylic methylene protons in the series further support is obtained for the structure of methylmelicopin as (12). The spectrum of diacetylmethyl-desgeranylmelicopin (36) exhibited a signal at 5.12 ppm; acetylating this compound afforded triacetylmethyl-desgeranylmelicopin (20), in the spectrum of which the signal had moved upfield to 4.95 ppm. This result would place the benzyl alcohol function ortho to the geranyl ether residue.

EXPERIMENTAL

Ultraviolet spectra were recorded in ethanol on a Perkin-Elmer Spectracord and infrared spectra were recorded on a Perkin-Elmer Infracord 137 and Model 221 instruments. The infrared absorption maxima refer to nujol mulls unless otherwise stated. Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer in deuteriochloroform solution (unless otherwise stated) with tetramethylsilane as internal reference by Mr. D.C. Dehlson of this Department. Chemical shifts are expressed in ppm from TMS and coupling constants are derived from first order considerations only.

Melting points were determined on a Kofler heating stage and are uncorrected. Analyses were carried out by Miss B. Stevenson and her staff of this Department, and by the CSIRO Microanalytical Laboratory, Melbourne. Molecular weights were determined by mass spectrometry by Dr. J. Shannon, Division of Coal Research, CSIRO. Silica gel used for chromatography was 100/200 mesh from L. Light and Co., England. The alumina used was Spence Type H. Deactivated alumina was prepared by treatment with ethyl acetate for 3 days, washing with methanol, water, and methanol, then drying under

reduced pressure at room temperature.

All organic solutions were dried over anhydrous sodium sulphate. Light petroleum used was b.p. 60-80°.

The chemical shift (δ values) of each proton is listed using the following abbreviations:

s singlet
d doublet
t triplet
q quartet
ABq AB quartet
m multiplet

Each signal is described in terms of multiplicity, intensity, chemical shift (ppm from TMS), assignment (in brackets) and coupling constant (if present and observable) in that order. For example; d, 1, 6.9, (5'-H), 8.0 refers to a one-proton doublet at 6.9 ppm (J 8.0 c/s) assigned to the 5'-H.

Isolation of the Constituents

Extraction of the Bark

The finely ground bark (4.2 kg) was extracted by percolation with light petroleum, ether and methanol in turn at room temperature. The solvents were then removed under reduced pressure and the residues worked up as follows. (Flow sheet 1).

Light Petroleum Extract

The residual yellow oil (15.5 g) was dissolved in benzene (150 ml) and chromatographed on silica gel (1600 g). Yellow, crystalline material (14.2 g) eluted by benzene:ether (4:1) was recrystallised from methanol as yellow prisms of melibentin (6) (12.3 g), m.p. 134-135°.

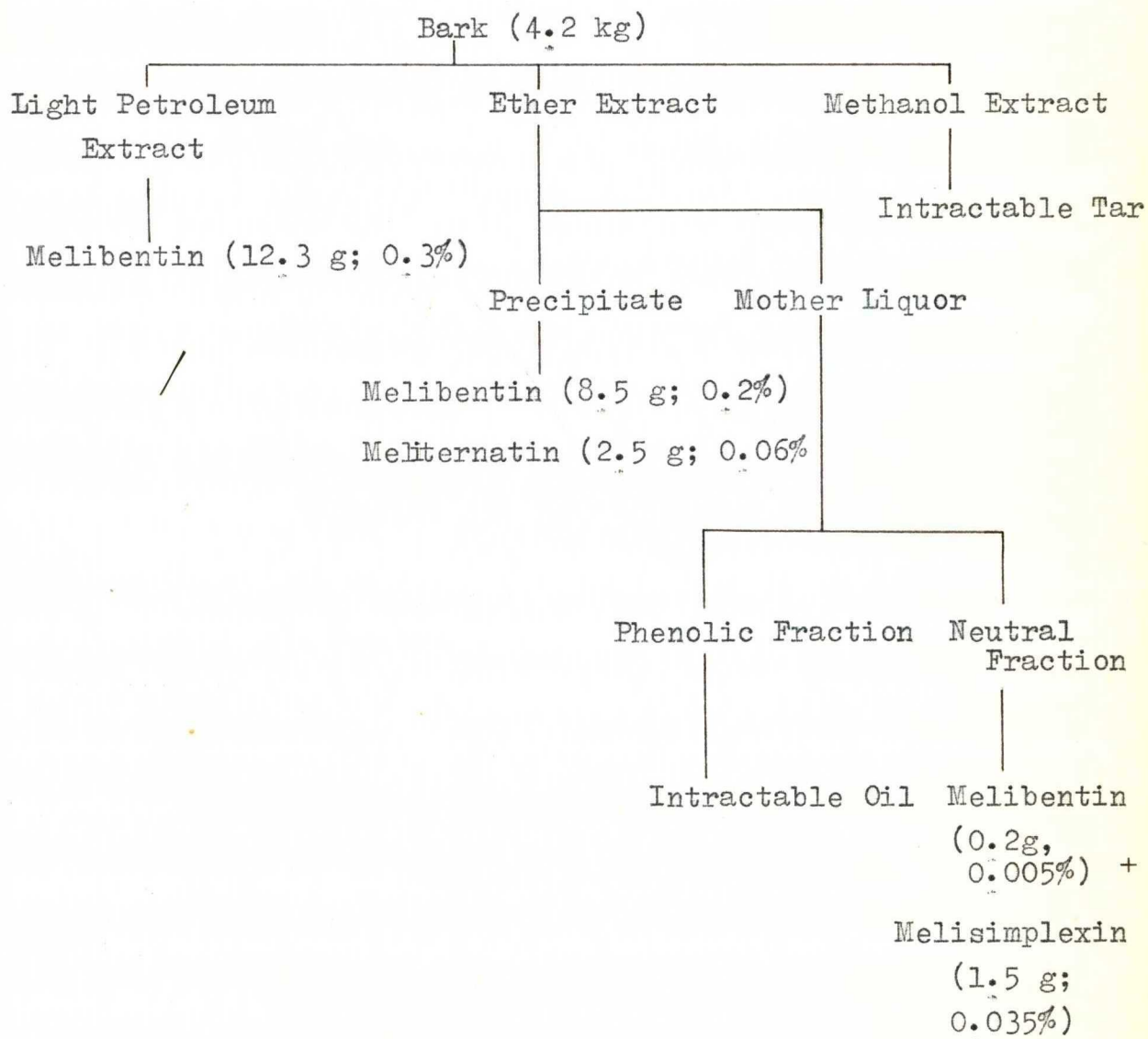
Ether Extract

Filtration of the concentrate gave a yellow solid (12 g) which was dissolved in benzene and chromatographed on silica gel (1 kg). Elution with benzene:ether (4:1) afforded melibentin (8.5 g), and with ether gave a cream powder (3 g). Meliternatin crystallised (from chloroform-methanol) as fine needles, m.p. 202-204°, mixed m.p., identical IR spectrum with an authentic specimen.

The original mother liquor was concentrated and the residue dissolved in ether. The solution was divided into phenolic and neutral fractions by extraction with 2% sodium

FLOW SHEET 1

Extraction of the Bark



hydroxide. Isolation of the base-soluble material gave a dark, brown gum (3 g) which was dissolved in benzene (50 ml) and chromatographed on deactivated alumina but no crystalline material was obtained. The neutral ethereal solution was concentrated to a yellow oil (4 g). This was dissolved in methanol and the solution kept at 5° overnight. Chromatography of a benzene solution of the precipitated solid separated two components. Melibentin (0.2 g) was eluted with benzene:ether (4:1) and melisimplexin (1.5g), m.p. 180-185°, was removed from the column with benzene:ether (2:1).

Methanol Extract

This extract afforded a dark tar. Attempts to isolate pure substances by chromatography or by chromatography of the fractions obtained by extraction in turn with aqueous sodium hydrogen carbonate, sodium carbonate and sodium hydroxide were fruitless.

Extraction of the Wood

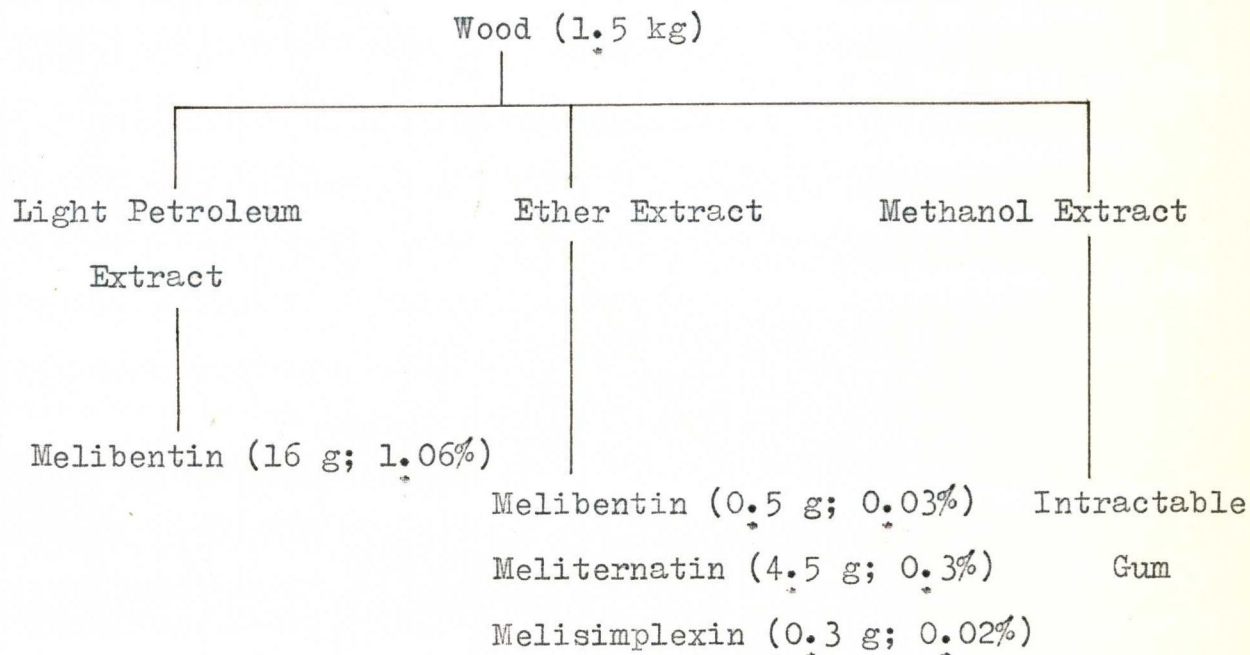
The milled wood (1.5 kg) was extracted as for the bark. (Flow sheet 2).

Light Petroleum Extract

The residue solidified on refrigeration and was shown to be substantially homogeneous by thin layer chromatography (t.l.c.). Crystallisation of the solid from methanol afforded

FLOW SHEET 2

Extraction of the Wood



yellow prisms, m.p. 134-135^o, found to be identical with melibentin by comparison with an authentic sample.

Ether Extract

Evaporation yielded a yellow solid (18 g) which was separated into its components by chromatography on silica gel. Melibentin (0.5 g), melisimplexin (0.3 g) and meliternatin (4.5 g) were isolated in turn by eluting with benzene:ether (4:1), benzene:ether (2:1) and ether respectively. Each was identified by m.p., mixed m.p. and spectral comparison with an authentic specimen.

Methanol Extract

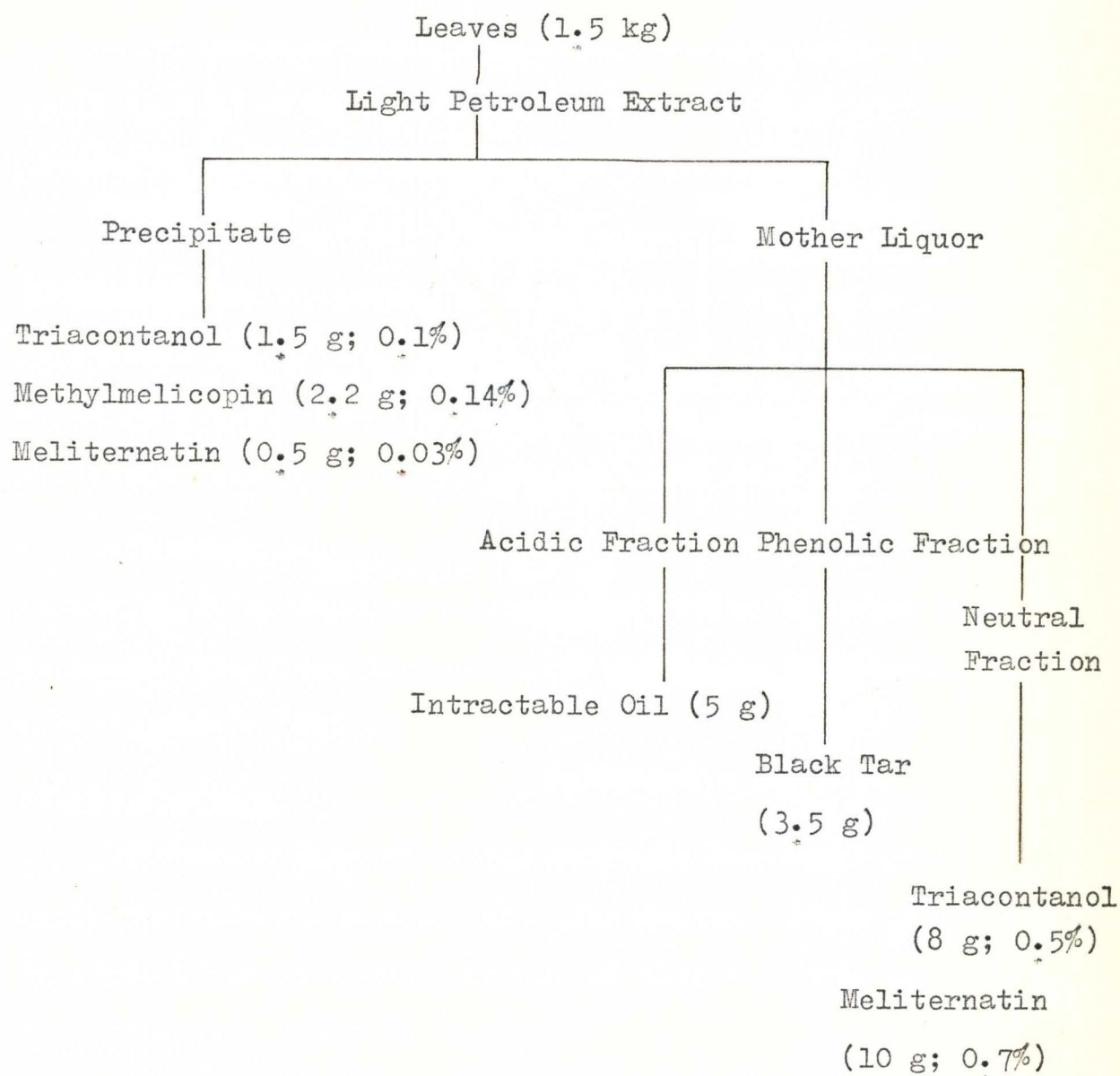
As with the bark, no crystalline material was obtained from this extract.

Extraction of the Leaves

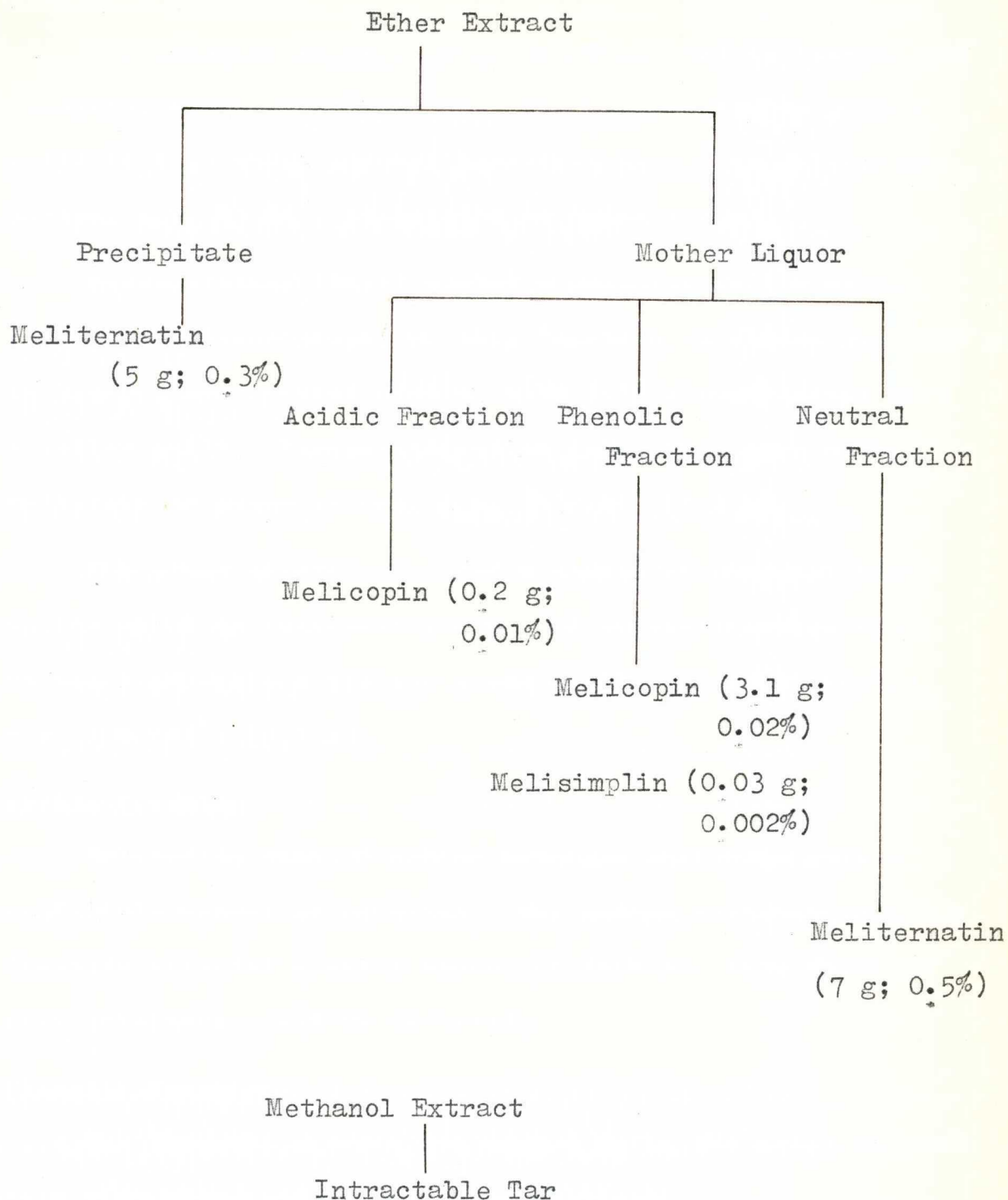
The finely ground leaves (1.5 kg) were exhaustively extracted with the same solvents as before. Solid material that precipitated on refrigeration of the concentrates was collected and purified as below. The filtrates were then concentrated, diluted with ether and separated into acidic, phenolic and neutral fractions by shaking with aqueous solutions of 5% sodium hydrogen carbonate, 5% sodium carbonate and 2% sodium hydroxide. (Flow sheet 3).

FLOW SHEET 3

Extraction of the Leaves



FLOW SHEET 3 (continued)



Light Petroleum Extract

Insoluble Material

The greenish solid (5.5 g) was dissolved in benzene and chromatographed on silica gel. The benzene eluate gave a solid (1.5 g) which crystallised from hexane as colourless plates, m.p. 85-86°, identified as triacontanol.

Benzene:ether (20:1) eluted a greenish-yellow solid, (3 g). Rechromatography of this fraction on silica gel (250 g) in benzene and careful elution with 3-5% ether-benzene gave a yellow solid. Recrystallisation from ether gave methylmelicopin as cream prisms, m.p. 103-104° (2.2 g).

The ether eluates afforded a series of greenish-black solids which on rechromatography and crystallisation from methanol-chloroform yielded cream needles of meliternatin, m.p. 202-204°; (0.5 g).

Acidic Fraction

Extraction with 5% sodium hydrogen carbonate gave a negligible amount of material. The sodium carbonate-soluble fraction afforded a small amount of dark oil from which no pure substance could be isolated.

Phenolic Fraction

The phenolic extract was worked up in the usual way but only black intractable tars were obtained.

Neutral Fraction

The concentrate (25 g) was extracted with hot benzene (200 ml) and the solution filtered from a grey residue. This on recrystallisation from chloroform-methanol gave off-white needles of meliternatin (10 g). The benzene-soluble fraction was chromatographed on alumina to yield further triacontanol, m.p. 85-86°, (8.0 g).

Ether Extract

Insoluble Material

The solid (7 g) was filtered through a short silica gel column in chloroform. Concentration of the first washings and further recrystallisation afforded meliternatin (5 g).

Acidic Fraction

Careful chromatography of the crude fraction on silica gel yielded a product (0.5 g) eluted by benzene-ether (5:1). This was redissolved in benzene and chromatographed on silica gel. Elution by benzene:ether (4:1) gave melicopin which crystallised from methanol as cream needles, m.p. 134-135° (0.2 g).

Phenolic Fraction

Chromatography of the fraction (5 g) on silica gel gave a bright yellow powder (0.03 g) and melicopin (3.1 g), on elution with benzene:ether (25:1) and benzene-ether (5:1)

respectively. The pigment melisimplin crystallised from acetone as yellow needles, 0.03 g, m.p. 227-235°.

Neutral Fraction

Meliternatin (7 g) was isolated from the insoluble portion of this fraction and purified by chromatography. Evaporation of the mother liquor gave only intractable oils.

Methanol Extract

Only tars were obtained from working up this extract in the usual way.

Identification of Known Compounds

Meliteratin (1)

Meliteratin crystallised as fine needles from methanol, m.p. 202-204° [Found: C, 61.3; H, 4.0; O, 34.4; OCH₃, 16.6%. Calc. for C₁₉H₁₄O₈: C, 61.6; H, 3.8; O, 34.6; OCH₃, 16.7%]. Light absorption: $\nu_{\max}$ 1640 (carbonyl), 930, 718 (methylene-dioxy) cm⁻¹, no hydroxyl absorption; $\lambda_{\max}$ 236, 369sh, 248 m μ (log ϵ 4.41, 4.11, 4.25); NMR spectrum: m, 1, 7.62, (6'-H), 8.0; s, 1, 7.54, (2'-H); d, 1, 6.90, (5'-H), 8.0; s, 1, 6.64, (8-H); s, 4, 6.02, (O₂CH₂); s, 3, 4.08, 3.87, (ArOCH₃).

Meliteratin dissolved in concentrated sulphuric acid to give a bright yellow solution. Treatment of an ethanolic solution of (1) with magnesium ribbon and hydrochloric acid gave a salmon pink colour. The flavone gave an intense green colour in the Labat test using gallic acid, whilst a purple colouration was obtained in the Hansen test using chromotropic acid.

The flavone had identical m.p., mixed m.p. and IR spectrum with an authentic sample of meliteratin.

Hydrolysis of Meliteratin

Meliteratin (0.4 g) and potassium hydroxide (0.8 g) were refluxed in aqueous ethanol (75%) under nitrogen for 4 hr. The solution was concentrated, acidified and extracted thoroughly

with ether (3 x 50 ml). Recovery of the acid fraction (0.15 g) with 5% sodium hydrogencarbonate and crystallisation from methanol gave needles of piperonylic acid, m.p. and mixed m.p. 225-227° (lit.⁹ m.p. 229°). Evaporation of the ether solution gave a crystalline phenol (0.23 g) which on recrystallisation from ethanol gave cream prisms of 2-hydroxy-,6-dimethoxy-4,5-methylenedioxyacetophenone (4), m.p. 142-144° (lit.⁹ m.p. 143-144°). This phenol gave a dark green ferric chloride test and a positive Labat test.

Light absorption: $\nu_{\max}$. 1590 (bonded carbonyl) cm^{-1} ;
 $\lambda_{\max}$. 244, 286, 350 μ ($\log \epsilon$ 4.06, 4.13, 3.69).

Melisimplexin (2)

Repeated recrystallisation from methanol afforded cream, flakey plates, m.p. 185-187°. M.p., mixed m.p. and IR absorption were identical with an authentic specimen of melisimplexin. [Found: C, 62.0; H, 4.6; OCH_3 , 41.2%. Calc. for $\text{C}_{20}\text{H}_{18}\text{O}_8$: C, 62.2; H, 4.3; OCH_3 , 40.2%]. Light absorption: $\nu_{\max}$. 1635 (carbonyl), 928 cm^{-1} (methylenedioxy), no hydroxyl absorption; $\lambda_{\max}$. 235, 336 μ ($\log \epsilon$ 4.30, 4.29); NMR spectrum: m, 1, 7.74, (6'-H); s, 1, 7.68, (2'-H); d, 1, 6.95, (5'-H), 8.0; s, 1, 6.81, (8-H); s, 2, 6.12, (O_2CH_2); s, 3, 4.06, 4.04, 3.98, 3.94 (ArOCH_3).

Treatment of the flavone with sulphuric and gallic acids gave a green colour whilst reduction with magnesium ribbon in hydrochloric acid gave a bright red colour. A ferric chloride test was negative.

Melisimplin (3)

Melisimplin crystallised as yellow needles from acetone, m.p. 227-235°. Light absorption: ν_{max} 1667 cm^{-1} (carbonyl). It was identified by direct comparison (m.p., mixed m.p. and IR comparison) with an authentic sample prepared by demethylating melisimplexin.

Demethylation of Melisimplexin

A solution of anhydrous aluminium chloride (0.495 g) in nitrobenzene (3 ml) was added to a solution of melisimplexin(2) (1.2 g) in nitrobenzene (9.6 ml) and the reaction mixture maintained at 105° for 1 hr. The cooled reaction mixture was poured into dilute hydrochloric acid and steam-distilled to remove nitrobenzene. The cooled solution was then filtered and the product recrystallised from acetone to give 5-hydroxy-3,6,7-trimethoxy-3',4'-methylenedioxyflavone(3) as yellow needles (0.9 g), m.p. 232-234°.

Triacontanol

The waxy solid crystallised from hexane as colourless plates, m.p. 85-86° (lit.¹ m.p. 85-86°). [Found: C, 82.1;

H, 14.0%. Calc. for $C_{30}H_{62}O$: C, 82.1; H, 14.2%.] Light
absorption: $\nu_{\max}$. 3540, 1034 cm^{-1} (primary hydroxyl).

Identification of Melibentin

Melibentin(7)

Melibentin crystallised from methanol as yellow prisms, m.p. 134-135°. [Found: C, 60.3; H, 4.8; O, 34.5; OCH₃, 36.9%. Calc. for C₂₁H₂₀O₉: C, 60.5, H, 4.8; O, 34.6; 5xOCH₃, 37.2%]. Light absorption: $\nu_{\max}$. 1645 (pyrone carbonyl), 2780, 926, 719 cm⁻¹ (methylenedioxy), no hydroxyl absorption; $\lambda_{\max}$. 240, 270sh, 340 m μ (log ϵ 4.38, 4.20, 4.35); NMR spectrum: m, 1, 7.74, (6'-H); s, 1, 7.63, (2'-H); d, 1, 6.9, (5'-H), 8.0; s, 2, 6.05, (O₂CH₂); s, 3, 4.10, 4.02, 4.00, 3.97, 3.90, (ArOCH₃).

Melibentin dissolved in concentrated sulphuric acid to give a bright yellow solution from which it was precipitated on dilution. A ferric chloride test was negative. Treatment of an ethanolic solution with some magnesium ribbon and concentrated hydrochloric acid gave a bright red lake in two minutes. A Labat test gave a green colour whilst a Hansen test was also positive (blue).

Hydrolysis of Melibentin

Melibentin (0.2 g) was dissolved in a solution of potassium hydroxide (0.4 g) in ethanol (5 ml of 80%). The solution was heated under reflux for 5 hr., concentrated to half volume and diluted with water (4 ml). Most of the

remaining alcohol was boiled off and more water was added (6 ml). Carbon dioxide was bubbled through the cold solution which was then extracted with ether (3 x 40 ml) (fraction 1). The aqueous phase was acidified with hydrochloric acid and re-extracted with ether (3 x 40 ml) (fraction 11). The organic solutions were washed with brine, dried and evaporated under reduced pressure.

Fraction 1: The fraction yielded a solid (0.056 g) which crystallised from ethanol as colourless needles, m.p. 225-227°. [Found: C, 57.7; H, 3.6%. Calc. for $C_8H_6O_4$: C, 57.8; H, 3.6%]. Light absorption: ν_{max} . 2400-2700 (acid hydroxyl), 1690 (acid carbonyl), 930 and 723 cm^{-1} (methylenedioxy). Identical IR absorption, m.p. and mixed m.p. were observed for an authentic sample of piperonylic acid (lit.⁹ m.p. 229°).

Fraction 11: The fraction afforded a bright yellow solid (0.1 g) which on crystallisation from benzene gave 2-hydroxy- ω ,3,4,5,6-pentamethoxyacetophenone(8), m.p. 85-87° (lit.²³ m.p. 86-87°). [Found: C, 54.4; H, 6.2; O, 39.5; OCH_3 , 56.0%. Calc. for $C_{13}H_{18}O_7$: C, 54.5; H, 6.3; O, 39.1, 5x OCH_3 , 54.2%]. Light absorption; λ_{max} . 222, 284, 385 $m\mu$ ($\log \epsilon$ 4.22, 4.09, 3.46) (neutral and acidic ethanol); 236, 276 $m\mu$ ($\log \epsilon$ 4.17, 3.68) (alkaline ethanol); NMR spectrum: s, 3, 4.09, 4.00, 3.85,

3.80, (ArOCH_3); s, 3, 3.52, ($-\text{CH}_2\text{OCH}_3$); s, 2, 4.65, ($-\text{COCH}_2$); s, 1, 11.17, ($-\text{OH}$).

Partial Demethylation of Melibentin

Melibentin (0.2 g) was heated under reflux for 14 hr. with a solution of anhydrous aluminium chloride (2.0 g) in absolute ether (12 ml). The orange solid which formed when the mixture was poured on to ice-water was collected and boiled for 20 min. with acetic acid (20 ml) and hydrochloric acid (10N, 10 ml). Dilution with water (200 ml) and filtration of the resulting precipitate furnished yellow crystals of desmethylnelibentin(5). This was purified by recrystallisation from methanol as fine needles, m.p. 148-149°. [Found: C, 59.7; H, 4.7%. Calc. for $\text{C}_{20}\text{H}_{18}\text{O}_9$: C, 59.7; H, 4.5%]. Light absorption; ν_{max} . 1630 (bonded carbonyl), 928 cm^{-1} , no hydroxyl absorption: λ_{max} . 260, 280, 345 $\text{m}\mu$ ($\log \epsilon$ 4.23, 4.24, 4.25) (neutral and acidic ethanol), λ_{max} . 294, 315 $\text{m}\mu$ ($\log \epsilon$ 4.32, 4.33) (alkaline ethanol); NMR spectrum: s, 1, 12.38 (OH), (disappearing on D_2O exchange); m, 1, 7.78 ($2'\text{-H}$); s, 1, 7.65, ($6'\text{-H}$); d, 1, 6.95, ($3'\text{-H}$), 8.0; s, 2, 6.09, (O_2CH_2); s, 3, 4.13, 3.98, 3.93, 3.90 (ArOCH_3).

Acetyl Desmethylnelibentin

The flavone (0.1 g) (5) was heated for 30 min on the steam bath in a mixture of pyridine (4 ml) and acetic

anhydride (2 ml). The product was precipitated on dilution with water and collected by filtration. Crystallisation from ether-light petroleum afforded cream needles, m.p. 156-157°. [Found: C, 59.1; H, 4.5%. Calc. for $C_{22}H_{20}O_{10}$: C, 59.4; H, 4.6%]. Light absorption: $\nu_{\max}$. 1760 (phenolic acetate), 1650 cm^{-1} (pyrone carbonyl), no hydroxyl absorption: $\lambda_{\max}$. 253, 264, 342 $m\mu$ ($\log \epsilon$ 4.49, 4.24, 4.34); NMR spectrum as in (5), except that the signal at 12.38 was replaced by a singlet of three-proton intensity at 2.49 ($ArOCOCH_3$).

Oxidation of Desmethylnelibentin

The phenol (5) (1 g) was suspended in ether (50 ml) and the mixture cooled to 5°. Concentrated nitric acid (10 ml) was added with stirring till the colour of the ether solution did not deepen any further. The ether layer was separated, washed with water and 2% sodium hydrogencarbonate, to remove and nitro-phenols, and brine. Evaporation of the dried solution gave a brown residue which on crystallisation from acetonitrile yielded the quinone (6) as fine, red needles, m.p. 197-205°. [Found: C, 59.4; H, 3.9%. Calc. for $C_{19}H_{14}O_9$: C, 59.1; H, 3.7%]. Light absorption: $\nu_{\max}$. 1685, 1660 (quinone carbonyl), 1630 cm^{-1} (pyrone carbonyl); $\lambda_{\max}$. 287, 325 $m\mu$ ($\log \epsilon$ 4.16, 4.23) (neutral acetonitrile), $\lambda_{\max}$. 276, 323 $m\mu$ ($\log \epsilon$ 4.19, 4.09) (alkaline acetonitrile); $\lambda_{\max}$. 269,

274, 337 mμ (log ε 4.44, 4.41, 4.07) (reacidified aceto-
nitrate).

Identification of Melicopin

Melicopin (11)

Melicopin crystallised from benzene as cream prisms and from methanol as needles, m.p. 133-134°, $[\alpha]_D^{20}$ (c, 2.13 in ethanol). [Found: C, 65.3; H, 7.6; O, 27.3; OCH_3 , 9.3%. Calc. for $\text{C}_{19}\text{H}_{26}\text{O}_6$: C, 65.1; H, 7.4; O, 27.4; 1xOCH_3 , 8.9%]. Light absorption: ν_{max} . 3420-3150 (hydroxyl), 1640 (bonded ester), 1100-1050 cm^{-1} (phenolic hydroxyl); λ_{max} . 232sh, 292, 325 $\text{m}\mu$ ($\log \epsilon$ 4.04, 4.26, 3.61) (neutral and reacidified ethanol); λ_{max} . 255, 362 $\text{m}\mu$ ($\log \epsilon$ 3.9, 4.46) (alkaline ethanol); NMR spectrum: s, 1, 13.25, (bonded OH) (disappearing on D_2O exchange); m, 1, 7.15, (OH) (disappearing on D_2O exchange); s, 1, 6.01, (Ar-H); m, 1, 5.1, 5.5, ($\text{C}=\text{CH}$); s(b), 2, 4.71, (ArCH_2); d, 2, 4.53, ($\text{ArO}-\text{CH}_2-$), 4; s, 4, 3.9, (ArCO_2CH_3 and ArCH_2OH) (signal integrated for 3 protons after deuteration); d, 4, 2.12, ($\text{C}=\text{C}-(\text{CH}_2)_2-\text{C}=\text{C}$); s, 3, 1.77, 1.67, 1.62 ($\text{C}=\text{C}-\text{CH}_3$). A reddish-purple colour was obtained with ferric chloride solution.

Geranyl Proton Signals in the NMR Spectrum of Collinin.

t, 1, 5.6 and m, 1, 5.2, ($\text{C}=\text{CH}$); d, 2, 4.70, $\text{ArO}-\text{CH}_2-$), 4; m, 4, 2.12, ($\text{C}=\text{C}-(\text{CH}_2)_2-\text{C}=\text{C}$); s, 3, 1.79, 1.68, 1.63, ($\text{C}=\text{C}-\text{CH}_3$).

Natural Methylmelicopin (12)

Methylmelicopin crystallised from ether as pale yellow prisms, m.p. 103-104°. [Found: C, 65.8; H, 7.6; O, 26.8; OCH_3 , 17.3% Calc. for $\text{C}_{20}\text{H}_{28}\text{O}_6$: C, 65.9; H, 7.7; O, 26.3; $2\times\text{OCH}_3$, 17.6%]. Light absorption: ν_{max} . 3500-3200 (hydroxyl), 1640 (bonded ester), 1250 cm^{-1} (aryl ether); λ_{max} . 233.5, 289, 333 $\text{m}\mu$ ($\log \epsilon$ 4.06, 4.25, 3.59) (neutral and acidic ethanol); λ_{max} . 233, 292, 348 $\text{m}\mu$ ($\log \epsilon$ 4.18, 4.01, 3.59) (alkaline ethanol); NMR spectrum: s, 1, 13.0, (bonded OH); s, 1, 5.98, (Ar-H); t, 1, 5.6 and m, 1, 5.1, (C=CH); s(b), 2, 4.7, (ArCH_2); d, 2, 4.61 (ArO-CH_2 -), 4; s, 3, 3.91, 3.80 (ArOCH_3 and ArCO_2CH_3); m, 1, 3.47, (ArCH_2OH); m, 4, 2.17, ($\text{C=C-(CH}_2\text{)}_2\text{-C=C}$); s, 3, 1.77, 1.67, 1.62, (C=C-CH_3). Spectrum integrated for two less protons after deuteration. A purple ferric chloride test was obtained.

Synthetic Methylmelicopin

1) Melicopin (0.1 g) was treated with an ethereal solution of diazomethane at 0°. Evaporation of the solvent afforded a yellow gum which could not be induced to crystallise. Chromatography on silica gel gave starting material in 70% yield.

2) Melicopin (0.2 g), potassium (0.2 g) and anhydrous benzene (30 ml) were heated together under reflux for 3 hr.

Methyl iodide (2 g) was added and the refluxing continued for a further 3 hr. The mixture was then cooled, filtered and the residue, after removal of the solvent, chromatographed on silica gel (10 g) in benzene. Benzene-ether (20:1) eluted a yellow oil (0.03 g) which crystallised on standing. After recrystallisation it was identical, (m.p., mixed m.p. and IR spectrum) with a sample of natural methylmelicopin.

3) A mixture of melicopin (0.2 g), freshly distilled methyl-iodide (2 ml), anhydrous potassium carbonate (2.5 g) and dry acetone (30 ml) was allowed to stand in an atmosphere of nitrogen at room temperature for 16 hr. The reaction mixture was filtered and the solvent removed under reduced pressure. The residual oil was extracted with ether and the extract washed well with 3N hydrochloric acid (1 x 50 ml) and brine. Evaporation of the dried solvent afforded the ether (12) as a yellow gum. It crystallised from ether as yellow prisms (0.15 g), m.p. 103-104°, (identical with natural methylmelicopin). [Found: C, 65.8; H, 7.9%. Calc. for $C_{20}H_{28}O_6$: C, 65.9; H, 7.7%].

Triacetylmelicopin (13)

A solution of melicopin (0.1 g) in pyridine (4 ml) and acetic anhydride (2 ml) was left at room temperature overnight. The reaction mixture was diluted with ice-water and

extracted with chloroform (3 x 50 ml). The organic layer was washed with water (3 x 30 ml), 3N hydrochloric acid (2 x 50 ml) and brine. Evaporation of the dried solution gave a gum which was chromatographed in benzene on silica gel. Fractions eluted with benzene and crystallised from light petroleum gave triacetylmelicopin (13) (0.08 g), as colourless needles, m.p. 59-60° [Found: C, 63.2; H, 6.9; CH₃CO, 26.6%. Calc. for C₂₅H₃₂O₉: C, 63.0; H, 6.7; 3xCH₃CO, 27.1%]. Light absorption: $\nu_{\max}$. 1770 (phenolic acetate), 1745 (aliphatic acetate), 1705 cm⁻¹ (aromatic ester), no hydroxyl absorption; $\lambda_{\max}$. 250, 300 m μ (log ϵ 3.6, 3.47) (neutral ethanol); $\lambda_{\max}$. 249, 330 m μ (log ϵ 3.99, 4.32) (alkaline ethanol); $\lambda_{\max}$. 231, 292, 325 m μ (log ϵ 4.1, 4.06, 3.6) (reacidified ethanol); NMR spectrum: s, 1, 6.61, (Ar-H); t, 1, 5.44 and m, 1, 5.06, (C=CH); s, 2, 5.03, (ArCH₂); d, 2, 4.52, (Ar-O-CH₂-); s, 3, 3.75, (ArCO₂CH₃); s, 3, 2.31, 2.27, (ArOCOCH₃); s, 3, 2.14, (ArCH₂OCOCH₃); d, 4, 2.08, (C=C-(CH₂)₂-C=C); s, 3, 1.70, 1.67, 1.62 (C=C-CH₃).

Diacetylmethylmelicopin (14)

Methylmelicopin (0.2 g) was dissolved in a mixture of pyridine (4 ml) and acetic anhydride (2 ml) and left at room temperature overnight. Excess reagent was decomposed

with ice and the product isolated as with (13). Evaporation gave a colourless oil which crystallised with difficulty, on standing at 0° for several days. Recrystallisation from light petroleum (b.p. $30-40^{\circ}$) at 0° afforded diacetylmethylmelicopin (14) as colourless plates (0.15 g), m.p. $41-43^{\circ}$, [Found: C, 63.9; H, 7.0%. Calc. for $C_{24}H_{32}O_8$: C, 64.2; H, 7.2%]. Light absorption: $\nu_{\max}$. 1775 (phenolic acetate) 1755 (aliphatic acetate), 1700 cm^{-1} (aromatic ester), no hydroxyl absorption; $\lambda_{\max}$. 228, 267, 310 $m\mu$ ($\log \epsilon$ 4.22, 3.83, 3.63) (neutral ethanol); $\lambda_{\max}$. 239.5, 293 $m\mu$ ($\log \epsilon$ 4.07, 4.20) (alkaline ethanol); $\lambda_{\max}$. 232, 287, 329 $m\mu$ ($\log \epsilon$ 4.05, 4.26, 3.60) (reacidified ethanol); NMR spectrum: s, 1, 6.71, (Ar-H); m, 2, 5-5.1, (C=CH-); s, 2, 5.03, (ArCH₂); m, 2, 4.58, (ArO-CH₂-); s, 3, 3.90, 3.80, (ArOCH₃ and ArCO₂CH₃); s, 3, 2.30, (ArOCOCH₃); s, 3, 2.13, (ArCH₂OCOCH₃); d, 4, 2.09, (C=C-(CH₂)₂-C=C); s, 3, 1.70, 1.63, 1.57, (3 C=C-CH₃).

Dimethylmelicopin (15)

Methylmelicopin (0.2 g) was refluxed in dry acetone (150 ml) with anhydrous potassium carbonate (2 g) and dimethyl sulphate (2 ml) for 6 hr, under nitrogen. The mixture was filtered and most of the acetone removed under reduced pressure. Water was then added and the mixture

extracted with ether (3 x 50 ml). The combined extract was washed once with 2N hydrochloric acid (20 ml), once with water, dried, and evaporated. The residue was chromatographed on silica gel (20 g) in benzene. The fraction eluted with benzene-ether (25:1) was concentrated to yield a colourless oil (0.13 g) which was identified spectroscopically as dimethylmelicopin (15). Light absorption: ν_{max} . 3540 (hydroxyl), 1695 cm^{-1} (aromatic ester); λ_{max} . 237sh, 272 $\text{m}\mu$ ($\log \epsilon$ 3.80, 3.39) (neutral and alkaline ethanol); NMR spectrum: s, 1, 6.30, (Ar-H); t, 1, 5.41 and m, 1, 5.09, (C=CH-); m, 2, 4.57, (ArO-CH₂-); s, 2, 4.53, (ArCH₂-); s, 6, 3.90 and s, 3, 3.81, (2ArOCH₃ and 1 CO₂CH₃); m, 1, 2.7-3.2, (OH) (disappearing after deuteration); d, 4, 2.08, (C=C-(CH₂)₂-C=C); s, 3, 1.71, 1.67, 1.59, (C=C-CH₃).

Tosylmethylemelicopin (17)

p-Toluene sulphonyl chloride (1 g) was added to a cooled solution of methylemelicopin (0.2 g) in pyridine (5 ml), and the reaction mixture kept at 0° for 16 hr. It was decomposed with ice and the resultant oil extracted with chloroform (3 x 50 ml). The extract was washed with saturated sodium hydrogen carbonate (2 x 40 ml), 3N hydrochloric acid (2 x 50 ml) and brine. Evaporation gave an amorphous solid which after chromatography on silica gel

(10 g) in benzene and elution with benzene-ether (99:1) yielded a crystalline fraction (0.085 g). Tosylmethylmelicopin (17) crystallised from ether as cream needles, m.p. 133-135°, [Found: C, 62.6; H, 6.9; O, 24.8% Calc. for $C_{27}H_{34}O_8S$: C, 62.5; H, 6.6; O, 24.7%]. Light absorption: $\nu_{\max}$. 1620 cm^{-1} (bonded aromatic ester); $\lambda_{\max}$. 218sh, 222.5sh, 227sh, 236sh, 290, 330 $\text{m}\mu$ ($\log \epsilon$ 3.53, 3.49, 3.42, 4.28, 4.35, 3.64) (neutral ethanol); $\lambda_{\max}$. 227.5sh, 236, 285, 325 $\text{m}\mu$ ($\log \epsilon$ 3.42, 4.30, 4.38, 3.76) (alkaline ethanol): NMR spectrum: s, 8, 7.5, ($\underline{\text{H}}\text{-ArSO}_3^-$); s, 1, 12.84 (bonded $\underline{\text{OH}}$) (disappearing after deuteration); s, 1, 5.98, ($\text{Ar-}\underline{\text{H}}$); s, 2, 5.20 ($\text{Ar-CH}_2\text{-Otosyl}$); m, 2, 5.1-5.5, (C=CH-); d, 2, 4.62 ($\text{ArO-CH}_2\text{-}$); s, 3, 3.91, 3.79 (ArOCH_3 and ArCO_2CH_3) s, 3, 2.44, ($\text{CH}_3\text{-Ar-SO}_3^-$); m, 4, 2.13, ($\text{C=C-(CH}_2\text{)}_2\text{-C=C}$); s, 3, 1.77, 1.69, 1.62, (C=C-CH_3).

Attempted Alkaline Hydrolysis of Methylmelicopin (12)

1) Methylmelicopin (0.1 g) was allowed to stand in 1% aqueous potassium hydroxide overnight at room temperature. The reaction mixture was steam distilled and the distillate extracted with ether (2 x 50 ml). No material was present in this extract. Acidification and extraction of the residue afforded an intractable oil.

2) 0.2N aqueous sodium hydroxide was added to a solution of methylmelicopin (0.1 g) in methanol (2 ml) under an atmosphere of nitrogen and the mixture kept at room temperature for 2 hr. The mixture was diluted, acidified with 2N hydrochloric acid and extracted with ether (2 x 30 ml). The reddish extract was washed with water (2 x 30 ml), dried and evaporated under reduced pressure to give a dark gum. Chromatography on silica gel in benzene afforded a series of gums.

3) A solution of (12) (0.3 g) in methanol (12 ml) and sodium carbonate (0.3 g) in water (5 ml) was left in an atmosphere of nitrogen at room temperature overnight. Since on contact with air the solution rapidly became purple, the nitrogen atmosphere was maintained until the reaction mixture had been acidified with 2N hydrochloric acid. Extraction with ether and working up in the normal way gave an intractable brown oil.

Acid Hydrolysis of Methylmelicopin

A suspension of methylmelicopin (0.2 g) in 2N hydrochloric acid (10 ml) was refluxed for 5 min. under a stream of nitrogen. The solution rapidly darkened. It was then steam distilled until the distillate was no longer cloudy. On cooling an amorphous dark solid separated from the residual

solution. It was filtered, allowed to dry and dissolved in benzene. Chromatography on silica gel and elution with benzene-ether (1:1) afforded a yellow gum. Crystallisation from benzene yielded the desgeranylated phenol (18) as yellow prisms (0.02 g), m.p. 181-183°. [Found: C, 52.6; H, 5.4%. Calc. for $C_{10}H_{12}O_6$: C, 52.6; H, 5.3%.] Light absorption: $\nu_{\max}$. 3500-3300 (hydroxyl), 1630 cm^{-1} (bonded aromatic ester); $\lambda_{\max}$. 233sh, 285, 335 $m\mu$ ($\log \epsilon$ 3.96, 4.20, 3.43) (neutral and reacidified ethanol); $\lambda_{\max}$. 245sh, 299, 327 $m\mu$ ($\log \epsilon$ 3.97, 4.05, 3.86) (alkaline ethanol); NMR spectrum (acetone): s, 1, 6.05, (Ar-H); m, 2, 4.77, (ArCH₂); s, 3, 3.89, (ArOCH₃); s, 3, 3.70 (ArCO₂CH₃); (OH masked by solvent).

Trimethyldesgeranylmelicopin (16)

A mixture of methyldesgeranylmelicopin (0.15 g) dry acetone (100 ml), anhydrous potassium carbonate (2 g) and redistilled dimethylsulphate (1 ml) was refluxed under nitrogen for 5 hr. The potassium carbonate was removed by filtration and washed well with acetone. The combined filtrate was concentrated to a small volume, diluted with water and made strongly alkaline with ammonia to decompose excess reagent. Extraction of the mixture with chloroform (2 x 50 ml) and evaporation of the dried solvent gave a

yellow residue which was chromatographed on silica gel in benzene. Elution with benzene-ether (5:1) yielded a colourless gum which on recrystallisation from cyclohexane afforded trimethyldesgeranylmelicopin (16) as colourless prisms (0.045 g), m.p. 77-78°. [Found: C, 56.4; H, 6.3; O, 37.6%. Calc. for $C_{12}H_{16}O_6$: C, 56.2; H, 6.3; O, 37.5%]. Light absorption: $\nu_{\max}$. 3540 (aliphatic hydroxyl), 1710 cm^{-1} (free aromatic ester); $\lambda_{\max}$. 280 $m\mu$ ($\log \epsilon$ 3.80) (neutral and alkaline ethanol); NMR spectrum: s, 1, 6.30, (Ar-H); m, 2, 4.52, (ArCH₂-); s, 3, 3.92 and s, 6, 3.82, (3ArOCH₃); s, 3, 3.73, (ArCO₂CH₃); m, 1, 3.32 (ArCH₂OH). After deuteration the multiplet at 3.32 disappeared and the signal at 5.45 became a 2-proton singlet.

Tritosylmethyldesgeranylmelicopin (18)

p-Toluene sulphonyl chloride (1 g) was added to a cooled solution of desgeranylmethylmelicopin (0.2 g) in pyridine (5 ml), and the reaction mixture kept at 0° for 16 hr. It was decomposed with ice and the precipitated tosylate extracted with chloroform (3 x 50 ml). The organic extract was washed with saturated sodium hydrogencarbonate (2 x 50 ml), 2N hydrochloric acid (2 x 40 ml) and brine (2 x 30 ml) in turn. Evaporation of the dried extract yielded a brown oil (0.3 g) which was chromatographed on silica gel (10 g) in benzene. Elution with benzene-ether

(100:1) and crystallisation from methanol gave tritosyl-methyl-desgeranyl melicopin as colourless prisms (0.25 g), m.p. 121-123°. [Found: C, 54.1; H, 4.4; O, 28.1%. Calc. for $C_{17}H_{16}O_8S$: C, 54.0; H, 4.4; O, 28.1%. Calc. for $C_{17}H_{16}O_8S$: C, 54.0; H, 4.4; O, 27.9%]. Light absorption: $\nu_{\max}$. 1725 cm^{-1} (aromatic ester), no hydroxyl absorption: $\lambda_{\max}$. 227, 263, 273 $\text{m}\mu$ ($\log \epsilon$ 4.59, 3.90, 3.82); NMR spectrum: m, 12, 7.9-7.3, (H-Ar-SO₃); s, 1, 6.74 (Ar-H); s, 2, 4.76, (Ar-CH₂); s, 3, 3.78, 3.68 (ArOCH₃ and ArCO₂CH₃); s, 9, 2.41, (3xAr-CH₃).

Triacetylmethyl-desgeranylmelicopin (19)

A mixture of desgeranylmethylmelicopin (0.15 g), acetic anhydride (5 ml) and pyridine (10 ml) was heated on the steam bath for 2 hr. Dilution with water gave an oily product which was worked up in the normal way (see 13) to give a yellow oil which was chromatographed on a silica gel column prepared in benzene. Elution with benzene-ether (100:1) afforded a colourless gum which despite repeated attempts could not be induced to crystallise. Light absorption: $\nu_{\max}$. 1770 (phenolic acetate), 1745 (aliphatic acetate), 1638 cm^{-1} (aromatic ester), no hydroxyl absorption; NMR spectrum: s, 1, 6.65, (Ar-H); s, 2, 4.95, (ArCH₂-); s, 3, 2.32, 2.27, 2.14, (2xArOCOCH₃ and 1xArCH₂OCOCH₃).

Acetylmethyl-desgeranylmelicopin (20)

Methyl-desgeranylmelicopin (0.2 g) was acetylated with acetic anhydride (10 ml) and pyridine (5 ml) at room temperature overnight. The mixture was diluted, basified with solid sodium hydrogencarbonate, and left at room temperature for 3 hr. The bright yellow solution was then extracted with ether (3 x 50 ml) and the extract washed with 2N hydrochloric acid and brine in the usual way. Evaporation and crystallisation from cyclohexane gave acetylmethyl-desgeranylmelicopin (20), as cream, rosettes, m.p. 145-146°. [Found: C, 53.6; H, 5.5% Calc. for $C_{12}H_{14}O_7$: C, 53.3; H, 5.2%]. Light absorption: $\nu_{\max}$. 3340 (hydroxyl), 1750 (aliphatic acetate), 1640 cm^{-1} (bonded aromatic ester); $\lambda_{\max}$. 233sh, 285, 387 $m\mu$ ($\log \epsilon$ 4.03, 4.27, 3.51) (neutral and reacidified ethanol); $\lambda_{\max}$. 241sh, 295 $m\mu$ ($\log \epsilon$ 4.06, 4.20) (alkaline ethanol); NMR spectrum: s, 1, 12.50, (bonded OH), s, 1, 6.06, (Ar-H); s, 2, 5.18, (ArCH₂-); s, 3, 3.90, 3.82, (ArOCH₃ and ArCO₂CH₃); m, 1, 3.5 (OH); s, 3, 2.19, (ArCH₂OCOCH₃). The spectrum integrated for two less protons after exchange with deuterium oxide.

Lithium Aluminium Hydride Reduction of (12)

A solution of lithium aluminium hydride (0.3 g) in ether (20 ml) was added slowly to a stirred solution of methylmelicopin (12) (0.3 g) in tetrahydrofuran (50 ml),

under an atmosphere of nitrogen. After the addition was complete, stirring was continued for 2 hr at room temperature. Excess reagent was decomposed with moist ether and the mixture treated with a slight excess of dilute hydrochloric acid. Extraction with chloroform (2 x 50 ml) and evaporation of the dried extract afforded a brown oil, which could not be induced to crystallise even after chromatography on silica gel. Light absorption: $\nu_{\max}$. 3400 (hydroxyl) 1630 cm^{-1} (bonded ester).

Treatment of the crude product (0.05 g) with acetic anhydride-pyridine for 24 hr. at room temperature yielded the acetate (23) (0.05 g), as colourless plates from cyclohexane, m.p. 173-175°. [Found: C, 67.9; H, 7.4%. Calc. for $\text{C}_{43}\text{H}_{54}\text{O}_{12}$: C, 67.7; H, 7.1%]. Light absorption: $\nu_{\max}$. 1770 (phenolic acetate), 1720 cm^{-1} (aromatic ester), no hydroxyl absorption; $\lambda_{\max}$. 233, 284 $\text{m}\mu$ ($\log \epsilon$ 4.38, 3.97); NMR spectrum: s, 2, 6.55, (Ar-H); m, 6, 5.1-5.6, (4 $\text{C}=\text{CH}-$ and 2 $\text{Ar}-\text{CH}_2-\text{Ar}$); s, 6, 3.92, 3.83, (2 ArOCH_3 and 2 ArCO_2CH_3) s, 6, 2.40, (ArOCOCH_3); d, 8, 2.15, ($\text{C}=\text{C}-(\text{CH}_2)_2-\text{C}=\text{C}$); s, 6, 1.73, 1.67, 1.62 (6 $\text{C}=\text{C}-\text{CH}_3$).

Tetrahydromelicopin (24)

A mixture of melicopin (0.2 g) and 5% palladium-charcoal (0.02 g) in absolute ethanol (100 ml) was shaken under hydrogen until uptake ceased (28 hr.). After removal of the catalyst

by filtration through celite, the solvent was reduced to a small volume (5 ml). Addition of water precipitated an amorphous solid. Crystallisation from aqueous ethanol gave tetrahydromelicopin (24) as cream needles, (0.1 g), m.p. 126-128°, mixed m.p. with melicopin 120-125°. [Found: C, 64.2; H, 8.5; O, 27.5% Calc. for $C_{19}H_{30}O_6$: C, 64.2; H, 8.5; O, 27.2%]. Light absorption: $\nu_{\max}$. 3400-3300 (phenolic hydroxyl) 1630 (bonded aromatic ester), peaks at 952, 863 cm^{-1} (see spectrum of (11)) were absent: $\lambda_{\max}$. 235, 290, 330 $m\mu$ (log ϵ 3.95, 4.25, 3.55) (neutral and reacidified ethanol); $\lambda_{\max}$. 240, 322 $m\mu$ (log ϵ 3.89, 4.40) (alkaline ethanol).

Tetrahydromelicopintriacetate (25)

Treatment of (24) with acetic anhydride-pyridine afforded the triacetate (25) as colourless needles from light petroleum (b.p. 30-40°) at 0°, m.p. 62-63°, mixed m.p. with triacetylmelicopin (13) 50-54°. [Found: C, 62.4; H, 7.4%. Calc. for $C_{25}H_{36}O_9$: C, 62.5; H, 7.6%]. Light absorption: $\nu_{\max}$. 1770 (phenolic acetate), 1745 (aliphatic acetate), 1705 cm^{-1} (aromatic ester), no hydroxyl absorption; $\lambda_{\max}$. 252, 303 $m\mu$ (log ϵ 3.6, 3.47).

Methylmelicopintetrabromide (26)

A solution of bromine (0.088 g) in chloroform (10 ml)

was added dropwise to an ice-cold solution of methylmelicopin (0.1 g) in chloroform (20 ml), until the colour of the bromine persisted. The reaction mixture was immediately diluted with water and the organic phase washed with saturated sodium bisulphite (1 x 20 ml) and brine. Evaporation of the dried solution gave a yellow oil which on crystallisation from methanol afforded methylmelicopintetrabromide (26), as cream plates (0.14 g), m.p. 141-143° [Found: C, 35.2; H, 4.1%. Calc. for $C_{20}H_{28}O_6Br_4$: C, 35.1; H, 4.1%]. Light absorption: ν_{max} . 3470-3330 (hydroxyl), 1630 cm^{-1} (bonded ester); λ_{max} . 233, 288, 329 $m\mu$ ($\log \epsilon$ 4.14, 4.33, 3.71) (neutral and reacidified ethanol); λ_{max} . 240sh, 292, 348 $m\mu$ ($\log \epsilon$ 4.27, 4.18, 3.74) (alkaline ethanol); NMR spectrum: s, 1, 13.10 (bonded $\underline{OH}$); s, 1, 6.00 ($Ar-\underline{H}$); m, 2, 5.5-5.8 ($-\underline{CHBr}-CBrCH_3-$), s(b), 2, 4.73 ($ArCH_2-$); d, 2, 4.67, ($ArOCH_2-$); s, 4, 3.93 ($ArOCH_3$ and $\underline{OH}$); s, 1, 3.82, ($ArCO_2CH_3$); m, 4, 2.43, ($-CBr-(CH_2)_2-CBr$); s, 3, 1.94, 1.81, 1.79, ($CBr-CH_3$). Spectrum integrated for two less protons after deuterating.

Identification of the Side Chain

Methylmelicopin (0.5 g) was distilled with constant boiling hydrochloric acid (15 ml) until it had all dissolved and no more oily droplets appeared in the distillate. The distillate was extracted with ether (3 x 30 ml). Evaporation

of the dried extract gave an oil (0.2 g), smelling of hops.

The oil was dissolved in ethanol (1 ml), thiourea (0.2 g) added and the mixture heated for 15 min. on the steam bath. A saturated solution of picric acid in ethanol (3 ml) was added, and the solution left to cool in ice for 30 min. The bright yellow precipitate on repeated crystallisation from aqueous ethanol gave S-geranylisothiuronium picrate as yellow needles, m.p. 137-138°. The substance was identical (mixed m.p. and IR spectrum) with an authentic sample of S-geranylisothiuronium picrate, prepared in the same way from geranyl chloride.

Hydrogenolysis of Methylmelicopin

An ethanolic solution of methylmelicopin (0.25 g) was shaken under hydrogen in the presence of 5% palladium charcoal (0.05 g) for 50 hr. at atmospheric pressure. The filtered solution was evaporated to dryness and the residue crystallised from benzene to give methyl-desgeranylmelicopin (18), as yellow prisms, m.p. 181-183°, identical with the phenol derived from acid hydrolysis of methylmelicopin.

Oxidation of trimethyl-desgeranylmelicopin (16)

1) The alcohol (16) (0.2 g) was dissolved in cold aqueous 0.04 N potassium hydroxide (4 ml) and aqueous potassium permanganate (5%; 8 ml). After standing at room temperature

for 24 hr all the potassium permanganate had been consumed. A further 5 ml of reagent was added but after 18 hr the colour of the permanganate still persisted. Acidification and extraction of the reaction mixture with ether yielded a dark intractable gum.

2) A slurry of "active" manganese dioxide (5 g), prepared by the procedure of Attenborough et al.³² and the alcohol (16) (0.2 g) in benzene was refluxed for 30 min. The mixture was filtered and the residue washed well with hot chloroform (30 ml). Evaporation of the solvent afforded a reddish-brown tar from which no pure substance could be isolated.

3) The alcohol (0.3 g) in pyridine (5 ml) was added in one portion to chromium trioxide (1.5 g) suspended in pyridine (30 ml) at 0°. The mixture was stirred for 30 min. and then allowed to remain at room temperature for 24 hr.

The mixture was diluted with ether and filtered. The filtrate was washed with 3N hydrochloric acid (3 x 50 ml) and brine and evaporated to give a colourless oil. Methylation with an ethereal solution of diazomethane and crystallisation of the product from ether afforded colourless prisms (0.02 g), m.p. 119-120°. This was identical (mixed m.p. and IR spectra) with an authentic specimen of dimethyl-2,4,6-trimethoxyisophthalate (28).

Dimethyl 2,4,6-trimethoxyisophthalate (28)

Diethyl 2,4,6-trihydroxyisophthalate (32) was prepared as described by Leuchs and Geserick³⁵. The crude product crystallised from ethanol as colourless needles, m.p. 108-9°. (lit.³⁵ m.p. 107-108.5°). Light absorption: ν_{max} . 3390 (hydroxyl), 1660 cm^{-1} (bonded aromatic ester); λ_{max} . 246, 265, 310 $\text{m}\mu$ ($\log \epsilon$ 4.56, 4.19, 3.62) (neutral and reacidified ethanol); λ_{max} . 261, 290 $\text{m}\mu$ ($\log \epsilon$ 4.45, 4.22) (alkaline ethanol).

The foregoing phenol (3.5 g) was treated with excess ethereal diazomethane for 16 hr. at 0°. Solvent was removed under vacuum and an IR spectrum on the crude product showed hydroxyl absorption (3430 cm^{-1}). It was then dissolved in acetone and refluxed with dimethyl sulphate (3 ml) and anhydrous potassium carbonate (4 g) for 3 hr. Working up the methylation in the normal way afforded diethyl 2,4,6-trimethoxyisophthalate (33) as colourless plates (3 g), m.p. 90-91° (lit.³⁶ m.p. 91-92°) from cyclohexane. Light absorption: ν_{max} . 1725 cm^{-1} (aromatic ester), no hydroxyl absorption.

Hydrolysis of the diester (33) was carried out using more severe conditions than those described by Leuchs³⁷. (33) (3 g) was refluxed in aqueous alcoholic 1N sodium hydroxide

(20 ml) for 6 hr. The product was worked up as usual and crystallised from water to give 2,4,6-trimethoxyisophthalic acid (34) as colourless needles (1.8 g), m.p. 255-258° (lit.³⁷ m.p. sinters at 20°).

Treatment of (34) with excess ethereal diazomethane afforded dimethyl 2,4,6-trimethoxyisophthalate (28) which crystallised from ether as colourless prisms, (1.3 g), m.p. 121-123° (lit.³⁷ 123°) [Found: C, 54.9; H, 5.7%. Calc. for $C_{13}H_{16}O_7$: C, 54.6; H, 5.7%]. Light absorption: $\nu_{\max}$. 1710 cm^{-1} (aromatic ester), no hydroxyl absorption: $\lambda_{\max}$. 250sh, 280 $m\mu$ ($\log \epsilon$ 3.86, 3.27), NMR spectrum: s, 1, 6.30, (Ar-H), s, 6, 3.88, 3.83, and s, 3, 3.81, ($3xAr-OCH_3$ and $2xArCO_2CH_3$).

Hydrogenolysis of Triacetylmelicopin

Triacetylmelicopin (0.3 g) and 10% palladium-charcoal (0.05 g) were shaken in ethanol (100 ml) under hydrogen until uptake ceased (3 hr.). The filtered mixture was evaporated under reduced pressure to a small volume (5 ml), diluted with water (100 ml), and extracted with ether (3 x 30 ml). Evaporation of the washed and dried extract gave a gum which was chromatographed on silica gel in benzene. Elution with benzene-ether (10:1) afforded the desgeranylated phenol (35),

which crystallised from ether-light petroleum as yellow prisms, m.p. 99-102°. [Found: C, 52.9; H, 4.9%. Calc. for $C_{15}H_{16}O_9$: C, 52.9; H, 4.7%]. Light absorption: $\nu_{\max}$. 1770 (aromatic acetate), 1745 (aliphatic acetate), 1635 cm^{-1} (bonded aromatic ester), no hydroxyl absorption; $\lambda_{\max}$. 217.5sh, 254, 315 $m\mu$ ($\log \epsilon$ 4.21, 3.76, 3.51) (neutral ethanol); $\lambda_{\max}$. 251sh, 319 $m\mu$ ($\log \epsilon$ 3.97, 4.24) (alkaline ethanol); $\lambda_{\max}$. 237sh, 287, 350 $m\mu$ ($\log \epsilon$ 3.90, 4.17, 3.54) (reacidified ethanol); NMR spectrum: s, 1, 11.70, (bonded OH) (disappearing after deuteration); s, 1, 6.72, (Ar-H); s, 2, 5.12, (ArCH₂-); s, 3, 3.73, (ArCO₂CH₃); s, 3, 2.40, 2.31, 2.18, (2xArOCOCH₃ and 1xArCH₂OCOCH₃).

Hydrogenolysis of Diacetylmethylmelicopin

Diacetylmethylmelicopin (0.3 g) was hydrogenated using palladium-charcoal as catalyst (0.03 g). The mixture was worked up as for (35) to give an oil which on crystallisation from acetone-ether yielded the desgeranylated phenol (36), as colourless needles (0.12 g), m.p. 139-144°. [Found: C, 53.9; H, 5.26%. Calc. for $C_{14}H_{16}O_8$: C, 53.8; H, 5.2%]. Light absorption: $\nu_{\max}$. 1765 (aromatic acetate), 1742 (aliphatic acetate), 1640 cm^{-1} (bonded aromatic ester), no hydroxyl group; $\lambda_{\max}$. 215, 231.5, 276, 325 $m\mu$ ($\log \epsilon$ 4.18, 4.02, 4.05, 3.69) (neutral ethanol); $\lambda_{\max}$. 238, 277, 358 $m\mu$

(log ϵ 4.18, 3.88, 3.86) (alkaline ethanol); $\lambda_{\max}$. 231.5sh, 284, 330 m μ (log ϵ 4.02, 4.20, 3.51) (reacidified ethanol); NMR spectrum: s, 1, 12.63 (bonded OH) (disappearing on deuteration); s, 1, 6.42, (Ar-H); s, 2, 5.12, (ArCH₂-); s, 3, 3.90, 3.75, (ArOCH₃ and ArCO₂CH₃); s, 3, 2.40 (ArOCOCH₃); s, 3, 2.19 (ArCH₂OCOCH₃).

Hydrogenolysis of Dimethylmelicopin

Hydrogenation of dimethylmelicopin (0.3 g) (15) in ethanol (50 ml) with 10% palladium-charcoal gave, on working up as before, an oil which was chromatographed in benzene in silica gel. Elution with benzene-ether (5:1) afforded the desgeranylated phenol (37) which crystallised from ether-light petroleum as pale yellow needles (0.12 g), m.p. 86-87°. [Found: C, 54.8; H, 5.8%. Calc. for C₁₁H₁₄O₆: C, 54.4; H, 5.8%]. Light absorption: $\nu_{\max}$. 3380 (free hydroxyl) 1640 cm⁻¹ (bonded ester); $\lambda_{\max}$. 218sh, 233, 281, 333 m μ (log ϵ 4.15, 4.13, 4.17, 3.62) (neutral and reacidified ethanol); $\lambda_{\max}$. 239sh, 279, 360 m μ (log ϵ 4.16, 3.91, 3.73) (alkaline ethanol); NMR spectrum: s, 1, 12.72 (bonded OH); s, 1, 6.29, (Ar-H); s, 2, 4.76, (ArCH₂-); s, 3, 4.04, 3.91, 3.77, (2ArOCH₃ and ArCO₂CH₃), m, 1, 3.64, (ArCH₂OH). Spectrum integrated for two less

protons after deuteration.

Attempted Cyclic Derivatives of Dimethyl-desgeranylmelicopin(37)

1. Reaction with Phosgene

Toluene (50 ml), saturated with phosgene at 5°, was added to a solution of the above diol (0.02 g) in a mixture of pyridine (2 ml) and chloroform (10 ml). After being kept at room temperature for 2 days the red mixture was washed successively with saturated potassium hydrogen carbonate, 3N hydrochloric acid and brine. Removal of the solvent from the dried solution afforded an intractable gum.

2. Reaction with Thionyl Chloride

Thionyl chloride (1 ml) was added to a cooled solution of (37) (0.2 g) in pyridine (5 ml) and the mixture kept at 5° overnight. Working up the reaction as above gave a dark tar from which no pure substance could be isolated.

3. Reaction with Benzaldehyde

A mixture of the diol (0.3 g) and benzaldehyde (0.3 g) which had previously been saturated with benzoic acid, was heated on the steam bath for 2 hr. The resulting yellow oil was treated with 5% sodium hydroxide (50 ml) and the aqueous solution extracted with ether (2 x 50 ml) to afford benzaldehyde (0.2 g). Extraction of the acidified aqueous solution yielded benzoic acid and unchanged starting material (0.28 g).

4. Reaction with Phosphoryl Chloride

A solution of phosphoryl chloride (1 ml) in pyridine (2.5 ml) was added to a solution of the diol (37) in pyridine (2.5 ml) at 0°. The reaction mixture was kept at 0° for 30 min. and then hydrolysed with ice-water. Barium hydroxide was added to reach pH 7, after filtering the solution was lyophilised. Extraction of the powdery product with acetone gave a solution which yielded a crystalline, hygroscopic precipitate on addition of ether.

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PART II
NMR STUDIES OF SOME
MODEL COMPOUNDS

INTRODUCTION

It was pertinent to the interpretation of the NMR spectra of melicopin and its derivatives to determine whether changing the substituent on the benzene ring from a hydroxyl to an acetoxy or methoxy group altered the chemical shift of the ring protons. It was assumed that any mesomeric effect would be reflected in the chemical shift of the ortho and para protons and to a lesser degree in that of the meta protons. On the other hand any differences in the chemical shift of the ortho proton alone could be interpreted as arising from inductive or magnetic anisotropy contributions of the substituent. A series of phenols were chosen as model compounds to study these chemical shift differences.

Current work in this field^{1,2,3} has shown the difficulty in assessing the correlation between proton resonance shifts and the electron-density distribution in aromatic systems. Numerous solvent⁴ and field effects⁵ due to ring substituents are known to make considerable contributions to the proton shifts. As it is difficult to estimate the magnitude of these effects in individual cases, a series of empirical values of this kind can be very useful. In order to minimise

any contribution from solvent and concentration effects, the spectra were run at uniform concentrations in dilute solutions in deuteriochloroform (see experimental).

A series of measurements by Rasmussen⁶ had indicated that acetylating a phenolic group will result in an upfield shift of an ortho methyl group. As this observation had possible application to the interpretation of the NMR spectra of phenolic compounds, it was decided to measure the displacements of the methyl proton resonance in the same model compounds.

RESULTS

The relevant portions of the NMR spectra of the model compounds investigated are shown on Fig. 1-6 and the chemical shift and spin-spin coupling constants obtained from them are summarised on Table 2.

The spectra were not rigorously analysed (Pople, Schneider and Bernstein)⁶ but the approximate treatment described below is believed to be sufficiently accurate for the present purpose.

Analysis of Spectra

- 1) 2,5-xylenol: The ring protons in this phenol and its derivatives (Fig. 1a-c) formed a reasonably well defined AMX pattern whose analysis was straightforward (Pople, Schneider and Bernstein⁷; Jackman⁸).
- 2) 3,5-xylenol: The ring protons of this phenol and its derivatives (Fig. 2a-c) give rise to ill-resolved AB₂ patterns. While accurate analysis of these spectra would not be trivial, the approximate chemical shifts of the A and B protons could be assigned with reasonable certainty on the basis of selecting centres of gravity of multiplets; as the data of interest concerned the relative shifts in the three derivatives whose spectra were of similar type, it was unlikely that any

error introduced affected the general validity of the conclusions.

- 3) thymol: This compound and its derivatives give rise to a very similar absorption pattern to that from 2,5-xyleneol (Fig. 3a-c) and its analysis follows in the same way.
- 4) 2,6-xyleneol: The ring protons in the acetate and methyl ether derived from this phenol showed accidental equivalence of their chemical shifts (Fig. 4a-c). The parent phenol gave rise to a well resolved AB_2 pattern which has been analysed by Fraser⁹.
- 5) p-cresol: The aromatic protons in this phenol and its derivatives (Fig. 5a-c) appeared as a well resolved $AA'BB'$ pattern. The spectrum of the parent phenol has been analysed by Sternhell et al.⁷⁰, in which attention was drawn to the non-equivalence of the signal widths.
- 6) p-nitrophenol: The ring protons in this phenol and its derivatives were easily distinguished as an $AA'BB'$ pattern (Fig. 6a-c) and its analysis was quite simple.

Benzylic Methyl Protons

The chemical shifts of these protons in the model compounds are shown on Table 3. In each case, they gave rise to three-proton singlets.

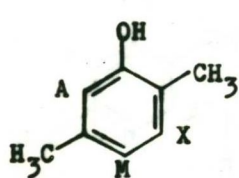


Fig. 1a

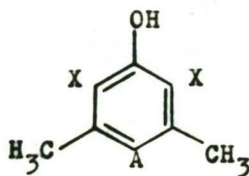
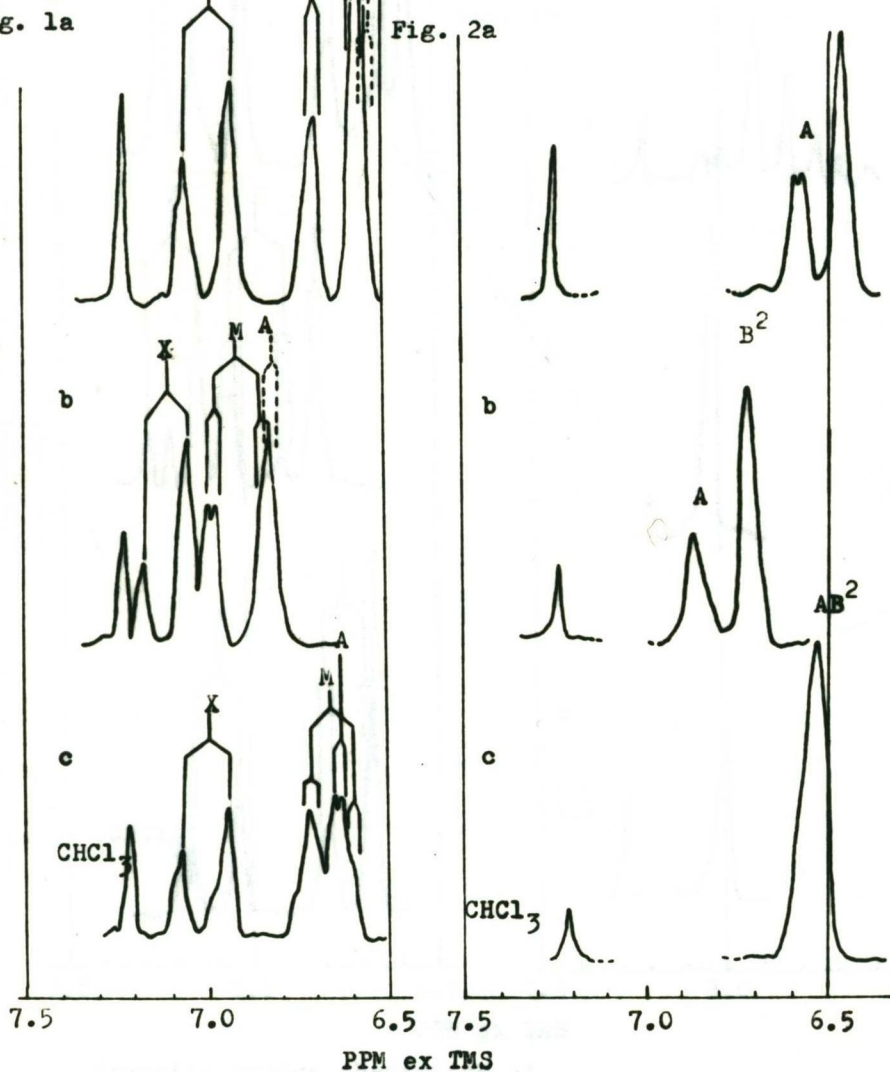
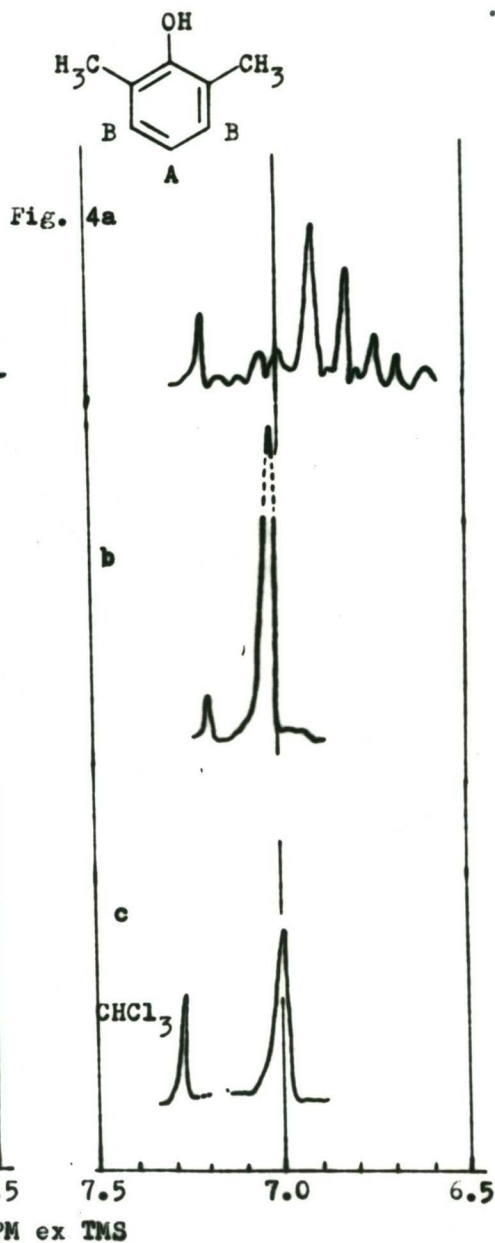
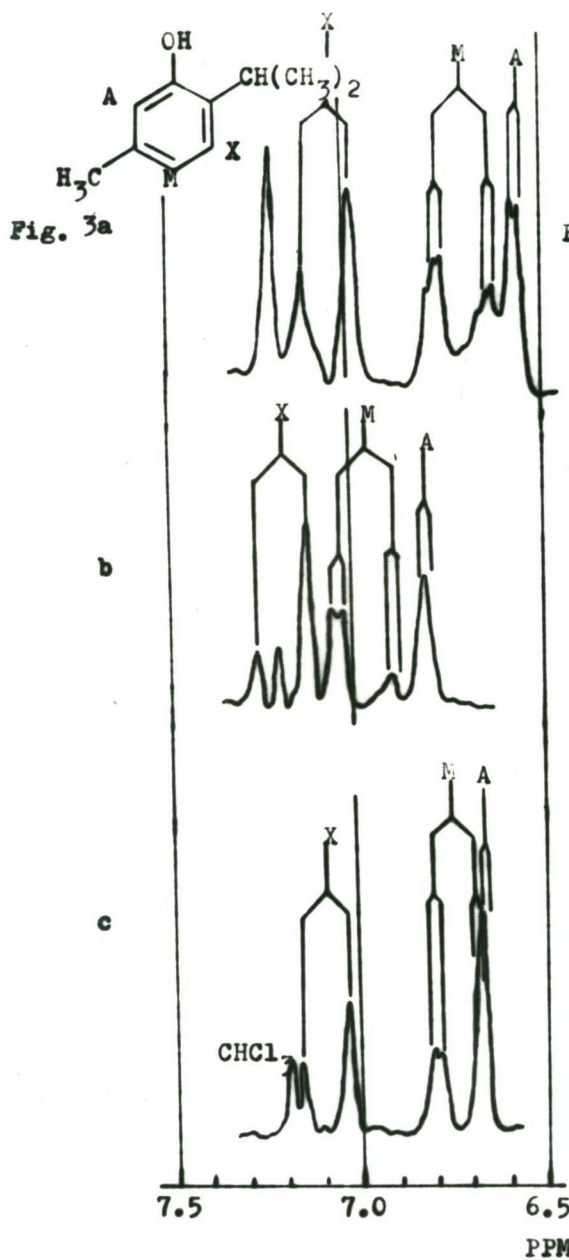


Fig. 2a

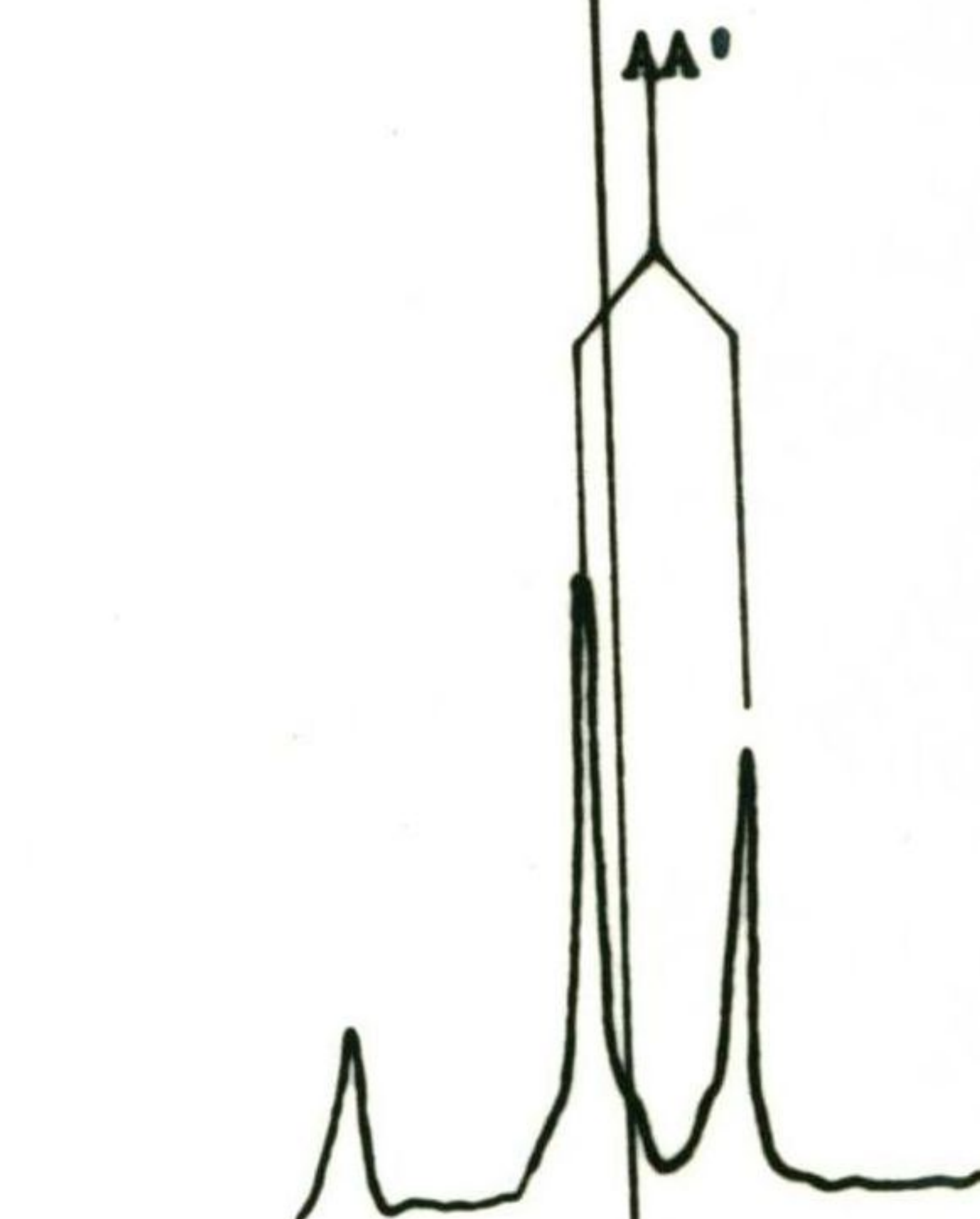
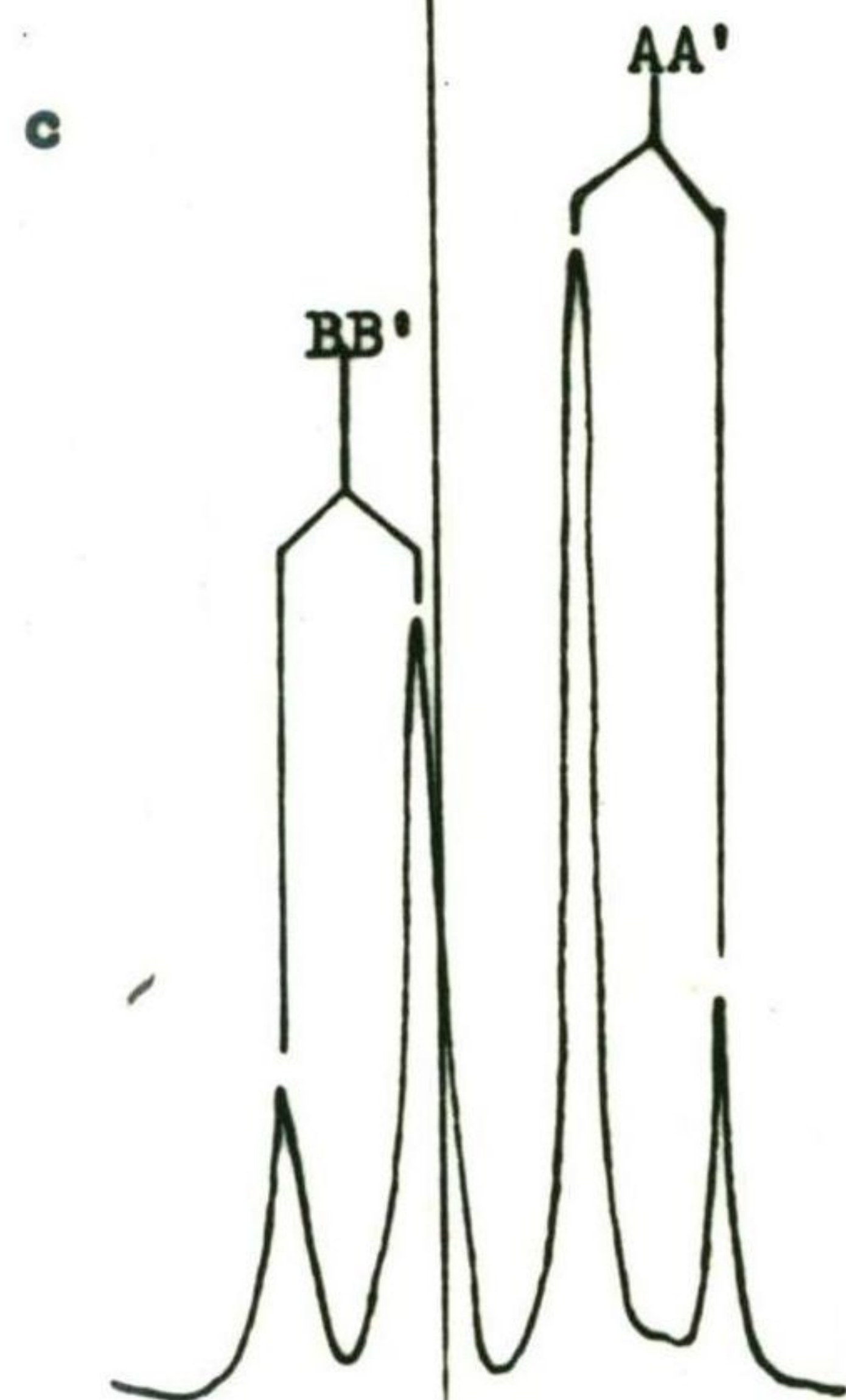
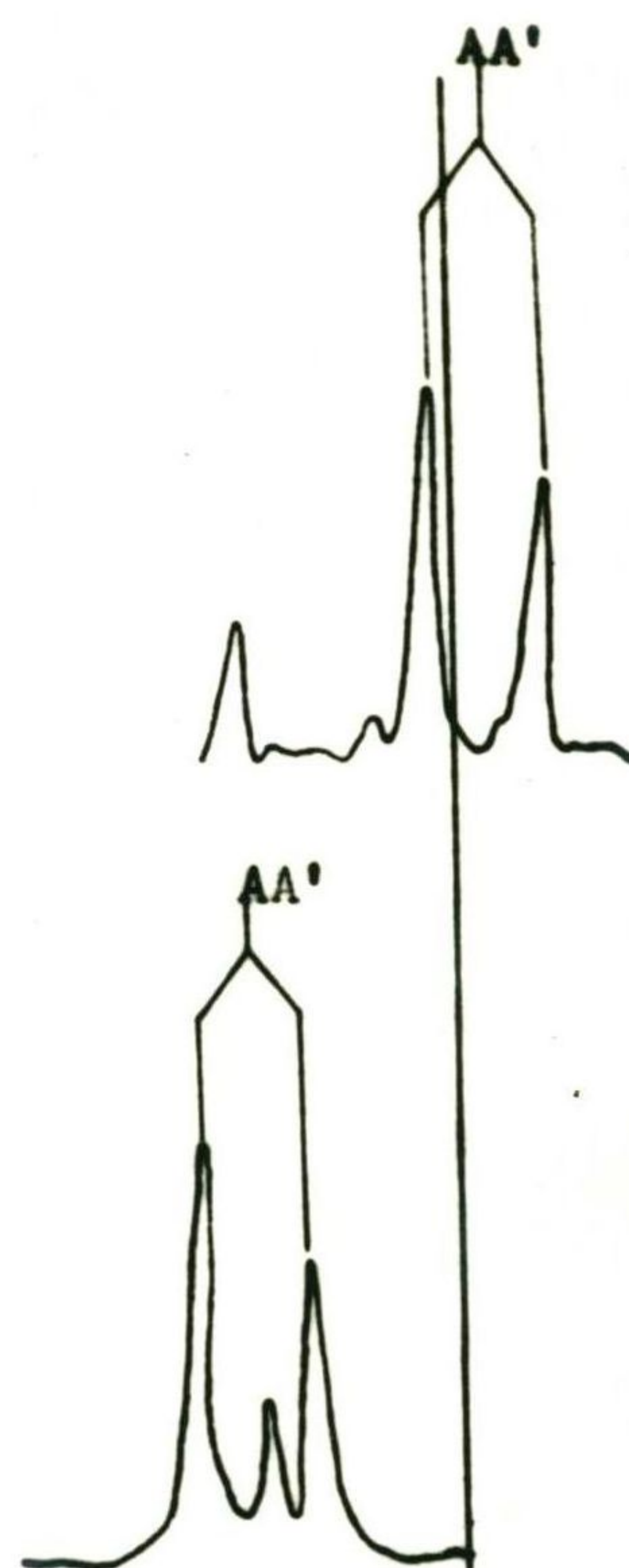
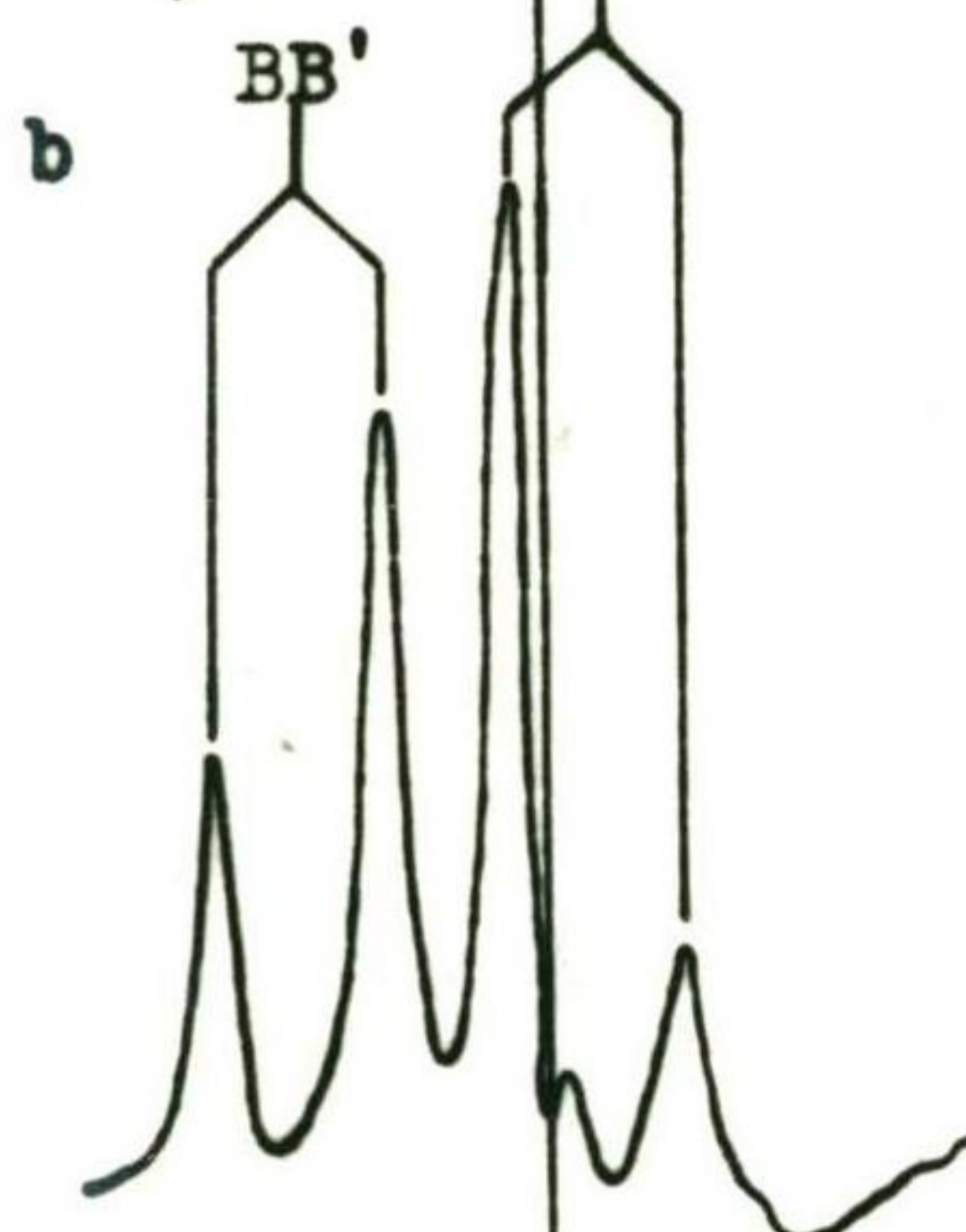
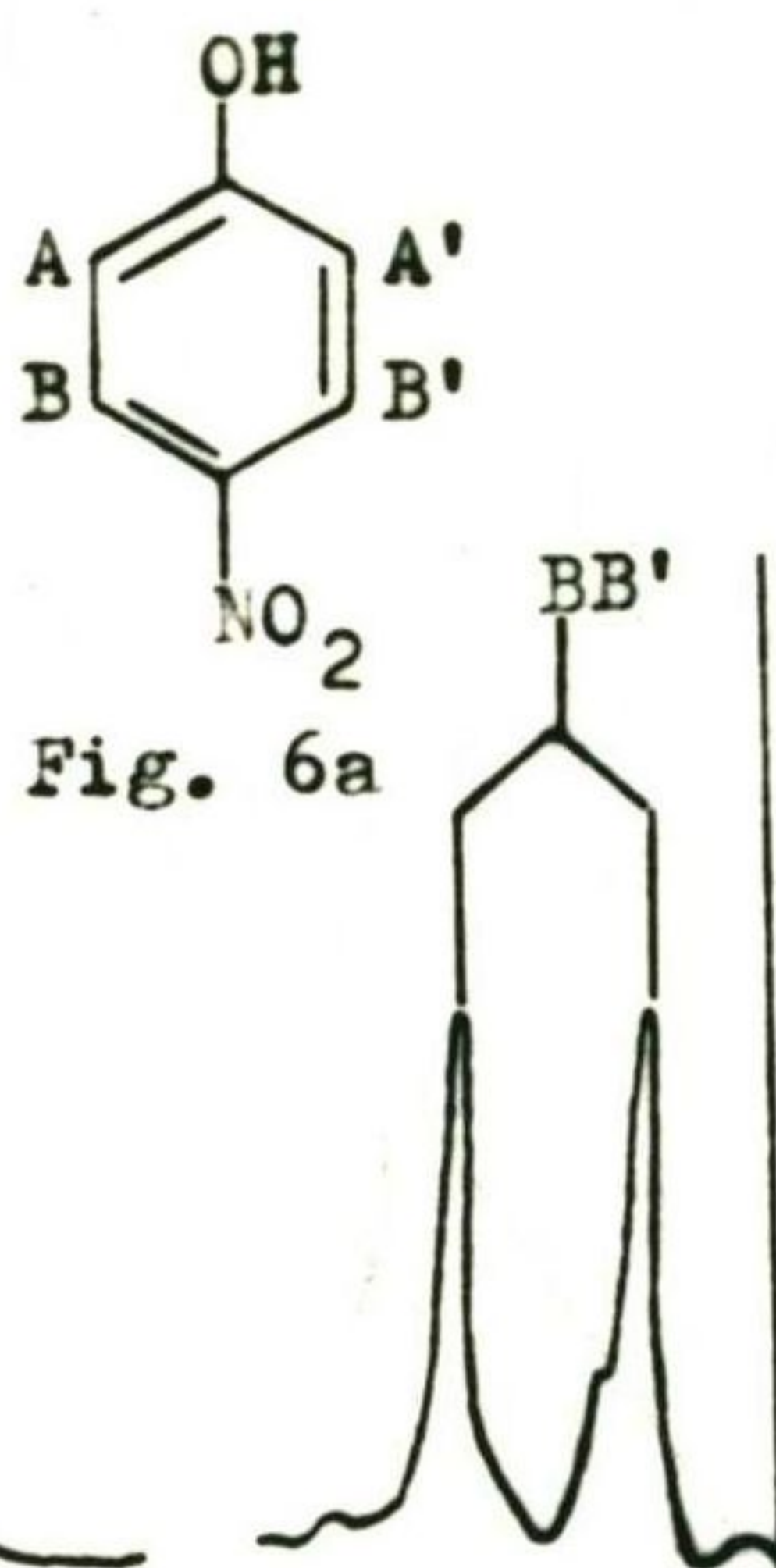
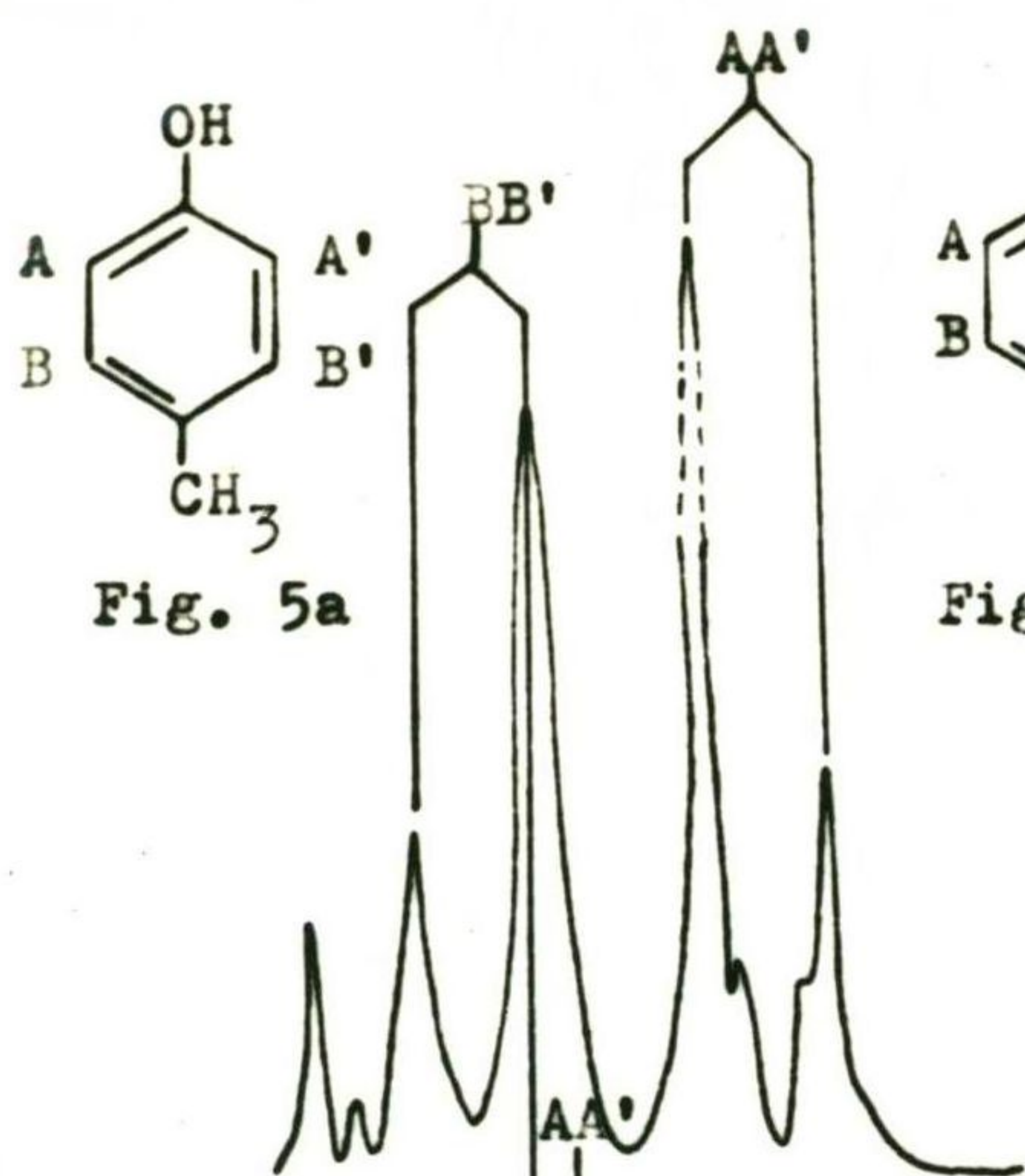


Aromatic proton absorption of
 1a 2,5-xyleneol, b acetate, c methyl ether, 2a 3,5-xyleneol, b acetate
 c methyl ether, as 5% solutions in deuteriochloroform.



Aromatic proton absorption of

3a thymol, b acetate, c methyl ether, 4a 2,6-xylenol, b acetate, c methyl ether, as 5% solutions in deuteriochloroform.



7.5

7.0

8.0

7.5

7.0

PPM ex TMS

Aromatic proton absorption of
 5a p-cresol, b acetate, c methyl ether 6a p-nitrophenol b acetate
 c methyl ether, as 5% solutions in deuterochloroform.

TABLE 2

Chemical shifts^a of aromatic protons in the model phenols and their derivatives.

Compound	<u>H_{ortho}</u>	<u>H_{para}</u>	<u>H_{meta}</u> ^b	<u>J_{ortho}</u>	<u>J_{meta}</u> ^c
2,5-Xylenol	6.58	6.66	7.02	7.5	2
Acetate	6.85(0.27) ^d	6.94(0.28)	7.10(0.08)	7.6	2.3
Methyl ether	6.74(0.16)	6.67(0.01)	7.01	7.4	
3,5-Xylenol	6.45 ^e	6.60			1.7
Acetate	6.73(0.28)	6.87(0.27)			1.9
Methyl ether	6.54-6.60*	6.54-6.60*			
Thymol	6.57	6.68	7.07	7.8	2
Acetate	6.84(0.27)	6.98(0.30)	7.16(0.09)	7.8	2
Methyl ether	6.67(0.10)	6.70(0.02)	7.08(0.01)	8.0	1.5
2,6-Xylenol	6.77		6.98		
Acetate	7.04*(0.27)		7.04*(0.06)		
Methyl ether	6.98*(0.11)		6.98*		
<u>p</u> -Cresol	6.76		7.09	8.0	
Acetate	7.02(0.26)		7.25(0.16)	8.0	
Methyl ether	6.84(0.08)		7.09	8.1	

Compound	<u>H_{ortho}</u>	<u>H_{para}</u>	<u>H_{meta}</u>	<u>J_{ortho}</u>
<u>p</u> -Nitrophenol	6.96	-	8.20	9.0
Acetate	7.25(p.29)	-	8.41(0.21)	9.0
Methyl ether	6.96	-	8.20	8.9

* superimposed on other signals

a in ppm ex TMS as internal reference

b Suffix refers to the relative position of the proton with respect to the phenolic group (or the derivative thereof).

c The coupling constants are in c/s and refer to the protons separated by two and three bonds respectively.

d Figures in parentheses refer to difference in the chemical shift with respect to the original phenol. All differences are towards low field. (paramagnetic).

e Estimated from centre of gravity of an unresolved multiplet; subject to an error of approximately ± 0.02 ppm.

TABLE 3

Chemical shifts^a of benzylic methyl protons in the model phenols and their derivatives

	<u>ortho</u> ^b	<u>meta</u>	<u>para</u>
2,5-Xylenol	2.21	2.25	
Acetate	2.11(+0.10) ^c	2.29(-0.04)	
Methyl ether	2.18(-0.03)	2.32(-0.07)	
3,5-Xylenol		2.27	
Acetate		2.28(-0.01)	
Methyl ether		2.29(-0.02)	
Thymol		2.28	
Acetate		2.29(-0.01)	
Methyl ether		2.31(-0.03)	
2,6-Xylenol	2.22		
Acetate	2.12(+0.10)		
Methyl ether	2.28(+0.06)		
2,4-Xylenol	2.20		2.22
Acetate	2.12(+0.08)		2.29(-0.07)
Methyl ether	2.19(+0.01)		2.25(-0.03)

- a in ppm ex TMS as internal reference
 - b refers to the relative position of the methyl group with respect to the phenolic group (or derivatives thereof).
 - c figures in parenthesis refer to differences in the chemical shift with respect to the original phenol: negative differences a downfield shift and positive differences an upfield shift.
-

DISCUSSION

Table 2 shows that acetylation of phenolic hydroxyl groups results in a paramagnetic shift of 0.28 ± 0.02 ppm for the ring protons in the ortho and para positions and a paramagnetic shift of 0.13 ± 0.05 ppm for the ring protons in the meta position. This suggests that mesomeric effects predominate in this case^{1,2}. A recent examination of the NMR spectra of the acetate and methyl ether of thymol by Cooke and Rae¹¹ fully supports these conclusions.

Methylation of the phenolic hydroxyl groups had little effect on the chemical shifts of the ring protons in the meta and para positions (Table 2) and appeared to cause a small, but apparently consistent, paramagnetic shift of the protons in the ortho positions (0.12 ± 0.04) which is therefore most likely due to inductive or anisotropic differences¹² between the hydroxyl and methoxyl groups. The partially anomalous result obtained for p-nitroanisole suggests the intervention of some other effect in the presence of a strong electron-withdrawing substituent.

Clearly, if the above data can be substantiated by further examples, such correlations could be of general value in the assignment of structures to unknown phenolic compounds.

Brown¹³ has recently examined the effect of salt formation on the NMR spectra of a number of phenols and noted the appearance of consistent effects. These results are considered complementary to those reported here.

Table 3 shows that acetylating a methyl-substituted phenol causes an upfield shift of the methyl proton resonance ($+0.1 \pm 0.02$ ppm) only when the methyl group is ortho to the phenolic group. Methyl groups in other positions were seen to undergo small paramagnetic displacements only.

The diamagnetic displacement, restricted to ortho substituents, could be due to contributions from electric field effects or from magnetic anisotropy differences in the hydroxyl or acetoxyl groups or from a displacement of the methyl group out of the plane of the ring. Intramolecular dispersions effects, which would be expected to cause a downfield shift^{14,15} are not apparent.

EXPERIMENTAL

The spectra were measured with a Varian A60 NMR spectrometer. All measurements of the aromatic proton absorption were made with 2% and 5% solutions in deuteriochloroform with tetramethylsilane as the internal reference. Within experimental error the 2 and 5% solutions gave identical results although the higher concentration was used in Table 2 and 3 and Fig. 1-6 to take advantage of the better signal to noise ratio. Each result reported is the average of at least two determinations. All chemical shifts were derived by direct measurement or by calculation⁸ of the centre of gravity of doublets.

Preparation of Model Compounds

1) 2,5-Xylenol

Reagent grade 2,5-xylenol was recrystallised from ethanol-ether to yield colourless prisms, m.p. 73-74° (lit.¹⁶ m.p. 75°).

Acetate

A mixture of 2,5-xylenol (1 g), pyridine (5 ml) and acetic anhydride (10 ml) was allowed to stand overnight at room temperature. The solution was then decomposed with ice and extracted with chloroform (2 x 75 ml). The extract was washed with 3N hydrochloric acid (2 x 50 ml) and brine

(1 x 100 ml) and after drying was concentrated under reduced pressure. The residual yellow oil was filtered through a short alumina column (5 g) in light petroleum and then distilled, b.p. 235-236°/760 mm (lit.¹⁶ b.p. 237°/760 mm).

Methyl ether

2,5-xyleneol (1 g) was refluxed for 4 hr. with dimethyl sulphate (2 ml) and anhydrous potassium carbonate in acetone (100 ml). The ether was isolated by evaporation of the acetone, dilution with water and extraction with ether. On evaporation, the washed and dried solution afforded a brown oil. This was purified by filtration through a short column of alumina (10 g) in light petroleum and then distilled, b.p. 205°/760 mm (lit.¹⁶ 198°/748 mm).

The derivatives of the other model compounds were prepared and purified as for 2,5-xyleneol. Their physical constants are listed in Table 4.

Table 4Physical Constants of Model Phenols and Derivatives

Compound	Physical Constants
3,5-Xylenol	m.p. 67-68° (lit. ¹⁶ 68°)
Acetate	b.p. 130°/20mm (lit. ¹⁶ 120°/11mm)
Methyl ether	b.p. 190° (lit. ¹⁶ 193°)
Thymol	b.p. 225-230° (lit. ¹⁶ 233-235°)
Acetate	b.p. 237-240° (lit. ¹⁶ 242-244°)
Methyl ether	b.p. 218-220° (lit. ¹⁶ 211°/745mm)
2,6-Xylenol	b.p. 200-202° (lit. ¹⁶ 203°)
Acetate	b.p. 190-194° (lit. ¹⁶ 194°)
Methyl ether	b.p. 180-182° (lit. ¹⁶ 182-183°)
2,4-Xylenol	m.p. 68° (lit. ¹⁶ 68°)
Acetate	b.p. 130-132°/26mm (lit. ¹⁶ 120°/11mm)
Methyl ether	b.p. 189-191° (lit. ¹⁶ 193°)
p-Cresol	b.p. 85-86°/10mm (lit. ¹⁶ 90°/11mm)
Acetate	b.p. 207-208° (lit. ¹⁶ 212-213°)
Methyl ether	b.p. 170-171° (lit. ¹⁶ 175-176°)
p-Nitrophenol	m.p. 110-112° (lit. ¹⁶ 114°)
Acetate	m.p. 80-81° (lit. ¹⁶ 81-82°)
Methyl ether	m.p. 51-52° (lit. ¹⁶ 54°)

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PART III

THE CHEMICAL CONSTITUENTS OF
THE WOOD OF GREVILLEA ROBUSTA

INTRODUCTION

Phenols bearing long unbranched aliphatic side chains form an expanding group of naturally occurring compounds. They have been found in four botanical families: the Anacardiaceae, the Ginkgoaceae and more recently the Proteaceae and the Gramineae. The structures of these phenols have several common features and it has already been noted by Birch and Donovan¹ and Robinson² that they provide a neat illustration of the acetate theory of biogenesis. All have the fundamental structural unit (1) in which the long aliphatic chain is attached meta to the phenolic group and the long chain always contains an odd number of carbon atoms.

A considerable amount of work has been done on the vesicant and irritant extracts of several species of the family Anacardiaceae and a number of alkyl phenols have been identified. Cardol, a resorcinol derivative, and anacardic acid, a salicylic acid derivative, both of which have $\underline{n}\text{-C}_{15}$ side chains, have been found together in cashew nut-shell oil; their chemistry has been reviewed by Dawson³. "Urushiol", the active principle of poison ivy, has been separated into five catechol derivatives (Symes and Dawson⁴) having different degrees of unsaturation in their $\underline{n}\text{-C}_{15}$ side chains. It should

be noted that it has been established that unsaturation in the side chain is essential for the development of vesicant action. Two related compounds, laccol and pellantjuic acid, which are catechol derivatives with $n\text{-C}_{17}$ chains, have been shown⁵ to co-occur in Rhus succeda. Tigaso oil, which is obtained as an exudate from Camptosperma brevipetiolata (Volk.) has been found⁶ to be rich in two related $n\text{-C}_{19}$ compounds: camptospermonol, which is 3-nonadec-10'-en-2'-onyl-phenol, and 5-hydroxy-5-(nonadec-10'-en-2'-only)-cyclohex-2-enone. Substances of this type may be important as intermediates in the biogenesis of plant phenols.

Bilobol has been isolated from Ginkgo biloba (class Gymnospermae) by Furukawa⁷ and identified as 5-pentadec-8'-enyl-resorcinol. A recent investigation of the nonsaponifiable fraction of wheat bran by Wenkert et al.⁸ has shown the presence of a mixture of 5-alkylresorcinols ($n\text{-C}_{17}$ to $n\text{-C}_{23}$). The vesicant principle of the seed pods of Grevillea pyramidalis (family Proteaceae) has been identified⁹ as 5-(10'-penta-decenyl) resorcinol.

Investigation of another member of the Proteaceae, G. robusta (A. Cunn.) has now led to the isolation of a new phenol, grevillol. G. robusta, commonly known as "Silky Oak", is native to northern New South Wales and Southern Queensland, but is now also grown along most of the south-eastern coastal

districts of Australia, either in timber plantations or as an ornamental tree. It is a moderately large tree, reaching under favourable conditions, 100 feet in height, with frond-like leaves and orange flowers (Fig. 7,8). It yields a gummy exudate containing approximately 6% resin whilst its bark has a high tannin content (2.5-6%). This gum has little commercial value¹⁰. Hurst¹¹ has recorded that sap has been reported to cause inflammation in the eyes of a gardener. Extracts of the plant have given negative antibiotic tests and grevillol itself showed little antibiotic activity (see appendix).

In Australia, timber from G. robusta has been used for making furniture and boxes. Another member of the Proteaceae, Oritis excelsa (R.Br.) is frequently sold and used commercially as genuine "Silky Oak". In fact, careful examination by a wood anatomist is needed to distinguish between the woods of the two trees. Because of this similarity, an examination of its constituents was considered worthwhile.





Fig. 7



Fig. 8

ISOLATION OF GREVILLOL

Extraction of the wood of G. robusta with benzene gave a dark gum which on distillation under reduced pressure afforded substantially pure grevillol in the remarkably high yield of 6.4%. Fractionation of another portion of the extract into acidic, phenolic and neutral fractions yielded only one other constituent, sitosterol, which was identified by m.p. mixed m.p. and IR spectral comparison with an authentic specimen. Lamberton⁶, in his investigation of Tigaso oil, emphasised the need for care in dealing with phenolic extracts, as distillation or treatment with alkali may drastically alter the nature of the original substances. To ensure that the isolation procedure had not generated grevillol from a precursor, a second sample of wood was extracted, twice at room temperature, with light petroleum, to remove dark amorphous material, and then with ether. By concentrating the ethereal extract at room temperature and careful precipitation with light petroleum, crude, semi-solid grevillol was obtained and recognised by its IR spectrum. Several repetitions of the processes of dissolution in ether and precipitation by light petroleum gave almost pure grevillol. It was therefore safe to conclude that grevillol was a genuine natural product and that some of it at least, was not an artifact.

An earlier examination of the wood of O. excelsa by Smith¹² had resulted in the isolation of basic aluminium succinate which was evidently responsible for the formation of white deposits on saws during milling of the timber. Extraction of the wood with ether afforded only a small amount of oil of negligible phenol content. The only isolable substance was sitosterol. This striking difference in their extractives may be useful in distinguishing between the two species.

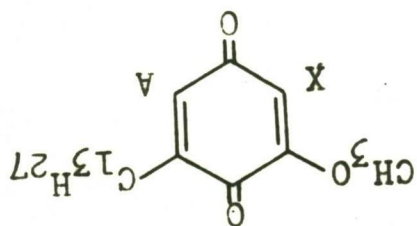
THE STRUCTURE OF GREVILLOL

Grevillol crystallised from light petroleum as woolly aggregates of colourless needles, m.p. $82-83^{\circ}$. It was a weak acid and dissolved in 5% sodium hydroxide. The structure of grevillol has been firmly established as 5-tridecyl-resorcinol (2) and its chemistry will be interpreted in terms of this structure.

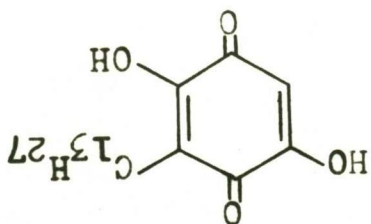
From elemental analysis of grevillol and its derivatives, the molecular formula was established as $C_{19}H_{32}O_2$. Mass spectrometry confirmed the molecular weight as 292, and showed that no higher homologues, at least, were present even in crude grevillol, isolated by a single distillation. Several qualitative tests suggested it was a dihydric phenol. A red colour with Candussio's reagent, and with chloroform and potassium hydroxide (Guareschi's test) confirmed the phenolic nature of the molecule whilst a positive vanillin-hydrochloric acid test indicated it was a meta dihydric phenol¹³. A white precipitate with mercuric nitrate suggested grevillol was an alkylresorcinol¹⁴. No colour was obtained with ferric chloride in aqueous ethanol; a negative ferric chloride test has been recorded⁹ for several other alkylresorcinols.

The IR spectrum of grevillol showed hydroxyl absorption at $\nu_{\max}$. $3200-3330\text{ cm}^{-1}$ and no carbonyl absorption. The

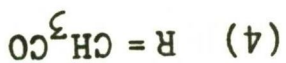
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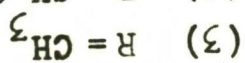
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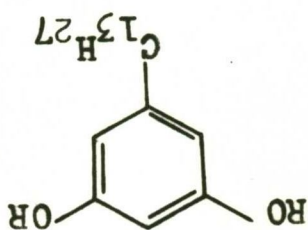
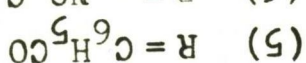
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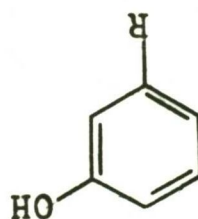
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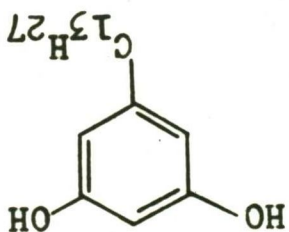
(5)



(1)



(2)



phenolic character of the compound was confirmed by the preparation of a crystalline methyl ether (3), diacetate (4), dibenzoate (5) and di-p-nitrobenzoate (6). The IR spectra of these esters revealed no hydroxyl absorption and a single band at approximately $\nu_{\max}$. 1770 cm^{-1} corresponding to a phenolic ester group. A six-proton singlet at 2.26 ppm in the NMR spectrum of diacetylgrevillol (4) indicated the presence of two equivalent acetoxyl groups.

The UV spectrum of grevillol was almost identical with that of orcinol⁸, suggesting that it also was a resorcinol derivative, and further evidence for this hypothesis was obtained by the observation that the addition of alkali caused the same bathochromic shift in each case, from $\lambda_{\max}$. 274, 281 m μ ($\log \epsilon$ 3.20, 3.19) to $\lambda_{\max}$. 290 m μ ($\log \epsilon$ 3.48).

The substitution pattern in grevillol was clearly demonstrated by IR spectroscopy. Bands in the region $1225\text{--}950 \text{ cm}^{-1}$ are known¹⁵ to be largely independent of the nature of substituents and thus to afford one of the best methods of determining the degree and type of substitution present. Peaks at 1150 and 1060 cm^{-1} (CS_2 solution) in the spectrum of dimethylgrevillol (3) demonstrated the presence of a 1:3:5 aromatic substitution pattern. The IR spectrum of dimethyl-orcinol possessed identical absorption in this region. The absorption patterns in the region $2000\text{--}1650 \text{ cm}^{-1}$ of the spectra of both ethers were also very similar.

In the NMR spectrum of grevillol, the aromatic protons gave rise to a three-proton singlet at 6.28 ppm. The aromatic protons of orcinol also resonated as a singlet at 6.28 ppm, the intensity of which was equal to that of the protons of the benzylic methyl group, absorbing at 2.18 ppm. In the spectra of the derivatives of grevillol, the aromatic protons appeared in each case as a three-proton singlet. In the diacetate (4), the dibenzoate (5) and the di-*p*-nitrobenzoate (6) the signal was shifted downfield to 6.97, 7.01 and 7.08 ppm respectively. The coincidence of the chemical shifts of these protons after esterification was consistent with the 1:3:5 substitution pattern of the nucleus. In the dimethyl ether (3) a three-proton singlet at 6.34 ppm again corresponded to the aromatic protons whilst the methoxyl protons appeared as a six-proton singlet at 3.76 ppm.

The presence of one methyl group in grevillol was shown by a three-proton multiplet centred at 0.82-1.05 ppm confirming that the side chain was unbranched. The benzylic protons gave rise to a two-proton multiplet, centred at 2.6 ppm, whilst the other methylene protons appeared as a twenty two-proton multiplet centred at 1.27 ppm.

Thus grevillol was 5-*n*-tridecylresorcinol.

Although the structure of grevillol could be confidently deduced without degrading the molecule, it was decided to carry out some degradative experiments on it which would serve as models for the degradation of similar but more complex phenols, of unknown structure, which have been isolated in this Department.

THE DEGRADATION OF GREVILLOL

Oxidation

Following the procedure of Backer and Haack⁵ in their work on hydrocardol, grevillol was oxidised with potassium permanganate in acetone and gave myristic acid in 85% yield. This was identified by m.p., and mixed m.p. with an authentic sample of myristic acid and of the S-benzylisothiuronium salts. The IR spectra were also respectively identical. Additional proof of its identity came from vapour phase chromatography (VPC) of the methyl ester of the degradation product. Using methyl palmitate and methyl myristate as reference compounds, the presence of n-C₁₃ chain was established and no evidence found for the presence of material with a longer chain.

In order to confirm the nature of the nucleus of grevillol, several oxidations of dimethylgrevillol (3) were attempted. Although resorcinol ethers substituted by unsaturated side chains have been smoothly oxidised to 3,5-dimethoxybenzoic acid with hot alkaline potassium permanganate^{5,9}, dimethylgrevillol was completely resistant to oxidation under these conditions.

It was then decided to attempt to introduce a carbonyl group in the α position and then degrade the ketone by

oxidation or by a Beckmann rearrangement of its oxime. Bible¹⁶ had reported that a benzylic methylene group is readily oxidised to a carbonyl group, particularly when it is para to a methoxyl substituent. Dimethylgrevillol was therefore treated with a chromic-acetic acid mixture at 50°. Chromatography of the crude product afforded starting material and a bright yellow crystalline product, m.p. 81-83°. The identification of this compound as 6-methoxy-2-tridecyl-p-benzoquinone (7) followed from its analysis, C₂₀H₃₂O₃, and spectral properties. The IR spectrum of (7) showed characteristic carbonyl absorption at $\nu_{\max}$. 1670 and 1645 cm⁻¹ whilst the UV absorption showed typical shifts from $\lambda_{\max}$. 266 m μ (log ϵ 3.87) to $\lambda_{\max}$. 297 m μ (log ϵ 3.75) on adding alkali and then to $\lambda_{\max}$. 274 m μ (log ϵ 3.35) on reacidifying the solution. The NMR spectrum of the quinone (7) possessed a three-proton singlet at 3.81 confirming that the compound had only one methoxyl group. Aromatic protons were absent, but the quinone protons were clearly visible as an AX pattern. The proton H_A appeared as a doublet of triplets (J_{AX} 2.3 c/s) at 6.46 ppm, whilst a doublet at 5.87 ppm (J_{AX} 2.3 c/s) was assigned to the H_X. The value of this coupling constant was consistent with the value found^{17,18} for this type of allylic long-range coupling. The

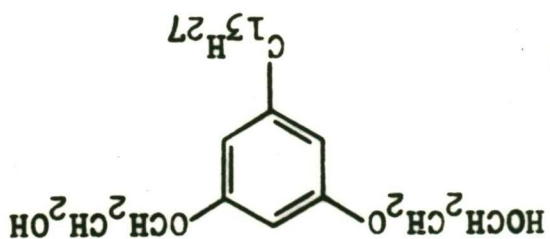
multiplet from the allylic protons appeared upfield at 2.42 ppm and the chemical shifts of the other side chain protons had the same values as in dimethylgrevillol.

This quinone (7) had already been obtained by Asano and Gamaguti¹⁹ in their synthesis of the naturally occurring quinone, rapanone (8). The literature m.p. of this compound, m.p. 81-83° agreed with m.p. obtained for the quinone from grevillol.

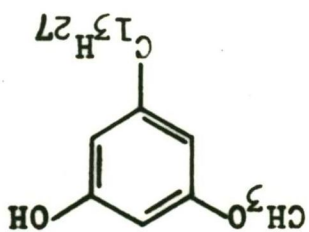
Another possible method for degrading the side chain involved introducing an acetoxyl group on the benzylic carbon atom. Treatment of dimethylgrevillol with lead tetraacetate in hot acetic acid²⁰ gave as the major product (85%) the above quinone (7). A second product which was separated from the quinone by chromatography on silica gel, was a yellow oil. Its UV absorption, $\lambda_{\text{max.}}$ 276 m μ ($\log \epsilon$ 3.90), suggested the presence of another substituent on the benzene ring whilst absorption in the IR at $\nu_{\text{max.}}$ 1765 cm⁻¹ was consistent with a phenolic acetate. Confirmation that acetoxylation had taken place in the benzene ring came from the NMR spectrum. A two-proton multiplet at 2.63 ppm showed that the benzylic hydrogens were still present whilst a multiplet at 6.33 ppm showed the presence of only two aromatic protons. A three-proton singlet at 2.24 ppm

corresponded to the protons of one acetoxyl group and two singlets of three-proton intensity at 3.87 and 3.80 ppm were attributable to the protons of the methoxyl groups. The NMR signals from the side chain protons were identical with those in the spectrum of grevillol. Free radical attack on dimethylgrevillol could lead to (9), or (10) or both. In order to check that the product of acetoxylation was homogeneous, it was converted to the phenol by treatment with lithium aluminium hydride. This gave a crystalline compound, $C_{31}H_{36}O_3$, with a sharp m.p. of 68-69°. It was identified as a phenol by a band at $\nu_{\max}$. 3510 cm^{-1} in the IR spectrum and by the shift of its UV absorption, $\lambda_{\max}$. 287 m μ ($\log \epsilon$ 3.59) to $\lambda_{\max}$. 302 m μ ($\log \epsilon$ 3.67) on the addition of alkali. In the NMR spectrum the three-proton singlet at 2.24 ppm due to the acetoxyl protons was replaced by a singlet at 5.27 ppm which was assigned to a hydroxyl proton. The structure of the phenol was deduced as (11) from a consideration of the chemical shifts of the methoxyl protons. If the hydroxyl substituent had been para to the side chain (12) the molecule would have been symmetrical and the methoxyl protons would have appeared as a six-proton singlet. Since the methoxyl protons absorbed as two three-proton singlets at 3.85 and 3.75 ppm the structure of the phenol was shown to

(13)

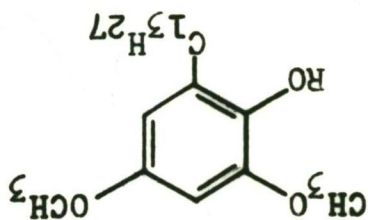


(14)



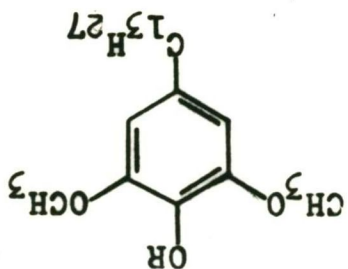
(11) $\text{R} = \text{H}$

(9) $\text{R} = \text{CH}_2\text{CO}$



(12) $\text{R} = \text{H}$

(10) $\text{R} = \text{CH}_2\text{CO}$



be (11) and its related acetate to be (9).

Several reductions were attempted in the hope of finding an efficient method of degrading the molecule so that some at least, of the ring atoms would be preserved. Birch and his collaborators²¹ have shown that many aromatic ring systems can be reduced by a sodium-ammonia-alcohol combination to hydroaromatic compounds. Dimethylgrevillol (3) was found to be completely inert to reduction using this system, presumably due to its insolubility in the reagents. In an attempt to overcome this problem a variety of co-solvents, including dioxan and tetrahydrofuran²², were added to the reaction mixture, but only starting material was isolated in each case. The reductions were repeated using di(β hydroxyethyl) grevillol (13) instead of dimethylgrevillol but no reaction took place. (13) was prepared by heating an alkaline solution of grevillol with ethylenechlorhydrin²³. Modifications of the conditions (Wild and Nelson²⁴) in which sodium is replaced by lithium were also tried unsuccessfully.

Benkeser et al.²⁵ have found that lithium in certain lower aliphatic amines is a potent reducing agent for aromatic ring systems. Recent work by these authors²⁶ has shown that by using only two equivalents of lithium in the

presence of alcohol, the lithium-amine combination can be used quite successfully to form dihydro-aromatics. As the merits of the more commonly used amines, methylamine, ethylamine and propylamine, have not been fully determined yet, all three were tried, in the presence of ethyl alcohol, with dimethylgrevillol. Although the ether (3) was soluble in ethylamine and propylamine no reduction of the aromatic ring took place. Reaction with the last amine system afforded, besides starting material, 5% of a phenol which was identified by its spectral properties as the monomethyl-ether of grevillol (14). Absorption at $\nu_{\text{max.}}$ 3350 cm^{-1} in the IR and a shift in the UV absorption from $\lambda_{\text{max.}}$ 273, 281 $\text{m}\mu$ ($\log \epsilon$ 3.20, 3.19) to $\lambda_{\text{max.}}$ 288 $\text{m}\mu$ ($\log \epsilon$ 3.45) on the addition of alkali were characteristic of a phenolic group. A three-proton singlet at 3.76 ppm showed the presence of only one methoxyl group whilst an envelope between 4.6-4.9 ppm, which disappeared on deuteration, corresponded to the hydroxyl proton. Aromatic absorption had broadened to a three-proton multiplet centred at 6.28 ppm. Signals from the side chain were identical with those in the spectrum of dimethylgrevillol. The structure of the phenol was therefore (14).

THE SYNTHESIS OF GREVILLOL

To confirm the structure of grevillol, it was synthesised. It was proposed to accomplish the synthesis by hydrogenolysis of the corresponding aryl alkyl ketone. The possibility of preparing 3,5-dimethoxyphenyl n-dodecyl ketone (15) by the action of n-dodecylmagnesium bromide on 3,5-dimethoxybenzamide was fully investigated. This method has been successfully used in the preparation of shorter chain homologues ($R=C_3-C_7$) by Suter and Weston²⁷. The amide, however, proved to be too unreactive, much unchanged starting material being present even after a protracted reaction time and only a small amount of the ketone was isolated.

The preparation of the ketone (15) was best effected by treating 3,5-dimethoxybenzoyl chloride with n-dodecylmagnesium bromide in the presence of ferric chloride as catalyst. This method has been investigated by Cason and Kraus²⁸ who have found that at -60° ferric chloride specifically catalyses the reaction of a Grignard reagent with an acid chloride in great preference to other carbonyl groups. In order to obtain the maximum yield of ketone (15) care was taken to keep the concentration of reactants as high as their solubility at -60° allowed and to use only 2 mole% of catalyst, as specified

by these authors. The reaction afforded a mixture of products which were separated by chromatography on silica gel. The main fraction (68%) was identified as the desired ketone (15), 3,5-dimethoxyphenyl n-dodecyl ketone, by its analysis and by the presence in the IR spectrum of a peak at $\nu_{\text{max.}} 1700 \text{ cm}^{-1}$.

The NMR spectrum of the ketone confirmed this assignment of structure. The signals from the ortho aromatic protons appeared as a well-resolved two-proton doublet ($J_{\text{meta}} 2.2 \text{ c/s}$) at 7.09 ppm whilst the para proton gave rise to a one proton triplet ($J 2.2 \text{ c/s}$) at 6.73 ppm. The signals from the two methoxyl groups appeared as a six-proton singlet at 3.83 ppm. The multiplet arising from the methylene protons α to the carbonyl group was centred at 2.82 ppm, due to shielding by the aromatic ring. A narrow, twenty-proton envelope at 1.32 ppm corresponded to the other methylene protons of the side chain, whilst the three-proton multiplet centred at 0.95 ppm was due to the protons of the terminal methyl group.

Hydrogenolysis of the ketone (15) was accomplished by treating it with hydrogen in the presence of palladium charcoal and a trace of sulphuric acid at 5 atmospheres, according to the method of Wenkert et al.⁸. The product of this reaction was identical (m.p., mixed m.p. and IR spectra)

with a sample of dimethylgrevillol (3). As ethers in this series do not always depress in a mixed m.p. determination it was necessary to dealkylate the synthetic ether. This was carried out by heating it with anhydrous pyridine hydrochloride. The resulting phenol was identical in all respects with natural grevillol.

Hydrogenation of 3,5-dimethoxyphenyl n-dodecyl ketone to the corresponding benzylic alcohol was rapidly effected by treatment with hydrogen in the presence of 5% palladium charcoal. Prolonged reaction times and pressures of 5 atmospheres did not result in hydrogenolysis of the hydroxyl group. The alcohol was identified as 1-(3,5-dimethoxyphenyl)-n-tridecanol (16) by the presence of hydroxyl absorption in the IR at $\nu_{\text{max.}} 3500 \text{ cm}^{-1}$ and from the NMR spectrum. The aromatic protons formed a pattern similar to that in the ketone (15); the ortho protons, no longer deshielded by the carbonyl group, appeared as a doublet at 6.51 ppm whilst the para proton gave rise to a triplet at 6.4 ppm. A broad envelope between 4.4-4.7 ppm which sharpened to a triplet on exchange with deuterium oxide was assigned to the benzylic proton and a one-proton envelope at 1.8 ppm, which disappeared on deuteration, corresponded to the hydroxyl proton.

Treatment of the alcohol (16) with p-toluene sulphonyl chloride and chromatography of the product afforded a colourless oil. The IR spectrum possessed absorption at $\nu_{\text{max.}}$ 965 cm^{-1} , characteristic of a disubstituted double bond²⁹, and no hydroxyl absorption. This data suggested that the tosyl group had been eliminated during the work-up to give a substituted styrene (17). Reduction of the olefin with hydrogen and palladium charcoal catalyst gave a crystalline product which was shown to be identical with dimethylgrevillol in the usual way.

EXPERIMENTAL

Isolation of Grevillol

1) Milled wood (200 g) was extracted in a Soxhlet apparatus with benzene. The cooled extract was filtered from a little black tar and concentrated to a dark reddish-brown gum (approx. 15.0 g). Distillation of the gum afforded a slightly reddish-brown oil, b.p. 200-210°/0.35 mm (10.1 g), which solidified completely on cooling.

2) The bulk of the wood (10 kg) was exhaustively extracted in the cold with ether and the concentrated extracts fractionated in the usual way by extraction with aqueous alkaline solutions. 5% Sodium hydrogen carbonate and 5% sodium carbonate removed only traces of dark material which were discarded. Acidification and recycling of the 5% sodium hydroxide-soluble material furnished a reddish gum (750 g) which was distilled as above to give crude grevillol as a waxy solid (640 g).

The neutral fraction (18.7 g) was chromatographed on alumina in benzene. The fraction eluted with benzene-ethyl acetate (4:1) yielded on rechromatography sitosterol (1.2 g), m.p. 137-138°. This was identified by mixed m.p., and its IR spectrum which was identical with that of an authentic specimen. Other fractions from the chromatography proved to

be intractable.

3) To show that distillation had not changed a precursor to grevillol, wood (500 g) was extracted at room temperature twice with light petroleum and then several times with ether. The light petroleum extract contained only dark amorphous material. The ethereal extracts were concentrated at room temperature and treated carefully with light petroleum. Several crops of a reddish-brown sticky solid were obtained. Several reprecipitations of these fractions from ether with light petroleum eventually gave a series of solids each of which had an IR spectrum essentially identical with that of pure grevillol.

Grevillol (2)

The distilled material was recrystallised three times from light petroleum to yield clusters of small, colourless needles, m.p. $82-83^{\circ}$, unchanged by recrystallisation from benzene. [Found: C, 77.9; H, 11.3%, M.Wt., 292 (mass spectrometry). Calc. for $C_{19}H_{32}O_2$: C, 78.0; H, 11.0%, M.Wt. 292]. Light absorption: $\nu_{\max}$. 3200-3330 (hydroxyl), 1600 cm^{-1} (aromatic); $\lambda_{\max}$. 274, 281 $m\mu$ ($\log \epsilon$ 3.2, 3.19) (neutral and acidic ethanol); $\lambda_{\max}$. 290 $m\mu$ ($\log \epsilon$ 3.48) (alkaline ethanol); NMR spectrum: s (b), 3, 6.28, (Ar-H); s, 2, 5.22 (OH) (disappearing on D_2O exchange); m, 2, 2.6, (Ar-CH₂); m, 22,

1.27, ($-\text{CH}_2-$); m, 3, 0.9, ($\text{C}-\text{CH}_3$).

The results of several colour tests have been described above.

Extraction of *O. excelsa*

Preliminary tests showed that benzene, ether and acetone extracted only traces of material.

The milled wood (500 g) was exhaustively extracted with methanol at room temperature. The extract was concentrated and then shaken with ether and water. The ether layer was divided into acidic, phenolic and neutral fractions in the usual way. The first two fractions yielded only traces of intractable tars and thin layer chromatography showed no evidence of the presence of grevillol or similar compound. The neutral fraction was dissolved in benzene and chromatographed on alumina. Elution with benzene-ethyl acetate (4:1) afforded sitosterol (0.3 g), m.p. $137-138^\circ$, identified by mixed m.p. and IR spectral comparison with an authentic specimen.

Dimethylgrevillol (3)

Grevillol (2.1 g) and dimethyl sulphate (10 g) in methanol (10 ml) were added slowly to a saturated solution of potassium hydroxide (4.5 g) in methanol at 0° . The reaction mixture was refluxed for 1 hr, then a further 5 g

dimethyl sulphate and potassium hydroxide (2.5 g) added and the refluxing continued for 2 hr. The methanol was removed by distillation, 100 ml of water was added and the aqueous suspension extracted with ether. The dried solution was evaporated and the residue distilled under reduced pressure to yield a pale yellow liquid, b.p. 175-178°/0.02 mm. The crude ether in light petroleum was filtered through an alumina column and the resulting solid recrystallised from light petroleum at 0° to give colourless plates, m.p. 40-41° [Found: C, 79.0; H, 11.3%. Calc. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3%]. Light absorption; $\nu_{\max}$. 1250 cm^{-1} (aryl ether), no hydroxyl absorption: $\lambda_{\max}$. 272, 279 m μ (log ϵ 3.21, 3.22); NMR spectrum: s, 3, 6.34, (Ar-H); s, 6, 3.76, (Ar-OCH₃); m, 2, 2.5, (Ar-CH₂-); s, 22, 1.25, (-CH₂-); m, 3, 0.89, (-C-CH₃).

Diacetylgrevillol (4)

Grevillol (1 g) was refluxed for 5 min. with acetic anhydride (5 ml) and pyridine (10 ml). The solution was diluted with water, extracted with chloroform and the organic phase washed in turn with 2N hydrochloric acid (3 x 20 ml), water, dilute sodium hydrogen carbonate and water. The extract was dried and evaporated. The residue, diacetylgrevillol (4), crystallised from ethanol as colour-

less needles, m.p. 48° . [Found: C, 73.4; H, 9.9%. Calc. for $C_{23}H_{36}O_4$: C, 73.4; H, 9.9%]. Light absorption: $\nu_{\max}$. 1768 cm^{-1} (phenolic acetate), no hydroxyl absorption; $\lambda_{\max}$. $259\text{ m}\mu$ ($\log \epsilon$ 3.38); NMR spectrum: m, 3, 6.97, (Ar-H); m, 2, 2.4-2.8, (Ar-CH₂-); s, 6, 2.26, (Ar-O-COCH₃); s, 22, 1.27, (-CH₂-); m, 3, 0.91 (-C-CH₃).

Dibenzoylgrevillol (5)

Grevillol (1 g) was shaken with a mixture of 5% sodium hydroxide (40 ml) and redistilled benzoyl chloride (4 ml). Sodium hydroxide (50 ml) was added to destroy excess reagent and the reaction mixture then extracted with ether. Evaporation of the washed and dried extract gave a gum which crystallised from light petroleum (b.p. $30-40^{\circ}$) at 0° as colourless rosettes, m.p. $42-43^{\circ}$. [Found: C, 79.4; H, 8.3%. Calc. for $C_{33}H_{40}O_4$: C, 79.2; H, 8.1%]. Light absorption: $\nu_{\max}$. 1730 cm^{-1} (phenolic benzoate), no hydroxyl absorption; $\lambda_{\max}$. $233\text{ m}\mu$ ($\log \epsilon$ 4.45); NMR spectrum: m, 4, 8.17, (ortho benzoate protons); m, 6, 7.56, (benzoate protons); s, 3, 7.01, (Ar-H); m, 2, 2.68, (Ar-CH₂-); s, 22, 1.27, (-CH₂-); m, 3, 0.91, (C-CH₃).

Di-(p-nitrobenzoyl)grevillol (6)

Grevillol (0.5 g) was heated on a steam bath with p-nitrobenzoyl chloride (2.6 g) and pyridine (5 ml) for 1 hr.

The red reaction mixture was diluted with water (20 ml) and allowed to stand for 1 hr. to decompose excess reagent. The ester was extracted with chloroform (100 ml) and the organic layer washed with 3N hydrochloric acid, saturated sodium carbonate and brine. Evaporation of the dried extract yielded an orange gum (0.7 g) which was filtered through an alumina column in benzene. Concentration of the eluate and recrystallisation of the resulting solid from ether-ethanol yielded cream needles, m.p. 80-81°. [Found: C, 67.2; H, 6.6; N, 4.7%. Calc. for $C_{33}H_{38}O_8N_2$: C, 67.1; H, 6.5; N, 4.7%]. Light absorption: $\nu_{\max}$. 1750 cm^{-1} ($CHCl_3$) (phenolic benzoate), no hydroxyl absorption; $\lambda_{\max}$. 260 $m\mu$ ($\log \epsilon$ 3.46); NMR spectrum: s, 8, 8.33, (aromatic p-nitrobenzoate protons); s, 3, 7.08 (Ar-H); m, 2, 2.68, (Ar-CH₂-); s, 22, 1.26, (-CH₂-); m, 3, 0.9, (C-CH₃).

Oxidation of Grevillol

Grevillol (2 g) was dissolved in purified acetone (150 ml) and to the solution was added slowly with stirring potassium permanganate (6 g) in acetone (400 ml). At first the permanganate was decolourised in the cold, but later the solution was warmed to accelerate the reaction. Excess permanganate was decomposed with a little alcohol and the mixture was filtered. The precipitate was suspended in

water (200 ml) and decomposed with sodium bisulphite and sulphuric acid. The mixture was extracted with ether (3 x 50 ml), the extract washed with brine, dried and evaporated to give a brown, crystalline solid. Recycling the crude product by extraction with 5% sodium carbonate and reacidification failed to decolourise it. The acid was therefore chromatographed on silica gel. The fraction (85% yield) eluted with light petroleum-ether (20:1) crystallised from pentane as colourless plates, 51-53°, identified as myristic acid by a mixed m.p. with an authentic specimen and comparison of IR spectra. The S-benzylisothiouronium salt, m.p. 139-140°, prepared in the usual manner was also identical (mixed m.p. and IR spectrum) with an authentic specimen.

VPC of the Ester of Oxidation Product

Samples of myristic, palmitic and the acid from the foregoing oxidation were converted to their respective methyl esters by treatment with excess ethereal diazomethane.

VPC was carried out using a Perkin Elmer Model 154 Vapour Refractometer; the chromatograms were obtained using a metre column of silicone on celite and with helium as the carrier gas. With the column maintained at 206-208° and evacuated to 8 mm Hg methyl myristate and methyl palmitate

had retention times of 1 min. 10 sec. and 2 min. 54 sec. respectively. The unknown ester had a retention time of 1 min. 10 sec. and a mixed sample with methyl myristate, one peak was obtained after 1 min. 9 sec.

Oxidation of Dimethylgrevillol

- 1) Dimethylgrevillol (1.7 g) was stirred with water (6 ml) and pyridine (2 ml) at 40-45°. Powdered potassium permanganate (6.0 g) was added over a period of 1 hr. and the solution then refluxed for 3 hr. Only starting material (1.65 g) was obtained on working up the reaction mixture.
- 2) A solution of dimethylgrevillol (1.01 g) in acetic acid (25 ml) was cooled and treated with a solution of anhydrous chromic acid (1.23 g) in water (0.2 ml) and acetic acid (4 ml). The mixture was kept at room temperature overnight, and then heated at 50° for 3 hr. After dilution with water, the mixture was shaken with ether. The organic phase was extracted with saturated sodium hydrogen carbonate (2 x 50 ml), washed twice with brine, dried and evaporated under reduced pressure. The residual oil was chromatographed on a column of silica gel (40 g), prepared in light petroleum. Elution with light petroleum-ether (5:1) afforded a bright yellow solid in 18% yield. Recrystallisation of this from aceto-

nitrile afforded 6-methoxy-2-tridecyl-p-benzoquinone (7) as lemon yellow needles, m.p. 82-83° (lit.¹⁹ 82-83.5). [Found: C, 75.3; H, 9.9; O, 14.9; OCH₃, 9.2%. Calc. for C₂₀H₃₂O₃: C, 75.0; H, 10.0; O, 14.9; OCH₃, 9.3%]. Light absorption: $\nu_{\max}$. 1670, 1650 (quinone carbonyl), 1590 cm⁻¹ (double bond); $\lambda_{\max}$. 266 m μ (log ϵ 3.87) (neutral ethanol), $\lambda_{\max}$. 297 m μ (log ϵ 3.75) (alkaline ethanol); $\lambda_{\max}$. 274 m μ (log ϵ 3.35) (reacidified ethanol); NMR spectrum: d of t, 1, 6.46, (H_A), 2.3; d, 1, 5.87, (H_X), 2.3; s, 3, 3.81, (C=C-OCH₃); m, 2, 2.42, (C=C-CH₂-); s, 22, 1.24 (-CH₂-); m, 3, 0.90, (C-CH₃).

Acetoxylation of Dimethylgrevillol

A suspension of lead tetra-acetate (5.0 g) in a solution of dimethylgrevillol (3.3 g) in glacial acetic acid (7.5 ml) was stirred at 80° for 16 hr. The colourless solution became bright yellow and finally dark brown. Excess oxidizing agent was decomposed with glycerol and most of the solvent removed under reduced pressure. The oil obtained on dilution with water was extracted with ether (3 x 40 ml) and the extract washed well with water, 3N sodium carbonate (3 x 50 ml) and brine. The dried extract (anhydrous potassium carbonate) was evaporated to give a brown oil, which was dissolved in light petroleum and chromatographed on silica gel (100 g). The fraction eluted with light petroleum-ether (25:1), an orange oil (0.3 g) was identified as 5-(tridecyl)-6-acetoxyresorcinol.

dimethyl ether (9). Light absorption: $\nu_{\max}$. 1765 cm^{-1} (phenolic acetate); $\lambda_{\max}$. $276\text{ m}\mu$ ($\log \epsilon$ 3.90); NMR spectrum: m, 2, 6.28, (Ar-H); s, 3, 3.8, 3.7, (Ar-OCH₃); m, 2, (Ar-CH₂-); s, 3, 2.24, (ArOCOCH₃); s, 22, 1.27, (-CH₂-); m, 3, 0.91, (C-CH₃).

The yellow crystalline solid eluted with light petroleum-ether (5:1) was identified as the quinone (2.1 g) by m.p., mixed m.p., and comparison of IR spectra.

Lithium Aluminium Hydride Reduction of (9)

To a solution of the foregoing acetate (0.2 g) in ether (50 ml) was added a slurry of lithium aluminium hydride in ether. The reaction mixture was stirred for 1 hr. at room temperature, diluted with water and acidified with 3N sulphuric acid. The crude product was extracted into ether and the extract washed with brine, dried and evaporated under reduced pressure. The residue (0.17 g) was chromatographed on alumina and the fraction eluted with ether crystallised from pentane (b.p. $30-40^{\circ}$). 6-Hydroxydimethylgrevillol formed colourless needles, m.p. $68-69^{\circ}$, [Found: C, 75.0; H, 10.8%. Calc. for $\text{C}_{21}\text{H}_{36}\text{O}_3$: C, 75.2; H, 11.0%]. Light absorption: $\nu_{\max}$. 3510 cm^{-1} (hydroxyl); $\lambda_{\max}$. $287\text{ m}\mu$ ($\log \epsilon$ 3.59) (neutral and acidic ethanol); $\lambda_{\max}$. $302\text{ m}\mu$ ($\log \epsilon$ 3.67) (alkaline ethanol); NMR spectrum: m, 2, 6.38, (Ar-H); s, 1, 5.27 (OH) (disappearing

on D_2O exchange); s, 3, 3.85, 3.75, ($Ar-OCH_3$); m, 2, 2.6, ($Ar-CH_2-$); s, 22, 1.25, ($-CH_2-$); m, 3, 0.93, ($C-CH_3$).

Attempted Reductions of Dimethylgrevillol

- 1) Dimethylgrevillol (2 g) in ether (5 ml) and absolute ethanol (3 ml) was added to liquid ammonia (250 ml) and reduced with sodium (1.2 g). A heavy white precipitate was present throughout the addition. The reaction mixture was worked up by adding crushed ice as rapidly as was safe, and removing the ammonia. Acidification and extraction with ether gave starting material as the only isolable product.
- 2) The above procedure was repeated using tetrahydrofuran (5 ml) and then digol (5 ml) in place of ether. Starting material was recovered again.
- 3) To a solution of dimethylgrevillol (1.7 g) in digol (5 ml) was added liquid ammonia with stirring and to the mixture was added lithium wire over 10 min. After stirring for a further 10 min. absolute alcohol (3 ml) was added dropwise over a period of 20 min. Working up the reaction mixture in the usual way gave only unchanged starting material.
- 4) Di-(β hydroxyethyl)grevillol was prepared by heating a solution of grevillol (8 g) in aqueous sodium hydroxide solution (400 ml; 5%) with ethylene chlorohydrin (16 ml) at

80° for 1 hr. The cold solution was extracted with chloroform, (3 x 20 ml) the extract dried (potassium carbonate) and the solvent removed under reduced pressure.

The crude ether was treated as in (1), (2) and (3) but no reduction products could be isolated.

5) Lithium metal (0.042 g) was added to a solution of dimethylgrevillol (0.58 g) in propylamine (20 ml) and ethanol (0.3 ml). Stirring was continued for 10 hr. after the addition was complete. Working up the reaction mixture in the usual way gave an amorphous solid. Chromatography on silica gel in light petroleum afforded starting material (eluted with light petroleum) and a phenol (eluted with light petroleum-ether (5:1)). Crystallisation from light petroleum gave colourless prisms, (0.03 g), m.p. 62-63° of monomethylgrevillol (14). Light absorption: $\nu_{\max}$. 3350 cm^{-1} (hydroxyl); $\lambda_{\max}$. 273, 281 μ ($\log \epsilon$ 3.20, 3.19) (neutral and reacidified ethanol); $\lambda_{\max}$. 288 μ ($\log \epsilon$ 3.49); NMR spectrum: as for (3) except for one singlet at 3.76 ppm (Ar-OCH_3) and a multiplet at 4.6-4.9 ppm (OH).

3,5-Dimethoxybenzoic Acid

Dimethyl sulphate (13.4 g; 0.1M) was added to a solution of 3,5-dihydroxybenzoic acid (5 g) in 20% sodium hydroxide (30 ml). On warming, a vigorous reaction ensued. When this

subsided a further 3.5 g of sodium hydroxide was added and the solution refluxed for 1 hr. The reaction mixture was acidified and extracted with ether (2 x 50 ml). The crude acid was recycled and finally recrystallised from water to yield colourless prisms, m.p. 185-186° (lit.⁸ 185-186°) (2.4 g; 42%).

3,5-Dimethoxybenzamide

A mixture of 3,5-dimethoxybenzoic acid (1.82 g) and redistilled thionyl chloride (10 ml) was refluxed for 30 min. Excess reagent was removed by distillation with benzene (2 x 50 ml). The residue was dissolved in ether and treated with a stream of ammonia at 0°. The precipitated amide was filtered and washed well with ether and ice water (50 ml) to remove ammonium chloride. The product crystallised from water as colourless needles, m.p. 145-146 (lit.²⁸ 147°).

3,5-Dimethoxyphenyl n-Dodecyl Ketone (15)

A solution of dodecyl bromide (24.9 g) in dry ether (150 ml) was added over 1 hr. to dry magnesium turnings. A crystal of iodine was added to initiate the reaction and the mixture was heated under gentle reflux with rapid stirring throughout the addition. Refluxing was continued for a further 3 hr. to complete the formation of the Grignard

reagent.

(1) To a solution of n-dodecylmagnesium bromide in tetrahydrofuran (150 ml) was added, with stirring 4.6 g of 3,5-dimethoxybenzamide. The reaction mixture was refluxed for 50 hr. under nitrogen, and then decomposed with ice and 5% sulphuric acid. The resulting material (4.5 g) was identified as starting material.

(2) 3,5-Dimethoxybenzoyl chloride (from 1.82 g of acid) was dissolved in a mixture of dry ether (3.3 ml) and dry toluene (1.7 ml) in a 100 ml, two-necked flask equipped with a sealed stirrer, a dropping funnel and a condenser which was attached to a nitrogen line. A nitrogen atmosphere was maintained throughout the procedure. The solution was cooled to -60° and to it added anhydrous ferric chloride (0.33 g) dissolved in toluene (1 ml) and ether (2 ml). Then there was added dropwise, with stirring, an ethereal solution (10 ml) of n-dodecyl magnesium bromide (prepared from 3.36 g of n-dodecyl bromide) over 1 hr. The mixture was stirred for 30 min. after completion of the addition. The contents of the flask were then decomposed with ice and acidified to Congo red. The aqueous phase was separated and extracted thoroughly with ether (3 x 15 ml). The original organic phase and the extracts were washed with water, brine and

then dried and evaporated under reduced pressure to give an amber-coloured oil (3.9 g). This was chromatographed on alumina (20 g) and the fraction eluted with light petroleum-benzene (10:1) was crystallised from methanol to yield 3,5-dimethoxyphenyl dodecyl ketone (1.7 g), m.p. 52-53°, as colourless plates. [Found: C, 75.8; H, 10.5%. Calc. for $C_{21}H_{34}O_3$: C, 75.4; H, 10.3%]. Light absorption: $\nu_{\max}$. 1700 cm^{-1} (ketone), no hydroxyl peak; $\lambda_{\max}$. 262, 316 $\text{m}\mu$ ($\log \epsilon$ 3.80, 3.37); NMR spectrum: d, 2, 7.09, (ortho Ar-H), 2.2; t, 1, 6.73, (para Ar-H), 2.2; s, 6, 3.83, (ArOCH₃); m, 2, 2.82, (ArCOCH₂-); s, 20, 1.32, (-CH₂-); m, 3, 0.95, (C-CH₃).

Hydrogenolysis of (15)

The ketone (15) (0.2 g) was dissolved in warm ethyl acetate (100 ml) containing two drops of concentrated sulphuric acid and 20% palladium-charcoal (0.1 g). The mixture was shaken under hydrogen at 5 atmospheres for 5 hr. The mixture was filtered and the filtrate reduced to 10 ml., diluted with ether and washed with water. The dried organic layer gave a colourless solid which was identical (m.p., mixed m.p. and IR spectra) with a sample of dimethylgrevillol (3).

Demethylation of Synthetic Dimethylgrevillol

Dry hydrogen chloride was passed into a stirred solution

of anhydrous pyridine (20 g) in absolute ether (100 ml). After 3 hr. the solvent was removed under vacuum at room temperature, leaving colourless crystals of pyridine hydrochloride.

The ether (0.15 g) was heated with pyridine hydrochloride (3 g) in an atmosphere of nitrogen (bath temperature of 265°) for 8 hr. After cooling, the residue was shaken with a mixture of 2N aqueous sodium hydroxide solution (100 ml) and ether (250 ml). The red, basic aqueous solution was then thoroughly re-extracted with ether (3 x 250 ml) and the combined ether extracts washed with 2N hydrochloric acid (2 x 75 ml) and then with brine. The solvent was removed from the dried solution leaving a brown solid (0.075 g) which crystallised from light petroleum as colourless needles, identified as grevillol (2), by m.p., mixed m.p. and IR spectral comparison.

Hydrogenation of 3,5-Dimethoxyphenyl Dodecyl Ketone

The ketone (15) (0.2 g) and 10% palladium-charcoal catalyst (0.02 g) were shaken in ethanol under hydrogen for 1 hr. An oily residue was obtained on working up the reaction mixture in the normal way. Chromatography on alumina (10 g) in light petroleum afforded a crystalline solid on elution with ether-benzene (10:1). The product,

1-(3,5-dimethoxyphenyl)-n-tridecanol (16), crystallised from pentane at 0° as colourless needles, m.p. 53-54°.

[Found: C, 74.7; H, 10.5%. Calc. for $C_{21}H_{36}O_3$: C, 74.9; H, 10.8%]. Light absorption: $\nu_{\max}$. 3500 cm^{-1} (hydroxyl), no carbonyl absorption; $\lambda_{\max}$. 273, 279 $\text{m}\mu$ ($\log \epsilon$ 3.26, 3.27) (neutral and alkaline ethanol); NMR spectrum: d, 2, 6.51, (ortho Ar-H), 2.1; t, 1, 6.40, (para Ar-H), 2.1; m, 1, 4.4-4.7, (Ar-CH₂OH-); s, 6, 3.81, (Ar-OCH₃); m, 1, 1.8 (OH) (disappearing on D₂O exchange); s(b), 20, 1.3 (-CH₂-); m, 3, 0.93, (C-CH₃).

Conversion of (16) to Dimethylgrevillol

p-Toluene sulphonyl chloride (1 g) was added to a solution of the foregoing alcohol (0.1 g) and pyridine (3 ml). The reaction mixture was kept overnight at 0° and then worked up by dilution with water and extraction with chloroform. The extract was washed with 3N hydrochloric acid and brine and then dried and evaporated to give a mobile oil. Chromatography on alumina in light petroleum and elution with light petroleum-ether (100:1) gave crude 5-(tridec-1-enyl)dimethylresorcinol (17) (0.09 g) as a gum. Light absorption: $\nu_{\max}$. 965 cm^{-1} (disubstituted double bond); no hydroxyl absorption; $\lambda_{\max}$. 273, 280 $\text{m}\mu$ ($\log \epsilon$ 3.18, 3.15).

A solution of (17) in ethyl acetate (30 ml) and 10% palladium charcoal catalyst (0.01 g) absorbed 7.0 ml of hydrogen at S.T.P., equivalent to approximately one double bond per molecule. Filtration, and evaporation of the solvent afforded a crystalline solid, identified as dimethylgrevillol by m.p., mixed m.p. and IR spectral comparison.

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APPENDIX

BIOLOGICAL ACTIVITY OF GREVILLOL

The antibiotic activity of grevillol was investigated by assessing the inhibition of the growth rate of a representative series of fungi and bacteria. Grevillol was included in Czapek-Dox agar at a concentration of 100 µg/ml. The plates were inoculated with the fungi and bacteria listed below, and then incubated at 25°C until such time as the organism had developed far enough to detect any antibiotic effects of grevillol.

The data in this appendix was kindly supplied by
Mr. G. Evans, Faculty of Agriculture, University of Sydney.

Test Organism

Grevillol 100 µg/ml
Growth rate as % of control

1. BACTERIA

<u>Bacillus subtilus</u> (G)	80
<u>Streptococcus</u> (G)	100
<u>Staphylococcus citreus</u> (G)	50
<u>Pseudomonas aeriginosa</u> (G)	100
<u>Escherichia coli</u> (G)	50
<u>Corynebactium sp.</u> (G)	

2. FUNGI

<u>Alternaria solani</u>	100
<u>Aspergillus candidus</u>	100
<u>Aspergillus niger</u>	100
<u>Aspergillus repens</u>	100
<u>Botrytis allii</u>	100
<u>Cunninghamella elegans</u>	100
<u>Curvularia ramosa</u>	90
<u>Gloeosporium lunatum</u>	20
<u>Glomerella sp.</u>	100
<u>Fusarium culmorum</u>	100
<u>Helminthosporium sativum</u>	100
<u>Muco mucedo</u>	100
<u>Mycosptaerella sp.</u>	100

Test Organism

Grevillol 100 μ g/ml
Growth rate as % of control

<u>Neurospora crassa</u>	50
<u>Paecilomyces varioti</u>	100
<u>Penicillium claviforme</u>	100
<u>Penicillium gladioli</u>	100
<u>Penicillium notatum</u>	100
<u>Sclerotinia libitiana</u>	0
<u>Scopulariopsis brevicaulis</u>	100
<u>Sclerotinia aestivalis</u>	20
<u>Trichoderma viride</u>	100

SUMMARY

Part I of this thesis is concerned with the isolation and identification of the constituents of Melicope broadbentiana (Bail.). The wood and bark were found to contain two known flavones, meliternatin and melisimplexin, and a new flavone melibentin. Melibentin was shown to be 3,4'-methylenedioxy-3,5,6,7,8-pentamethoxyflavone. The leaves contained two known flavones, melisimplin and meliternatin, tricontanol and two related phenolic compounds melicopin and methylmelicopin. Melicopin, $C_{19}H_{26}O_6$, was shown to contain two phenolic hydroxyl groups, a benzyl alcoholic group, an aromatic methoxycarbonyl group and an O-geranyl side chain. Methylmelicopin was a monomethyl derivative of melicopin. The inter-relationship of these functional groups was deduced from chemical and spectroscopic evidence. The skeleton of the molecule followed from the degradation of methylmelicopin to dimethyl 2,4,6-trimethoxyisophthalate. The structure of melicopin was thus shown to be methyl 4,6-dihydroxy-2-geranyloxy-3-hydroxymethylbenzoate and methylmelicopin to be the 4-methyl ether.

Part II contains an NMR study of some phenols and their acetyl and methoxyl derivatives. The information obtained contributed to the elucidation of the structure of melicopin.

Part III deals with the structural determination of the phenolic compound, grevillol, the major constituent of the wood of Grevillea robusta (A. Cunn.). Elementary analysis and spectral evidence allowed the assignment of its structure as 5-n-tridecylresorcinol. Confirmation of this assignment was obtained by its synthesis. A series of degradation experiments were also carried out on grevillol as models for work on more complex phenols which have been isolated in the Department.