NOVEL COMPONENTS OF ACACIA MEARNSII

by

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CONTENTS.

SUMMARY.

From the complex mixture of flavonoid components present in the heartwood of the black wattle tree (Acacia mearnsii) four new compounds have been isolated. These include a novel dioxane-linked dimeric proanthocyanidin based on leucofisetinidin. This is the first proanthocyanidin of its type to be isolated from a natural source. Isolation was achieved by standard countercurrent partition separation and preparative paper chromatographic techniques. The compound, which forms an anthocyanidin, was identified by micro-degradation, and n.m.r. and mass spectrometry. Comparison of the spin-spin coupling constants of the heterocylic rings' protons with those of authentic monomeric flavan-3,4-diols showed that the heterocyclic rings of the two symmetrical flavan moeities have 2,3-trans-3,4-cis configurations. Dreiding models suggest that the central dioxane ring has a half-boat conformation while the heterocyclic rings of the two flavan units have five point coplanar conformations. Two possible absolute configurations have been proposed.

A diastereoisomeric form of mollisacacidin, viz, 2S:3S:4S-(+)-7,3',4'-trihydroxy-2,3-cis-flavan-3,4-cis-diol, has also been isolated. The absolute configuration was established by its identity with the corresponding synthetic compound of known configuration. The presence of this compound in the heartwood follows the anticipated concurrence of diastereoisomers within the same plant source.

Re-examination of the water-insoluble components of the heartwood extract revealed the presence of novel blue-fluorescent compounds which could be separated from fisetin

and butein by developing the preparative paper chromatograms of the enriched heartwood extract in 25% acetic acid. One of these new components is 3-methoxyfisetin(7,3',4'-trihydroxy-3-methoxyflavone). Ultraviolet spectroscopy and mass spectrometry confirm the location of the methoxy group at C3. No other methoxylated flavonoids, apart from mearnsitrin, have previously been found in wattle. The other blue-fluorescent compound is a xanthone, tentatively described as 3,4,6-trihydroxyxanthone. A detailed analysis of the n.m.r. spectrum of the crystalline triacetate of the xanthone gives the phenolic hydroxy substitution pattern.

REVIEW OF THE CHEMISTRY OF THE FLAVONOID COMPONENTS OF ACACIA MEARNSII.

The heartwood extract of the commercially afforested black-wattle tree (Acacia mearnsii) is composed of a mixture of flavonoid compounds which are either analogues or polymers of the predominant component, (+) - mollisacacidin. The bark extract, on the other hand, consists mainly of high molecular weight tannins accompanied by closely related low molecular weight phenolic components based on four different patterns of hydroxy substitution.

A. Heartwood Components.

The following flavonoid components have been isolated from the heartwood of Acacia mearnsii:

(+)-mollisacacidin (+)-7,3',4'-trihydroxyflavan-3,4-diol

(+)-fustin (+)-7,3',4'-trihydroxyflavan-3-ol-4-one

fisetin 3,7,3',4'-tetrahydroxyflavone

(-)-butin (-)-7,3',4'-trihydroxyflavanone

butein 2,4-dihydroxyphenyl-3,4-dihydroxystyryl ketone

(-)- fisetinidol (-)7,3',4'-trihydroxyflavan-3-ol

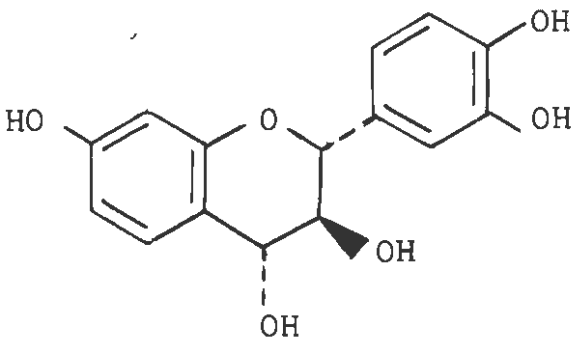
bileucofisetinidins

trileucofisetinidins

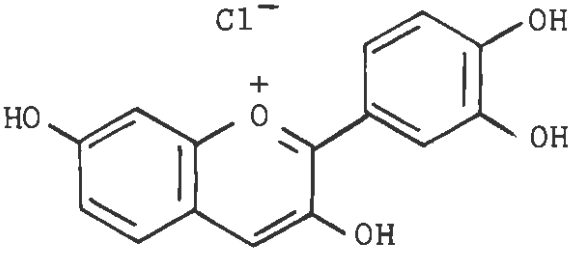
i) (+)- Mollisacacidin.

The major monomeric polyphenolic component of the heartwood is the leucoanthocyanidin, (+)-mollisacacidin (I), which when heated with mineral acid yields fisetinidin

chloride (II).



(I)



(II)

All leucoanthocyanidins on treatment with alcoholic mineral acid, yield red-coloured anthocyanidins as their flavylium salts. This reaction was used by Bate-Smith[1] to detect the presence of leucoanthocyanidins in plant extracts.

The first naturally occuring leucoanthocyanidin to be isolated was melacacidin[2,3] (7,8,3',4'-tetrahydroxy-flavan-3,4-diol). This confirmed the prediction by Robinson and Robinson[4] of the existence of this group of natural products.

(+)-Mollisacacidin, isolated by Keppler[5,6] in 1956 from Acacia mearnsii, was the first crystalline leucoanthocyanidin found. It was later also shown to be present in the closely related Acacia decurrens, A. dealbata, and A. pycnantha by Roux[7].

Elucidation of the structure of this compound[6] was by periodic acid oxidation of the trimethyl ether, showing an α,β-glycol group, and by treatment with potassium permanganate in acetone yielding 4-methoxy-salicylic acid and veratric acid. This evidence indicates a resorcinol type A-ring and a catechol type B-ring. Catalytic reduction of fustin isolated from Rhus glabra yielded racemic mollisacacidin. On the basis of the above evidence Keppler put forward the structure 7,3',4'-trihydroxyflavan-3,4-cis-diol.

In 1959 Clark-Lewis and Roux[8] showed that (+)-mollisacacidin was identical to gleditsin, the leucoanthocyanidin from Gleditsia japonica[9] and was the enantiomer of the laevorotatory leucoanthocyanidin from Schinopsis quebracho-colorado.[10,11]

In 1961 Brown and co-workers[12] showed that reduction of 2,3-trans-3-hydroxyflavanone with lithium aluminium hydride, sodium borohydride or hydrogen over palladium charcoal leads to a flavan-3,4-trans-diol while reduction with a mixture of lithium aluminium hydride and aluminium chloride yields a flavan-3,4-cis-diol. This cis-diol reacts with lead tetraacetate five times as fast as does the trans-diol.

This work laid the foundation for the preparation of synthetic isomers of mollisacacidin and the elucidation of the stereochemistry of (+)-mollisacacidin by Drewes and Roux[13,14,15] and Clark-Lewis and co-workers.[16,17]

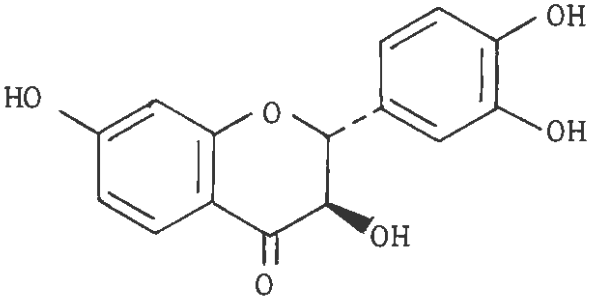
The trans-trans-and the trans-cis-isomers of trimethoxy-mollisacacidin were synthesised and comparison of the rates of oxidation with periodic acid and with lead tetraacetate with the methyl ether of naturally occurring (+)-mollisacacidin showed that (+)-mollisacacidin had a 3,4-trans-glycol system. This assignment was confirmed by nuclear-magnetic-resonance (n.m.r.) spectrometry by comparing the chemical shifts and coupling constants of the 3,4-diacetates of the methyl ethers of the natural and synthetic isomers. Further, using the relationship of Karplus[18] and Conroy[19] where the coupling constants are dependent on the dihedral angles of the proton to carbon bonds, it was shown that the protons of the heterocyclic ring were all-trans.

The isopropylidene derivatives of both (±)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-cis-diol and (±)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-trans-diol were identical and had a trans-cis-configuration. This involves the inversion of the 4-benzylhydroxy group of the trans-trans-isomer. The preferred inversion for the trans-trans-isomer assuming an all-equatorial orientation of substituents in the heterocyclic ring, is from equatorial to axial orientation. The above evidence indicates that (+)-mollisacacidin has a 2R:3S:4R-absolute configuration and can be defined as 2R:3S:4R-(+)-7,3',4'-trihydroxy-2,3-trans-flavan-3,4-trans-diol.

ii) (+)-Fustin.

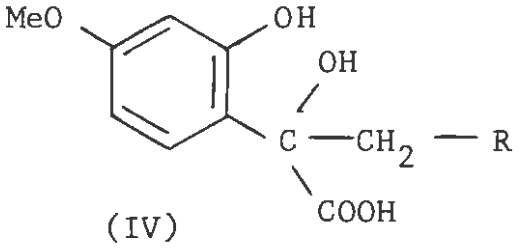
(+)-Fustin (III), a member of 2,3-dihydroflavonol group, was isolated from the heartwood of Acacia mearnsii

by Roux and Paulus[20].



(III)

Fustin was originally isolated from the heartwood of Rhus succedanae by Oyamada[21] who elucidated its structure as follows:[22,23] On heating 7,3',4'-trimethoxyfustin in sodium hydroxide, 7,3',4'-tri-methoxyfisetin and trimethylhazeic acid (IV) were produced.



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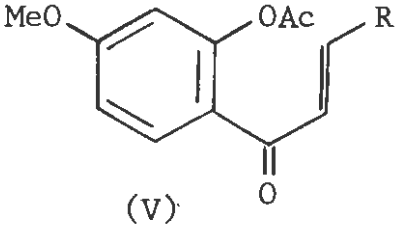
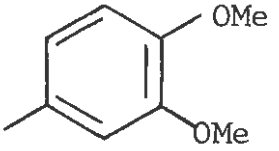


(V)

where R =

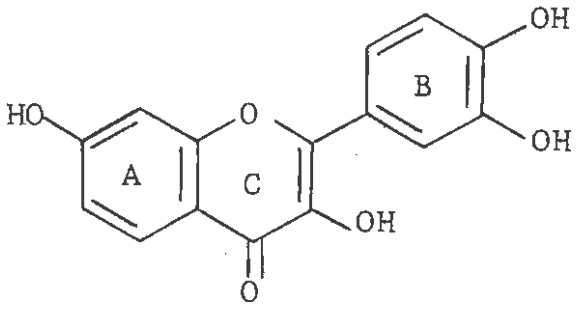


7,3',4'-Trimethoxyfustin was synthesised from 2-acetoxy-4-methoxyphenyl-3,4-dimethoxystyryl ketone (V) by adding bromine across the straight chain double bond, acetylating, and then ring closing in hydrochloric acid.

(+)-Fustin, on hydrogenation with platinum oxide, is converted to 2R:3S:4R-(+)-trihydroxy-2,3-trans-flavan-3,4-trans-diol[20].

iii) Fisetin.

Fisetin, 3,7,3',4'-tetrahydroxy flavone (VI), a widely occurring flavonol, was isolated from the heartwood by Roux and Paulus[20].



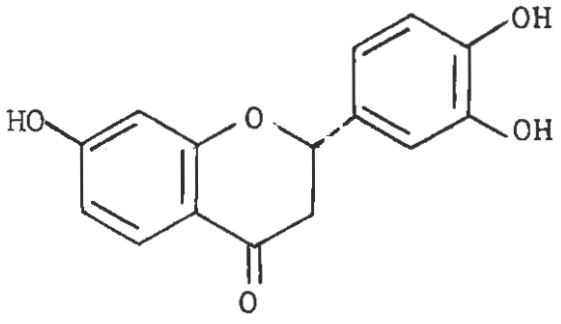
(VI)

Fisetin is yellow due to the conjugated C-ring and fluoresces yellow-green under ultraviolet light. It occurs in abundance in Rhus cotinus[24] and has been found in the wood of other Rhus species[21,25,26,27], and in the heartwood of Schinopsis lorentzii[28] and Gleditsia japonica[8].

Von Kostanecki[29] and Herzig[30] elucidated the complete structure of fisetin which was later proved by synthesis[31,32] using ω-methoxy-resacetophenone, veratric acid and potassium veratrate.[33]

iv) (-)-Butin and Butein.

(-)-Butin, (-)-7,3',4'-trihydroxyflavanone (VII), a flavanone, is isomeric with the chalcone, butein 2,4-dihydroxyphenyl-3,4-dihydroxystyryl ketone (VIII). These two compounds occur as minor components of the heartwood.



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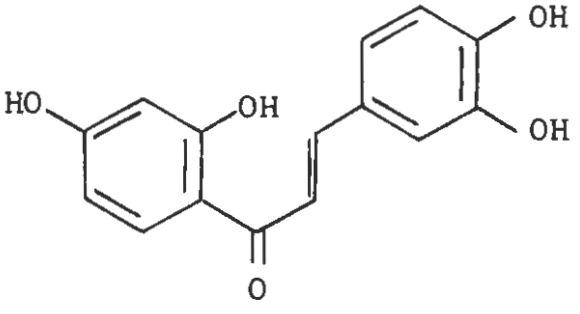


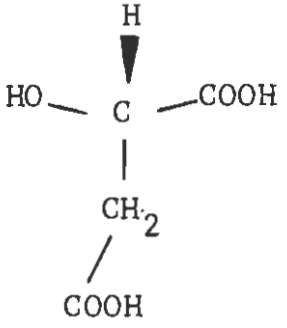
(VIII)

Butin was first isolated from the flowers of Butea frondosa[34]. It follows the general pattern of behaviour of flavanones in that it is converted by ring opening on boiling with potassium hydroxide into the chalcone, butein. In alcoholic mineral acid the reverse reaction takes place [35,36,37].

Optically active butin $[\alpha]_D$ -18.7° and the corresponding chalcone, butein, were isolated from the heartwood by Roux and Paulus[35] in 1961.

Arakawa and Nakazaki[38] showed that destructive ozonolysis of (-)-liquiritigenin, (-)-7,4'-dihydroxyflavanone,

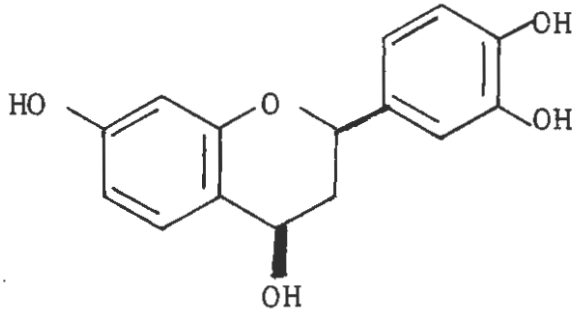
and (-)-hesperitin, (-)-5,7,3'-trihydroxy-4'- methoxy-flavanone, yielded L-malic acid (IX) of known absolute configuration, showing that these flavanones had a 2S-configuration.



(IX)

By analogy (-)-butin was thought to have a 2S-configuration which was independently confirmed by Roux and Paulus.[39] Elimination of the 3-hydroxy group of 2R:3S-(+)-7,3',4'-trihydroxyflavan-3-ol-4-one with deactivated zinc and hydrochloric acid yielded (-)-butin which can now be defined as 2S-(-)-7,3',4'-trihydroxyflavanone.

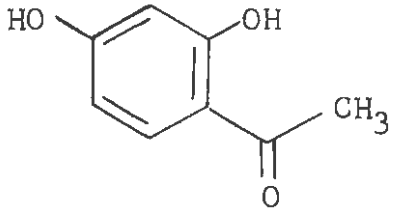
(-)-Butin is hydrogenated using platinum oxide in methanol[39] to 7,3',4'-trihydroxyflavan-4-ol (X)



(X)

Butein was first isolated by Perkin and Hummel[34] and occurs naturally in such flowers as yellow Dahlia variabilis[40,41] and in the Coreopsis spp.[41-45].

Butein is yellow due to resonance between the aromatic rings which are linked by a conjugated system and can be synthesised from resacetophenone (XI) and protocatechuic aldehyde (XII).



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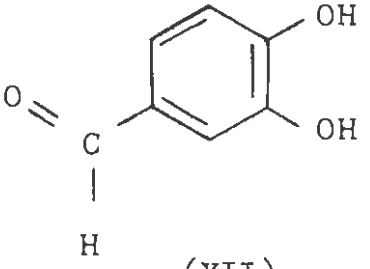
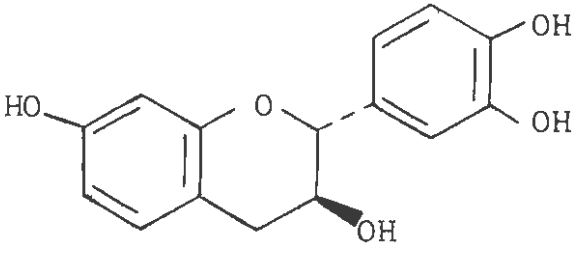


(XII)

v) (-)-Fisetinidol.

(-)-Fisetinidol (-)-7,3',4'-trihydroxyflavan-3-ol, (XIII) was first observed in Nature by Roux and Paulus[46] who extracted this catechin from the heartwood of Acacia mearnsii and proved its structure and stereochemistry.



(XIII)

It was synthesised by hydrogenation of (+)-mollisacacidin using palladium catalyst in dioxan as described by Weinges.[47,48] As the stereochemically interrelated substances (2S:3R:4S)-(-)-mollisacacidin, (2S:3S)(-)-fustin, and (2S:3R)-(+)-fisetinidol have the same absolute configuration as (2S:3R)-(-)-catechin[47,48,49] it follows that their enantiomers[8,20,50] have the same absolute configuration as (2R:3S)-(+)-catechin. (-)-Fisetinidol is designated (2R:3S)-(-)-7,3',4'-trihydroxy-2,3-trans-flavan-3-ol.

vi) The Polymeric Leucoanthocyanidins.

In 1962 Roux and Paulus[51] showed that the heartwood extracts contained a number of leucofisetinidins in addition to those already isolated and identified. These were polymeric leucofisetinidins with molecular weights up to three thousand.[52,53] The yields of fisetinidin chloride from these leucofisetinidins under the conditions of Pigman and co-workers[54] decrease with increasing molecular weight as seen in Table 1.

Table 1. Estimated yields of fisetinidin chloride from monomeric and polymeric leucofisetinidins

Compound	Percentage yield of fisetinidin chloride
monomeric leucofisetinidin	23.5
trimeric tannin	6.8
pentameric tannin	4.8
decameric tannin	4.9

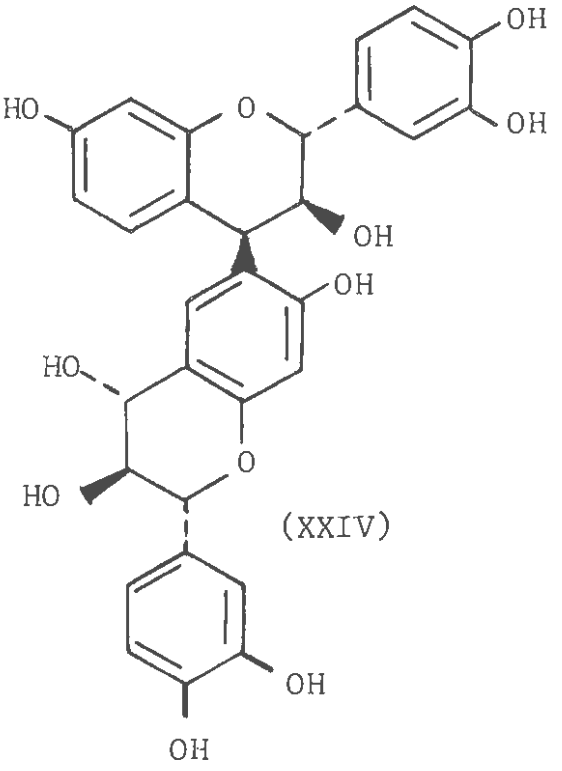
a) The Bileucofisetinidins.

One of the homogeneous tannin fractions mentioned above was further investigated by Roux and co-workers[55,56] who showed that this mixture could be resolved into three components by paper ionophoresis in borate buffer. When heated with mineral acid in alcoholic solution these components each formed fisetinidin chloride, fisetin and an additional anthocyanidin and flavonol. Alkali fusion indicated resorcinol and catechol nuclei for these dimers. They were isolated as their crystalline methyl ethers using thin-layer chromatography to separate the methylated tannin fraction.

The crystalline products were shown to be hexamethyl ethers of three diastereoisomeric bileucofisetinidins (XIV,XV,XVI).

Analysis of the high-resolution n.m.r. spectra of the hexamethyl ether triacetate derivatives of each diastereoisomer showed that they were made up of two flavan units linked via C4 of the one unit to C6 of the other and that the compounds had different stereochemistry of their heterocyclic ring systems. The presence of an ion at m/e 328 from a double retro Diels-Alder process in the mass spectrometer provides additional evidence of a 4-linkage to a resorcinol nucleus.

The assignment of accurate relative configurations and conformations of the heterocyclic rings of the three diastereoisomers was possible by using the spin-decoupling



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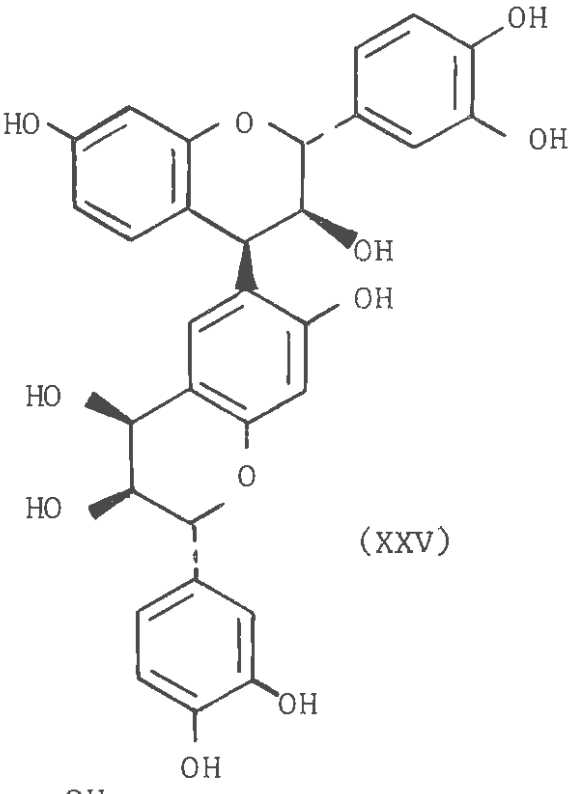

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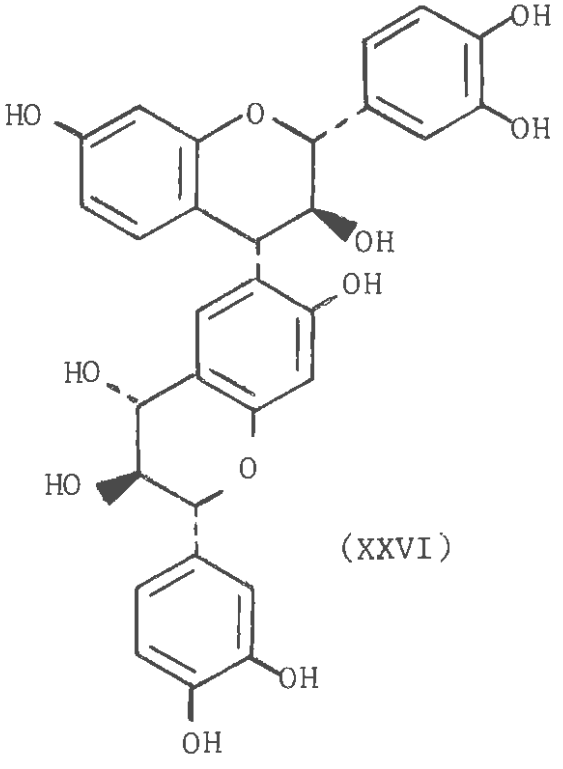


(XXVI)

technique to simplify the absorption patterns of the heterocyclic protons in the n.m.r. spectra.

Large coupling constants of the C-ring and F-ring protons of the hexamethyl ether triacetate of (XVI) indicates an all-trans configuration for this component. The coupling constants of the C-ring are greater than those of the corresponding F-ring because the bulky flavanoid substituent in the 4-position of the C-ring acts as a conformational anchor, and reinforces the effect of the 2-phenyl substituent of that ring thus reducing the possibility of conformational equilibrium of the C-ring. The coupling constants of the F-ring protons are similar to those of the flavan-3,4-diol analogue of similar trans-trans configuration.

These facts are in agreement with a half-chair conformation for the C-ring and a half-chair or 5-point coplanar conformation for the F-ring. The substituents of the heterocyclic rings have an all-equatorial orientation and therefore (XVI) has a 2R:3S:4S-2R:3S:4R absolute configuration.

For diastereoisomer (XIV) a 2,3-trans-3,4-cis: 2,3-trans-3,4-trans relative configuration was postulated with the heterocyclic rings in the half-boat (ring C) and half-chair or 5-point coplanar (ring F) conformations, while, for diastereoisomer (XV), a 2,3-trans-3,4-cis: 2,3-trans-3,4-cis relative configuration was postulated with the C- and F-ring conformations as in diastereoisomer (XIV). The coupling constants of the F-ring protons of (XV) were

similar to those of the flavan-3,4-diol analogue of similar trans-cis configuration.

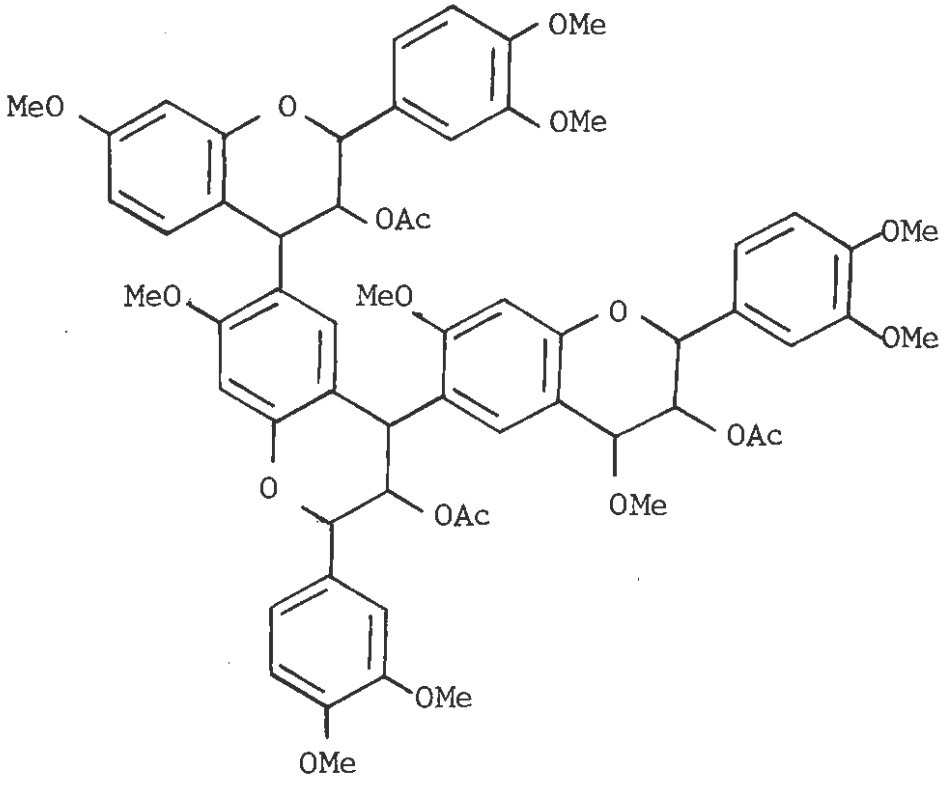
The substituents of the heterocyclic rings of (XIV) and (XV) have 2(eq):3(ax):4(eq)-2(eq):3(eq):4(eq) and 2(eq):3(ax):4(eq)-2(eq):3(eq):4(ax) orientations respectively.

Thus, the diastereoisomers (XIV) and (XV) have 2R:3S:4R-2R:3S:4R and 2R:3S:4R-2R:3S:4S absolute configurations respectivley.

b) Trileucofisetinidin.

Using paper chromatography Drewes and Roux[57] isolated a trimeric tannin mixture which was shown by paper electrophoresis to contain at least four components.

Methylation of the mixture and subsequent thin-layer chromatography yielded 4 fractions, one of which was isolated pure and had M^+ 974. The acetate of this compound was shown by mass spectrometry to be a decamethyl ether triacetate of trileucofisetinidin (XVII) made up of three C-15 units with links from the C4 of the heterocyclic ring on one unit to either the C6 or C8 of the resorcinol nucleus of the next unit. A methoxy group was proposed on the C4 of the terminal unit.



(XVII)

B. Components of the Bark Extract.

The following flavonoid components have been identified in the bark extract of Acacia mearnsii.

a) Flavan-3-ols:- (+)-catechin (+)-5,7,3',4'-tetrahydroxyflavan-3-ol , (+)-gallocatechin (+)-5,7,3',4',5'-pentahydroxyflavan-3-ol , (-)-fisetinidol (-)-7,3',4'-trihydroxyflavan-3-ol (-)-robinetinidol (-)-7,3',4',5'-tetrahydroxyflavan-3-ol .

b) Flavan-3,4-diols:- leucofisetinidin 7,3',4'-trihydroxyflavan-3,4-diol , leucorobinetinidin 7,3',4',5'-tetrahydroxyflavan-3,4-diol .

c) Flavanonols:-fustin 7,3',4'-trihydroxyflavan-3-ol-4-one dihydrorobinetin 7,3',4',5'-tetrahydroxyflavan-3-ol-4-one .

d) Flavonols:- fisetin 3,7,3',4'-tetrahydroxyflavone robinetin 3,7,3',4',5'-pentahydroxyflavone .

e) Flavonol glycosides:- myricitrin 3,5,7,3',4',5'- hexahydroxyflavone 3-rhamnoside , quercitrin 3,5,7,3',4'-pentahydroxyflavone 3-rhamnoside .

f) Flavanones:- butin 7,3',4'-trihydroxyflavanone .

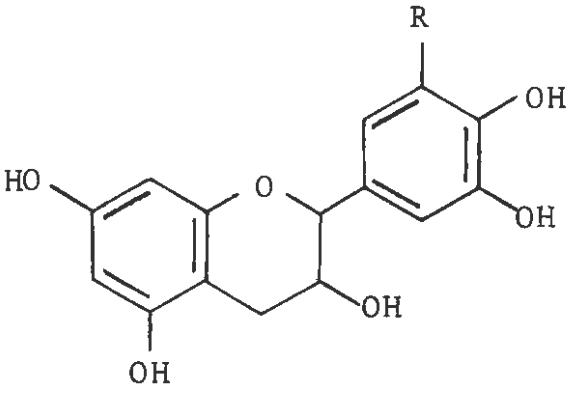
g) Chalcones;- butein 2,4-dihydroxyphenyl-3,4-dihydroxystyryl ketone , robtein 2,4-dihydroxyphenyl-3,4,5-trihydroxystyryl ketone .

h) Biflavanols:- leucofisetinidin -(+)-catechin; leucorobinetinidin -(+)-catechin; leucorobinetinidin-(+)-gallocatechin.

i) Flavan-3-ols.

(+)-Catechin (XVIII,R=H) and (+)-gallocatechin (XVIII, R=OH), (-)-fisetinidol (XIX,R=H), and (-)-robinetinidol (XIX,R=OH) were isolated from the bark tannins of Acacia mearnsii[58,59,60].

Catechin and gallocatechin are the 5-hydroxy analogues of fisetinidol and robinetinidol. Their pattern of hydroxy substitution is shown in Table 2.



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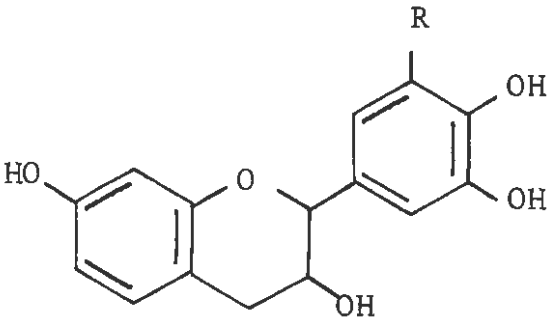


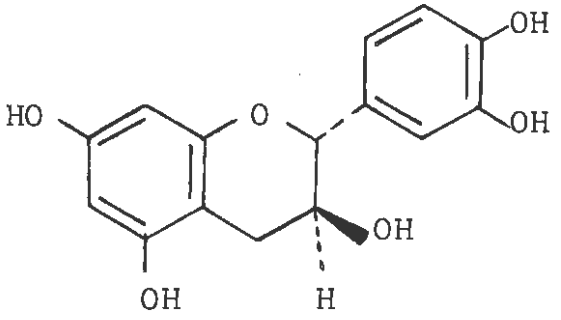
(XIX)

Table 2. Types of hydroxy substitution of the flavan-3-ols

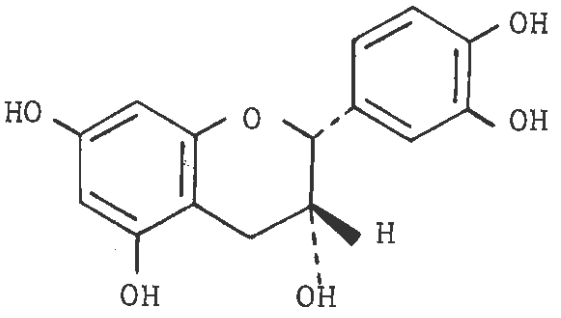
	Type of hydroxy substitution	
Flavan-3-ol	A-ring	B-ring
(+) catechin	phloroglucinol	catechol
(+)-gallocatechin	phloroglucinol	pyrogallol
(-)-fisetinidol	resorcinol	catechol
(-)-robinetinidol	resorcinol	pyrogallol

(+)-Catechin was shown by King, Clark-Lewis, and Forbes[61] and Whalley[62] to have a 2,3-trans-configuration.

The absolute configuration of (+)-catechin was established by Hardegger and co-workers[63] as follows: Ozonolysis of (+)-catechin, esterification of the resulting acid and reduction with lithium aluminium hydride yielded 2-deoxy-D-adonitol which was also obtained by reduction of 2-deoxy-D-ribose of known absolute configuration. (+)-Catechin is thus written in the form (XX)



(XX)



(XXI)

In this connection it is important to mention (-)-epicatechin. The absolute configuration of (-)-epicatechin is shown by writing the structure in the form (XXI) as shown by Hardegger and co-workers[64] and Birch, Clark-Lewis, and Robertson[65]. This means that these two isomers have the same configuration at C2 but opposite configurations at C3.

(+)-Catechin is therefore defined as 2R:3S-(+)-5,7,3',4'-tetrahydroxyflavan-3-ol and (-)-epicatechin as 2R:3R-(-)-5,7,3',4',-tetrahydroxyflavan-3-ol.

Weinges[47,48] showed that the absolute configuration of other catechins can be determined by comparison of molecular rotation of their derivatives with those of the derivatives of (+)-catechin and (-)-epicatechin.

Thus, it was shown that (+)-gallocatechin, (-)-fisetinidol, and (-)-robinetinidol which are all 2,3-trans catechins, had the same absolute configuration as (+)-catechin.

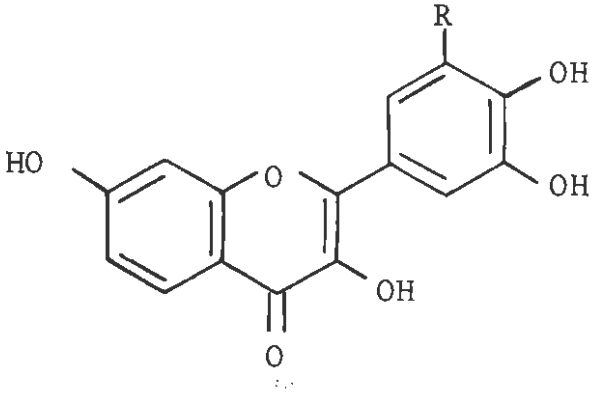
Freudenberg and Weinges[66] showed that hydrogenolysis of 5,7,3',4',-tetrabenzoxyflavan-3,4-diol in dioxan yielded racemic catechin. (+)-Catechin tetramethyl ether was synthesised by Clark-Lewis and Korytnyk[67] by hydrogenation of (+)-dihydroquercetin tetramethyl ether over Adam's catalyst.

Whalley[62] postulated the half-chair conformation for catechin but later work by Philbin and Wheeler[68] showed that the hetero-oxygen atom, the 3-carbon atom and the 4-carbon atom were in the same plane as the benzene ring, the heterocyclic ring taking up a sofa or 5-point coplanar conformation with the 2-phenyl group in the less restricted equatorial position.

The sofa or 5-point coplanar conformation was confirmed by Clark-Lewis, Jackman, and Spotswood[69] by n.m.r. spectral studies.

vi) Fisetin and Robinetin.

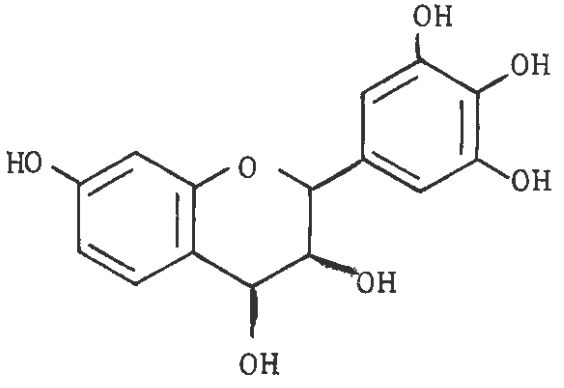
Two analogues of the flavonol type have been isolated from the bark extract. They are fisetin (XXII, R=H) and robinetin (XXII, R=OH) which were isolated by Roux.[70]



(XXII)

vii) Other flavonoid components.

Chromatographic evidence for the presence of (+)-mollisacacidin (I), (+)-1eucorobinetinidin (XXIII), and butin (VII) in the bark extract was shown by Drewes and Roux[60].

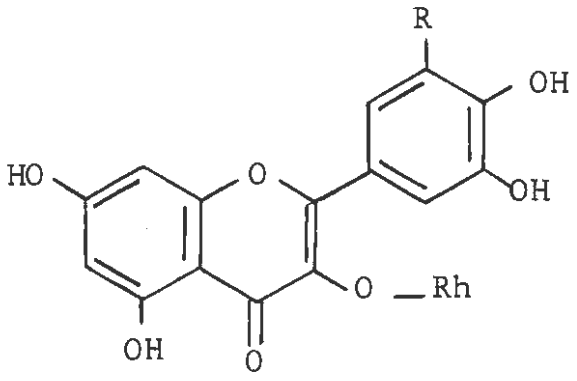


(XXIII)

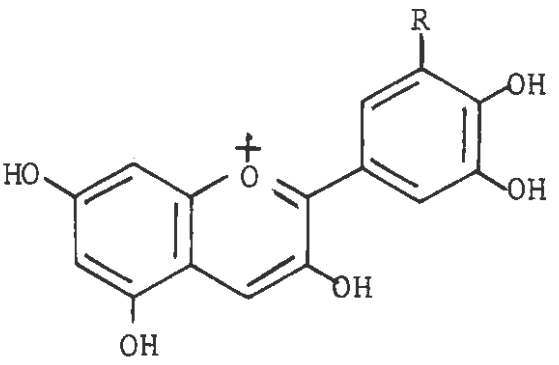
They further isolated butein (VIII) and robtein and identified these by paper chromatographic comparison and ultraviolet and infrared spectroscopic comparison with authentic reference compounds.

viii) Myricitrin and Quercitrin.

Myricitrin (XXIV, R=OH) and quercitrin (XXIV R=H), the 3-O-rhamnosides of myricetin (3,5,7,3',4',5'-hexahydroxyflavone) and quercetin (3,5,7,3',4'- pentahydroxyflavone) are related to the flavonoid groups based on delphinidin (XXV, R=OH) and cyanidin (XXV, R=H) respectively.



(XXIV)



(XXV)

Rh = Rhamnose

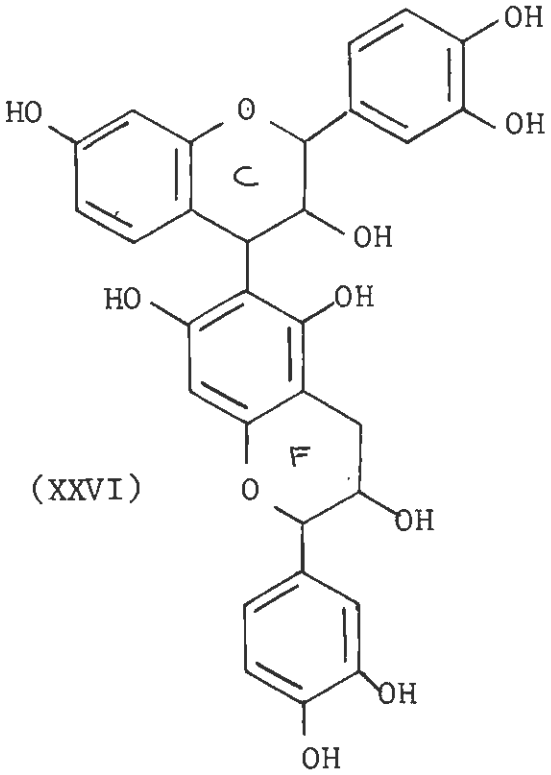
These two flavonol rhamnosides were isolated from fresh immature bark of Acacia mearnsii by Drewes and Roux[60] and to date represent the only two glycosides in the bark extract or heartwood.

The presence of a rhamnose residue in these glycosides is consistent with the finding of Stephen[71] who identified the sugar constituents of black-wattle gum.

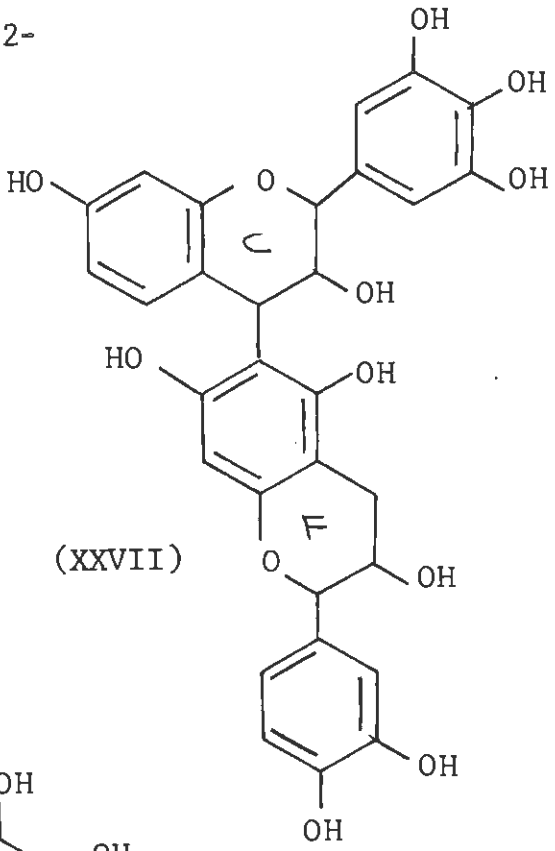
ix) The Biflavonols.

Three stereochemically similar biflavanols (XXVI, XXVII, XXVIII) were isolated from the bark tannins by Roux and co-workers[72,73]. These homologues, leucofisetinidin-(+)-catechin (XXVI), leucorobinetinidin-(+)-catechin (XXVII) and leucorobinetinidin-(+)-gallocatechin (XXVIII), are present in the bark in relatively high concentration.

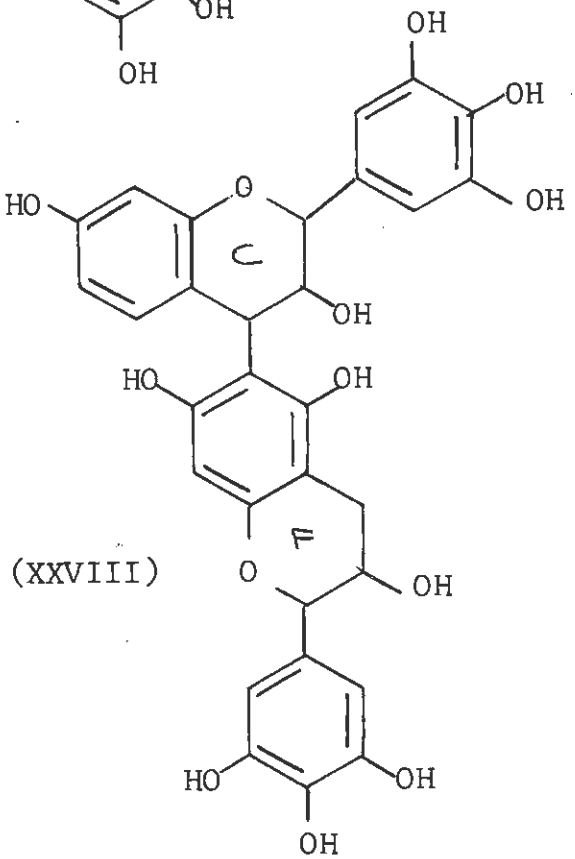
(XXVI)



(XXVII)



(XXVIII)



High resolution n.m.r. and mass spectra of the derivatives of these biflavanols showed that the heterocyclic C-andF-rings of the two units have 2,3-trans-3,4-trans : 2,3-trans configurations and that the constituent arrangement of these heterocyclic rings is all-equatorial.

The pattern of n.m.r. absorption shows that the flavanol units are either 4,6-or 4,8-linked; the former being preferred on the basis of the biflavanols' similarity to the all-trans bileucofisetinidin (XVI).

Large coupling constants of the C-ring heterocyclic protons favour the half-chair conformation for this ring which is stabilised by conformational anchors at C2 and C4.

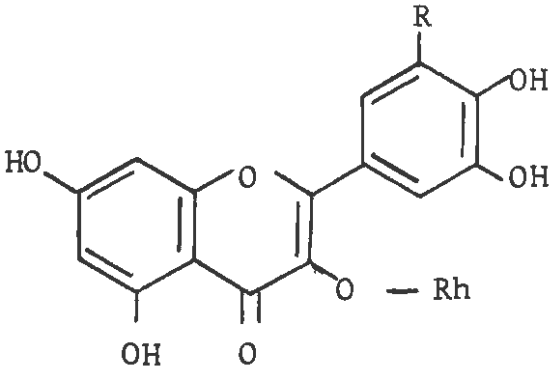
C. Components of the Leaves.

Five flavonol glycosides have been isolated from the leaves of Acacia mearnsii by MacKenzie[74]. They have been identified as

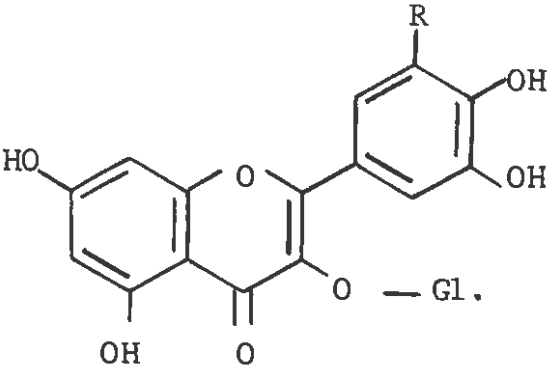
myricitrin (3,5,7,3',4',5'-hexahydroxyflavone 3-rhamnoside.)
quercitrin (3,5,7,3',4'-pentahydroxyflavone 3-rhamnoside)
cannabiscitrin(3,5,7,3',4',5'-hexahydroxyflavone 3-glucoside)
isoquercitrin(3,5,7,3',4'-pentahydroxyflavone 3-glucoside)
mearnsitrin(3,5,7,3',5'-pentahydroxy -4'-methoxyflavone 3-rhamnoside).

Myricitrin (XXIX, R=OH) and quercitrin (XXIX,R=H) (c.f. Saayman and Roux[75]) have previously been isolated from

the bark extract by Drewes and Roux[60]. Myricitrin and quercitrin are the 3-rhamnosides of the flavonols myricetin and quercetin while cannabiscitrin (XXX, R=OH) and isoquercitrin (XXX, R=H) are the 3-glucoside of these flavonols.



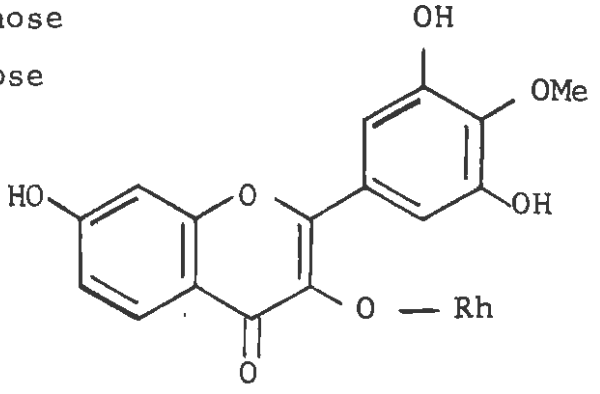
(XXIX)



(XXX)

Rh = Rhamnose

Gl = Glucose



(XXXI)

(+) Catechin and (+)-gallocatechin have been shown to be present in the leaf extracts by paper chromatographic comparison with authentic reference compounds[75].

Mearnsitrin (XXXI) is a partially methoxylated myricetin 3-rhamnoside[76]. The n.m.r. spectrum of mearnsitrin penta-acetate showed the presence of one methoxy group. The benzenoid proton absorption pattern was consisted with that of myricitrin with symmetrical substitution of the B-ring. KOH fusion of mearnsitrin yielded 4-methoxygallic acid. The above correlations indicate 4'-methoxy substitution of myricetin 3-rhamnoside.

EXPERIMENTAL AND RESULTS.

All nuclear magnetic resonance spectra were recorded at 100 Mc./sec. on a Varian high resolution HA 100 spectrometer using deuterochloroform as solvent and tetramethylsilane for the lock signal. Chemical shifts have been expressed as τ in p.p.m.

Mass spectra were recorded on an AEI MS9 double focussing spectrometer. Infrared spectra were recorded on a Beckman IR 12 spectrometer using KBr discs. Ultraviolet and visible spectra were recorded on a Beckman DB spectrophotometer.

Melting points were done on a Kofler hotstage attached to a Reichert "Thermopan" microscope. Microanalyses were by Drs. Weiler and Strauss, Microanalytical Laboratory, Oxford, England.

ISOLATION OF NOVEL COMPONENTS FROM BLACK WATTLE HEARTWOOD.

Extraction of Black Wattle Heartwood. The heartwood drillings (7.7 Kg.) from 12" diameter sections of a mature Black Wattle Tree (Acacia mearnsii) were extracted with acetone (30 l.) for 6 days at room temperature. The extract liquor was filtered and concentrated to dryness under reduced pressure at 50°. This yielded a light brown solid (300g) which was dewaxed by successive extractions with isohexane.

Initial enrichment of Extract. The wax-free heartwood extract (50 g.) was dissolved in the aqueous phase (1l.) of a 1:1 ($^{V}/v$) mixture of ethyl acetate and water. Preliminary enrichment was by partition separation between the ethyl acetate and water in five two-litre separating funnels. This fractionation was repeated and the combined contents of tubes 3,4 and 5 yielded an extract (100 g.) containing a high proportion of low molecular weight flavonoid components.

Separation of Components. Further separation was by partition separation in an automatic, 50 ml. underphase, Craig counter-current machine with water-butan-2-ol-isohexane (5:3:2 $^{V}/v$) as solvent. The enriched extract (100 g.) was dissolved in the aqueous phase and introduced into tubes 1 to 8 of the Craig machine. After 160 transfers the contents of every fifth tube was examined by two dimensional chromatography (in duplicate). The chromatograms were sprayed with ammoniacal silver nitrate and with toluene-p-sulphonic acid in order to locate the required components. The contents of various tubes were combined and the upper phases were added to the ethyl acetate extract of the aqueous phases. Concentration under reduced pressure yielded the following solid extracts from which the new compounds were isolated.

Tube	Yield	Containing
58-76	16.0 g	B
100-125	5.0 g	A
141-160	7.0 g	C,D,E.

Isolation and proof of structure of A. The concentrate (5.0 g) from tubes 100-125 was dissolved in acetone (250 ml.) and applied to 50 sheets of Whatman's No. 3 (46 x 57 cm.) paper and the chromatograms were developed in 2% (^{V}/v) acetic acid by upward migration. These were then dried in a current of warm air and side-strips (1 cm.) sprayed with ammoniacal silver nitrate and toluene-p-sulphonic acid to locate A. The reducing band (pink to toluene-p-sulphonic acid) at R_F 0.24-0.34 immediately behind fustin[20] was cut out and eluted with 70% (^{V}/v) ethanol. Concentration of the eluate under reduced pressure at 50° yielded a buff solid (500 mg.) which contained mainly A and fustin. Further purification was achieved by re-running the mixture on 10 sheets in the same solvent system. The material obtained after the second separation (105 mg.) was shown to be pure by two dimensional chromatography but failed to crystallise. A had R_F 0.80 in water-saturated butan-2-ol and R_F 0.28 in 2% (^{V}/v) acetic acid.

Anthocyanidin formation. A (1.2 mg.) was dissolved in 3N HCl-propan-2-ol (1:4 ^{V}/v) (2 ml.) and heated in a closed tube in boiling water for 90 minutes.[54] A reddish-pink colour developed. Some of the solution was spotted onto a strip of Whatman. No. 1. paper and the chromatogram was developed using 3N HCl-90% (^{W}/v) formic acid (1:1 ^{V}/v) as solvent.[77] The unknown anthocyanidin chloride had R_F 0.41 compared with fisetinidin chloride R_F 0.44 generated from mollisacacidin under the same conditions. Apart from

yielding pigments both compounds developed fisetin, R_F 0.14, which was detected by its bright yellow-green fluorescence on the paper chromatograms.

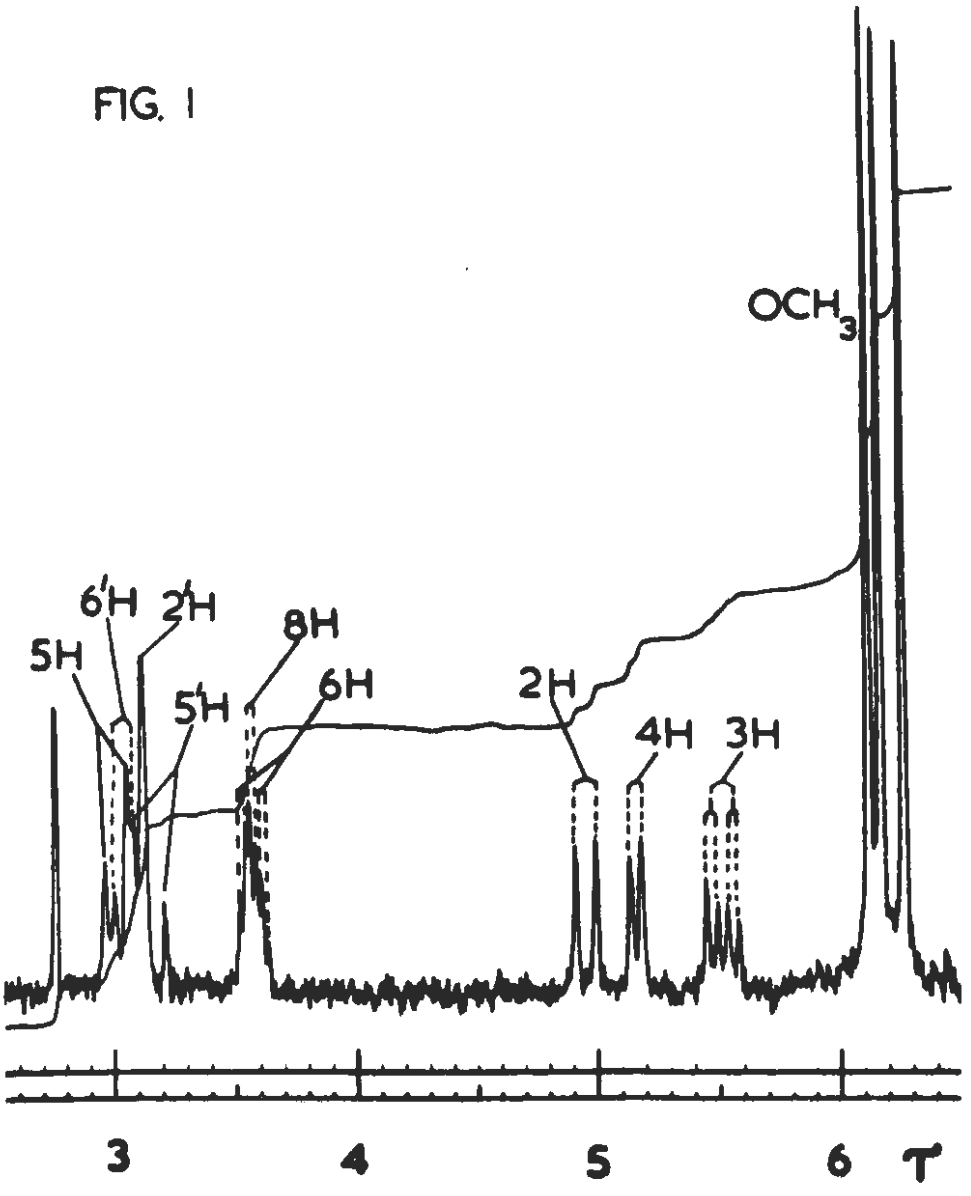
The products of pigment generation of both compounds in ethanol gave identical ultraviolet and visible spectra having absorption maxima at 280 mμ and 525 mμ.

Alkali degradation. KOH micro fusion of A (1.5 mg.) was carried out under the conditions described by Roux[78]. The phenolic and acidic fractions were examined both by paper chromatography (Whatmans No. 1) and by thin layer chromatography (Merck Kieselgel PF_{254}) with reference phenols and acids. The paper chromatograms were developed with butan-1-ol-acetic acid-water (6:1:2 $^{v}/v$) and the thin layer chromatograms with benzene-acetic acid-methanol (90:6:2 $^{v}/v$). Chromatograms of the phenolic fraction were sprayed with bis-diazotised benzidine, while those of the acidic fraction were sprayed with ferric alum. The degradation products were identified as ℮-resorcylic acid, protocatechuic acid and resorcinol.

Hexamethyl ether of A. A (78 mg.) was dissolved in methanol (100 ml.) and treated with diazomethane, generated from N-nitrosomethylurea (5 g.), at -10°. After 48 hours the reaction mixture was concentrated under reduced pressure. The yellowish residue crystallised as rosettes of needles (36 mg.) m.p. 175°, $[\alpha]_D^{20} = +102°$ (c 0.12 in acetone) from alcohol. (Found: C, 68.8; H, 5.5. $C_{36}H_{36}O_{10}$ requires C, 68.8; H, 5.7%). The n.m.r. spectrum and accurate mass (M^+ 628.233269) are in agreement with the analysis.

Treatment of the methyl ether of A (16 mg.) with acetic anhydride (0.25 ml.) and pyridine (0.25 ml.) at 60° for 12

FIGURE 1. N.m.r. Spectrum of Hexamethyl Ether of A. Dioxane-linked Dimeric Leucofisetinidin.



hours yielded a white solid (14 mg.) which was recovered from water. Crystallisation from ethanol gave white needles (11.5 mg.) m.p. 175°. This was found to be identical to the starting material. A mixed m.p. showed no depression and both compounds had R_F 0.49 by t.l.c. on silica gel developed in benzene-acetone (93:7 v/v). Trimethoxyleucofisetinidin in this system has R_F 0.05.

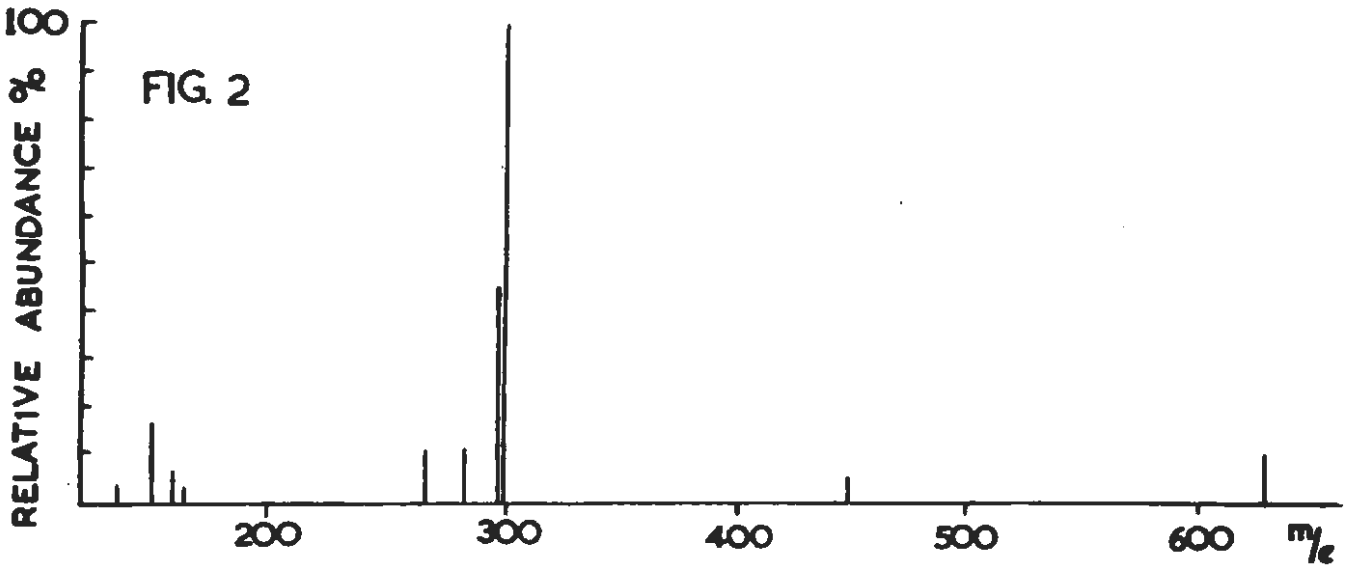
When examined by paper ionophoresis under the conditions described by Roux and co-workers[56,79], the hexamethyl ether showed no migration.

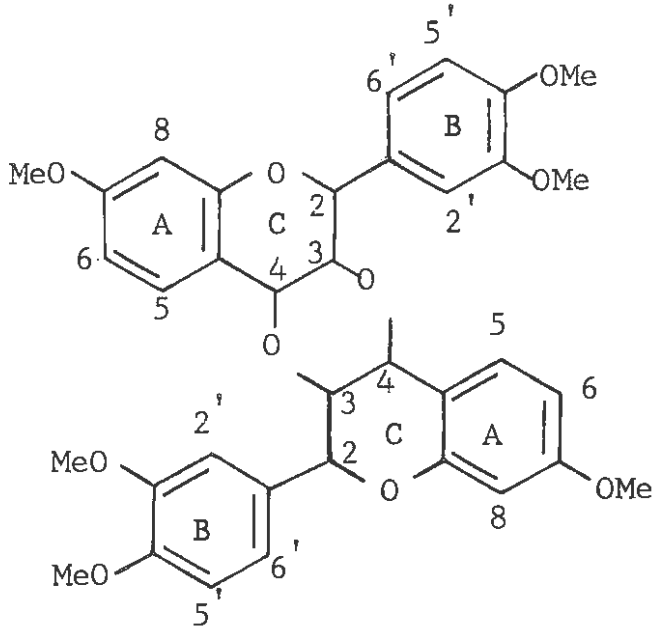
Hexaacetyl Derivative. A (20 mg.) was treated with acetic anhydride (0.25 ml.) and pyridine (0.25 ml.) and left to stand in a stoppered vessel overnight at 30°. The white, amorphous hexaacetate m.p. 115-118° was recovered from water. The n.m.r. and mass spectra confirmed the presence of six acetyl groups.

Nuclear magnetic resonance spectrum of the hexamethyl ether of A. An analysis of the n.m.r. spectrum of the hexamethyl ether of A (c.f. Figure 1.) is given below. The proposed structure of the hexamethyl ether of A is given by (1).

Methoxy proton resonances for 18 protons are at τ 6.24, 6.15, and 6.10.
Benzenoid proton resonances are as follows: 5H, τ 3.00; 6H, τ 3.56; 8H, τ 3.57; 2'H, τ 3.11; 5'H, τ 3.16; 6'H, τ 3.04.
Heterocyclic proton resonances are as follows: 2H, τ 4.95; 3H, τ 5.50; 4H, τ 5.16.
Coupling constants are $J_{5,6}$ = 8.8, $J_{6,8}$ = 2.9, $J_{3;4'}$ = 7.7 c./sec., and $J_{2,3}$ = 8.6, $J_{3,4}$ = 4.6 c./sec.

FIGURE 2. Mass Spectrum of Hexamethyl Ether of A - Dioxane-linked Dimeric Leucofisetinidin.





(I)

Mass spectra of Derivatives of A. An accurate mass measurement of the molecular ion of the hexamethyl ether of A, using the 613.9995 peak of heptacosafluorotributylamine as reference, gave M^+ 628.233269 compared with 628.230850 for $C_{36}H_{36}O_{10}$.

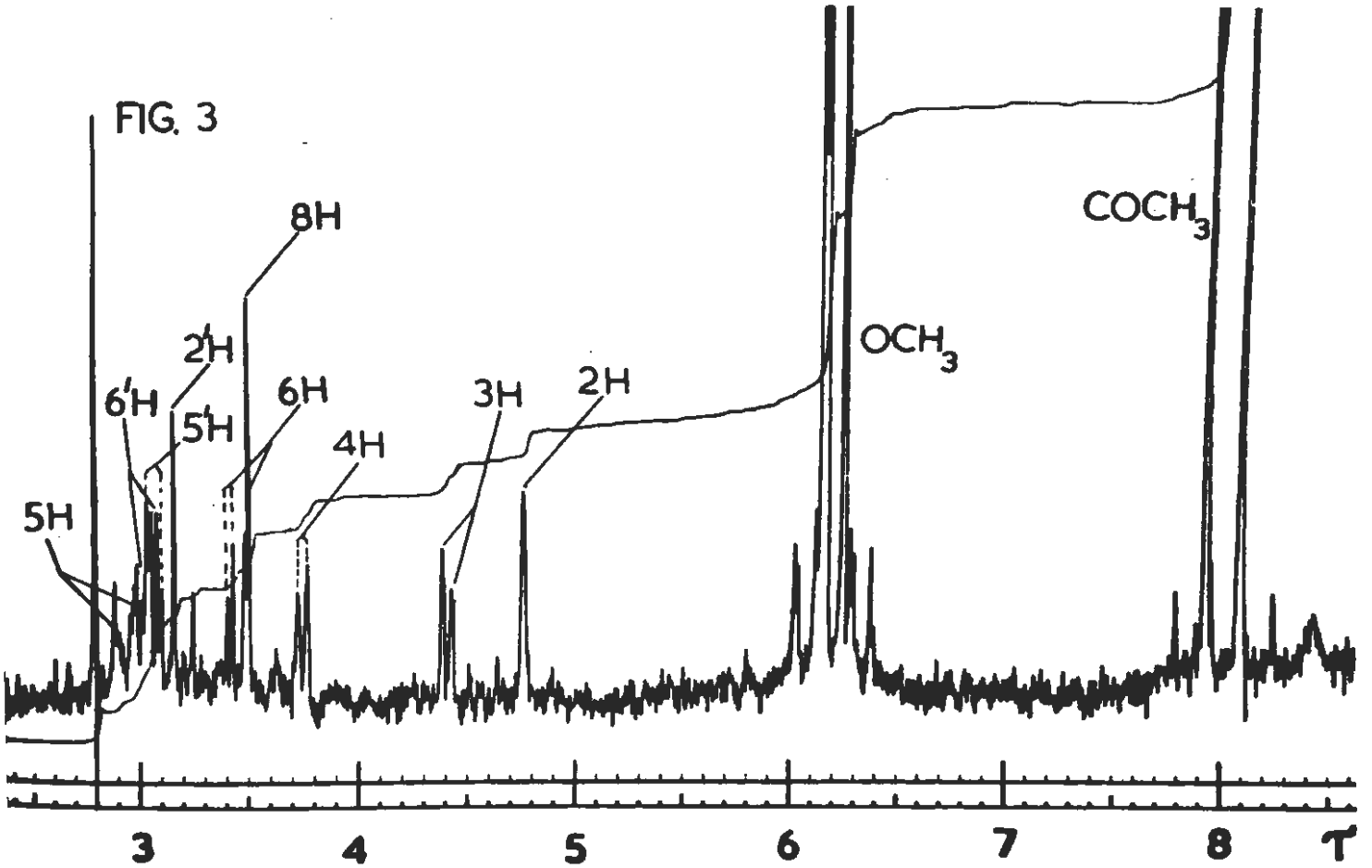
The hexamethyl ether of A gave a mass spectrum (c.f. Figure 2) with molecular ion peak at $^m/e$ 628 (relative abundance 10%) and prominent peaks at $^m/e$ 449 (5%), 298 (100%), 297 (44%), 283 (12%), 267 (10%), 165 (3%), 161 (6%), 151 (17%), 137 (3%).

The hexaacetate of A gave a mass spectrum with a molecular ion peak at $^m/e$ 796 (relative abundance 8%) and prominent peaks at $^m/e$ 754 (7%), 712 (5%), 670 (3%), 628 (2%), 586 (2%), 561 (4%), 544 (2%), 519 (4%), 477 (3%), 435 (2%), 382 (25%),

381 (19%), 340 (55%), 339 (32%), 298 (60%), 297 (48%), 286 (40%), 256 (100%), 255 (95%), 244 (31%), 225 (49%), 152 (22%), 147 (22%), 137 (35%), and 123 (74%).

Isolation and identification of B. The solids (16.0 g.) from tubes 58-76 were dissolved in dilute acetone (425 ml.) and applied to 85 sheets of Whatman No. 3 paper. The chromatograms were developed in 2% ($^{v}/v$) acetic acid by upward migration. They were dried in a current of warm air and side strips (1 cm.) were sprayed with ammoniacal silver nitrate and toluene-p-sulphonic acid. The reducing band (pink to toluene-p-sulphonic acid), running between dimeric and trimeric leucofisetinidins[51], at R_F 0.34-0.46 was cut out for each sheet. The strips were eluted with 70% ($^{v}/v$) ethanol and the combinded eluates concentrated. The resulting solids (1.3 g.) were dissolved in acetone (125 ml.) and applied to 25 sheets of Whatmans No. 3 paper and the above purification procedure was repeated. The resulting material (350 mg.), a mixture of B and dimeric and trimeric leucofisetinidin, was streaked onto 8 sheets of prewashed Whatmans No. 3 paper and developed by descending partition chromatography using water-saturated butan-2-ol as solvent. The strongly reducing band identified by spraying side-strips with ammoniacal silver nitrate was cut from each sheet and eluted with 70% ($^{v}/v$) ethanol. The combined eluates were concentrated under reduced pressure to a small volume. B crystallised out and was obtained as white needles (27 mg.) m.p. 136-138°, $[\alpha]_D^{20}$ = + 49° (c 0.4 in acetone/water). A two dimensional chromatogram with synthetic (+) - 7,3',4'-trihydroxy-2,3-cis-flavan-3,4-cis-diol[80] showed concurrence.

FIGURE 3. N.m.r. Spectrum of the Diacetate of the Trimethyl Ether of B.
(+)-7,3',4'-Trimethoxy-2,3-cis-flavan-3,4-cis-diacetate.



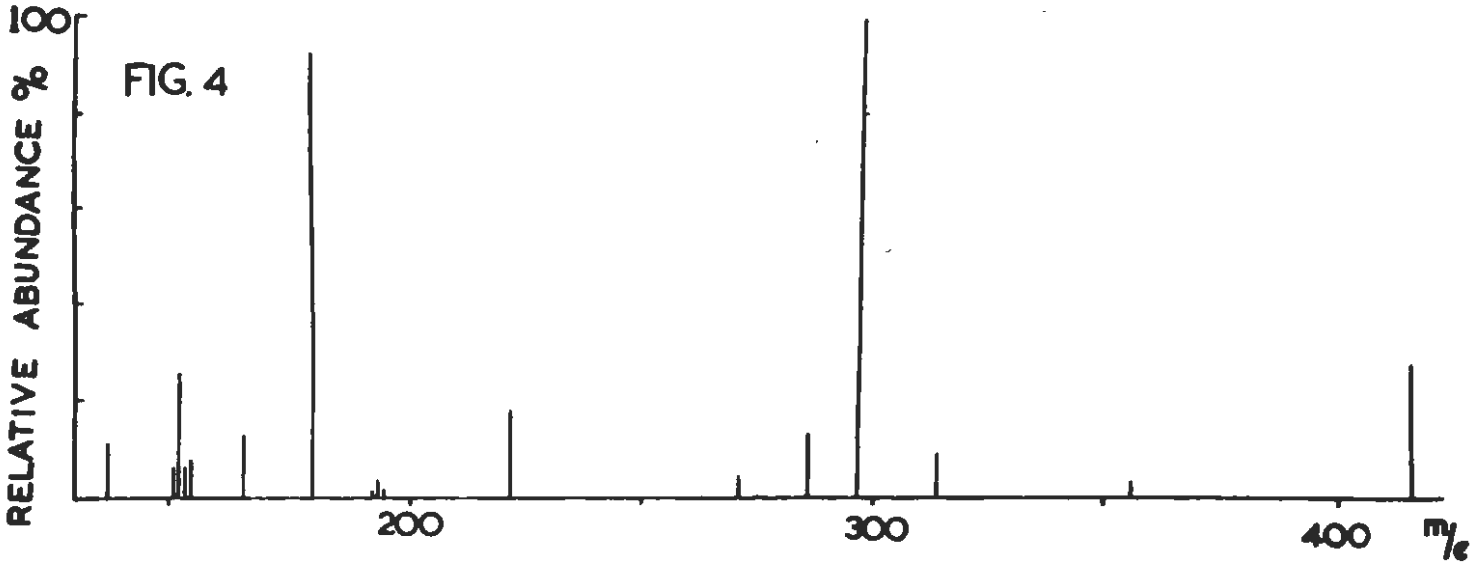
Trimethyl ether of B. Crystalline B (19.5 mg.) was dissolved in methanol (100 ml.) and methylated with diazomethane as before. The reaction mixture was concentrated under reduced pressure yielding an amorphous yellow residue (14.1 mg.) From ethanol white crystals (11.0 mg.) m.p. 127-9° were obtained. A mixed m.p. with synthetic (+)-7,3',4'-trimethoxy-2,3-cis-flavan-3,4-cis-diol[80] gave no depression.

Trimethyl ether diacetate of B. Crystalline trimethyl ether (11 mg.) was treated with acetic anhydride (0.2 ml.) and pyridine (0.2 ml.) at room temperature for 16 hours. The product recovered from water, was crystallised from ethanol to give white needles (9 mg.) m.p. 152°. (Found C, 63.2; H 5.9. Calc. for $C_{22}H_{24}O_8$: C, 63.5; H, 5.8%).

The n.m.r. spectrum of the diacetate confirms its 2,3-cis-3,4-cis configuration.

N.m.r. spectrum of the trimethyl ether diacetate of B. An analysis of the n.m.r. spectrum of the trimethyl ether diacetate of B (c.f. Figure 3.) is as follows:
Acetoxy proton resonances are = 3 OAc, τ 8.10 and 4 OAc, τ 7.92.
Methoxy proton resonances are = 7 OMe, τ 6.22 and 3',4' OMe, τ 6.12.
Benzenoid proton resonances are as follows: 5H, τ 2.92; 6H, τ 3.45; 8H, τ 3.46; 2'H, τ 3.15; 5'H, τ 3.06; 6'H, τ 3.02.
Heterocyclic ring proton resonances are as follows:
2H, τ 4.75; 3H, τ 4.40; and 4H, τ 3.74.

FIGURE 4. Mass Spectrum of the Diacetate of the Trimethyl Ether of B.
(+)-7,3',4'-Trimethoxy-2,3-cis-flavan-3,4-cis-diacetate.



Coupling constants are $J_{5,6} = 7.8$, $J_{6,8} = 2.8$, $J_{5',6'} = 7.4$ c./sec. and $J_{2,3} = 1.0$, $J_{3,4} = 4.3$ c./sec.

Mass spectrum of the trimethyl ether diacetate of B. The mass spectrum (c.f. Figure 4) shows the molecular ion at $^{m}/e$ 416 (relative abundance 28%) and prominent peaks at $^{m}/e$ 356 (2%), 314 (9%), 297 (100%), 286 (14%), 271 (4%), 222 (19%), 195 (1%), 194 (3%), 193 (2%), 180 (92%), 165 (13%), 153 (8%), 152 (6%), 151 (27%), and 137 (12%).

Isolation and proof of structure of C. The solids from tubes 141-160 were dissolved in acetone (225 ml.) and applied to 45 sheets of Whatmans No. 3 paper. The ascending chromatograms were developed in 25% ($^{v}/v$) acetic acid. The air-dried chromatograms, viewed under ultraviolet light, showed three blue fluorescent bands (Band C, $R_F = 0.38$; Band D, $R_F = 0.27$; and Band E, $R_F = 0.25$.) Band C became yellow when fumed with ammonia. It gave a black colour with ammoniacal silver nitrate and deep yellow with toluene-p-sulphonic acid. Band C was cut out from each sheet and eluted with 90% ($^{v}/v$) ethanol. The concentrate (180 mg.) of the combined eluates, (containing a white fluorescent impurity), was reapplied to 20 sheets and the adsorption separation repeated to yield compound C which crystallised from dilute ethanol in needles (45 mg.) m.p. 268-270° (Found C, 63.8; H, 4.6; OMe, 8.4. $C_{16}H_{12}O_6$ requires C, 64.0; H, 4.1; OMe, 10.3%).

Colour reactions. C with ethanolic Mg and HCl gave a cherry-red colour, with ferric alum a green colour and with bis-diazotised benzidine a yellow colour.

Alkali degradation. KOH fusion of C (1.0 mg.) by the method described previously, gave ε-resorcylic acid and

protocatechuic acid as products of degradation.

Ultraviolet and infrared spectra. The main peak in the ultraviolet spectrum of C is at 350 mμ. This was unaffected by the addition of aluminium chloride to an ethanolic solution of the compound. It underwent a bathochromic shift of 13 mμ on the addition of boric acid-sodium acetate . The carbonyl absorption band in the i.r. spectrum is at 1607 cm^{-1}.

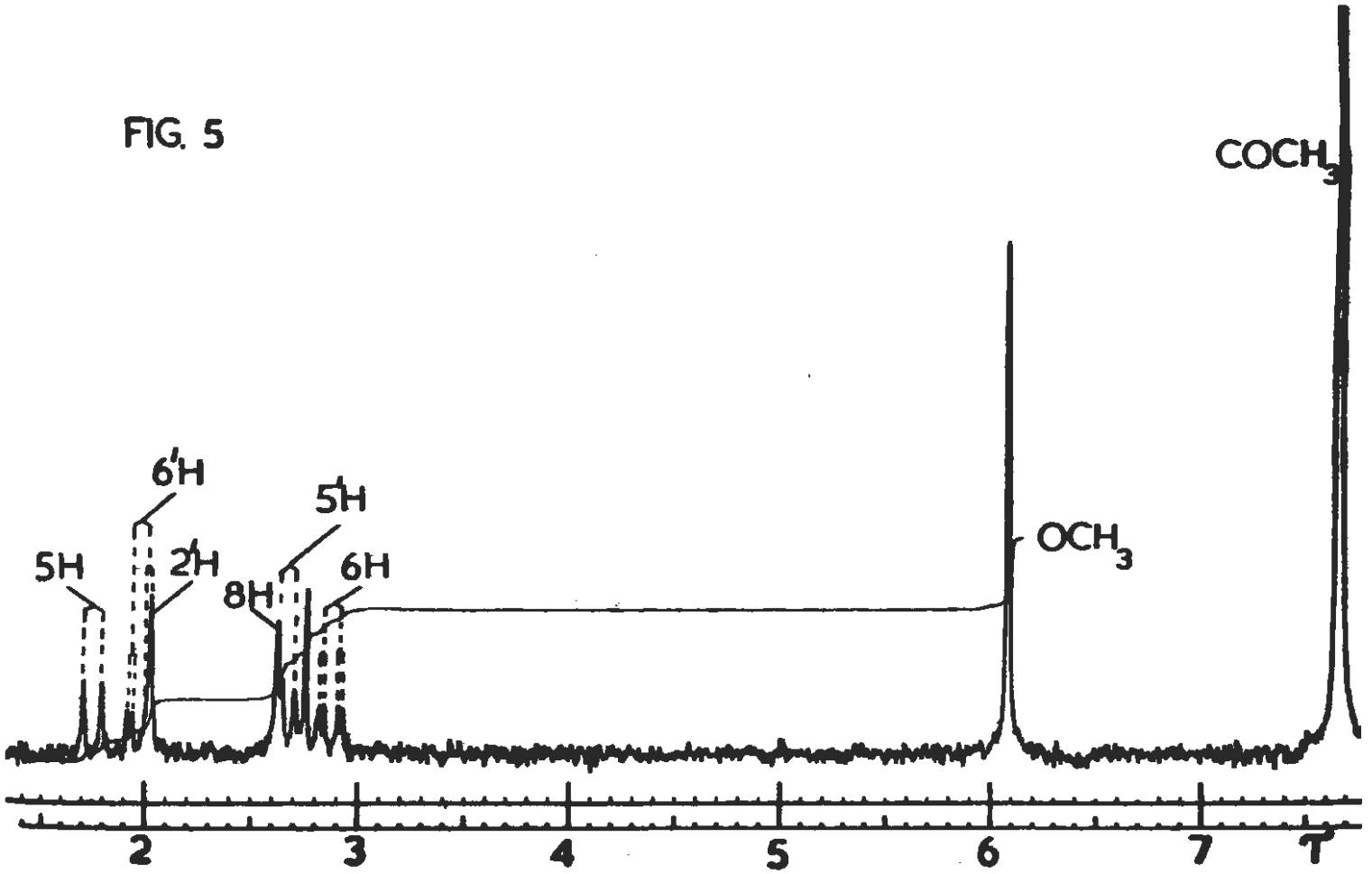
Triacetate of C. C (25 mg.) was treated with acetic anhydride (0.25 ml.) and pyridine (0.25 ml.) for 16 hours at 30°. The amorphous white powder (27 mg.), recovered from water, crystallised from methanol as white prisms (20 mg.) m.p. 145-147° (Found: C, 61.7; H, 4.4. $C_{22}H_{18}O_9$ requires C, 62.0; H, 4.2%). The n.m.r. and mass spectra are in agreement with the analysis.

Methyl ether of C. C (20 mg.) was dissolved in methanol (100 ml.) and treated with ethereal diazomethane as before. Concentration of the reaction mixture under reduced pressure yielded an amorphous white powder (27 mg.) which crystallised from methanol as elongated prisms (21 mg.) m.p. 152° (Found: C, 66.6; H, 5.2. Calc. for $C_{19}H_{18}O_6$: C, 66.7, H, 5.3%). A mixed m.p. with authentic tetra-O-methylfisetin gave no depression, m.p. 152°, and comparison of the n.m.r. and mass spectra of these two compounds showed complete identity.

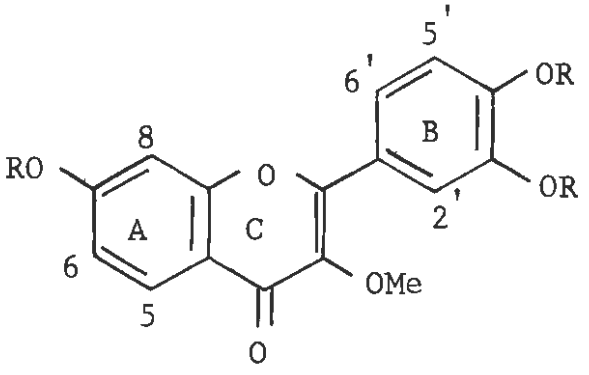
Authentic tetra-O-methylfisetin. Methylation of fisetin (3,7,3',4' - tetrahydroxyflavone) (Koch and Light) (25 mg.) by the above method yielded tetra-O-methylfisetin (3,7,3',4' - tetramethoxyflavone) as elongated rods (19 mg.) m.p. 152°.

N.m.r. spectra of derivatives of C. Table 1 gives the analyses of the n.m.r. spectra of the triacetate of C

FIGURE 5. N.m.r. Spectrum of the Triacetate of C - 7,3',4'-Triacetoxy-3-methoxyfisetin.



(c.f. Figure 5), the methyl ether of C (c.f. Figure 6), and authentic tetra-O-methyl-fisetin (c.f. Figure 7). Structures of the derivatives of C are given by (III).



(III)

Triacetate of C: R = Ac

Methylether of C: R = Me

FIGURE 6. N.m.r. Spectrum of the Trimethyl Ether of C - 3,7,3',4'-Tetramethoxyfisetin.

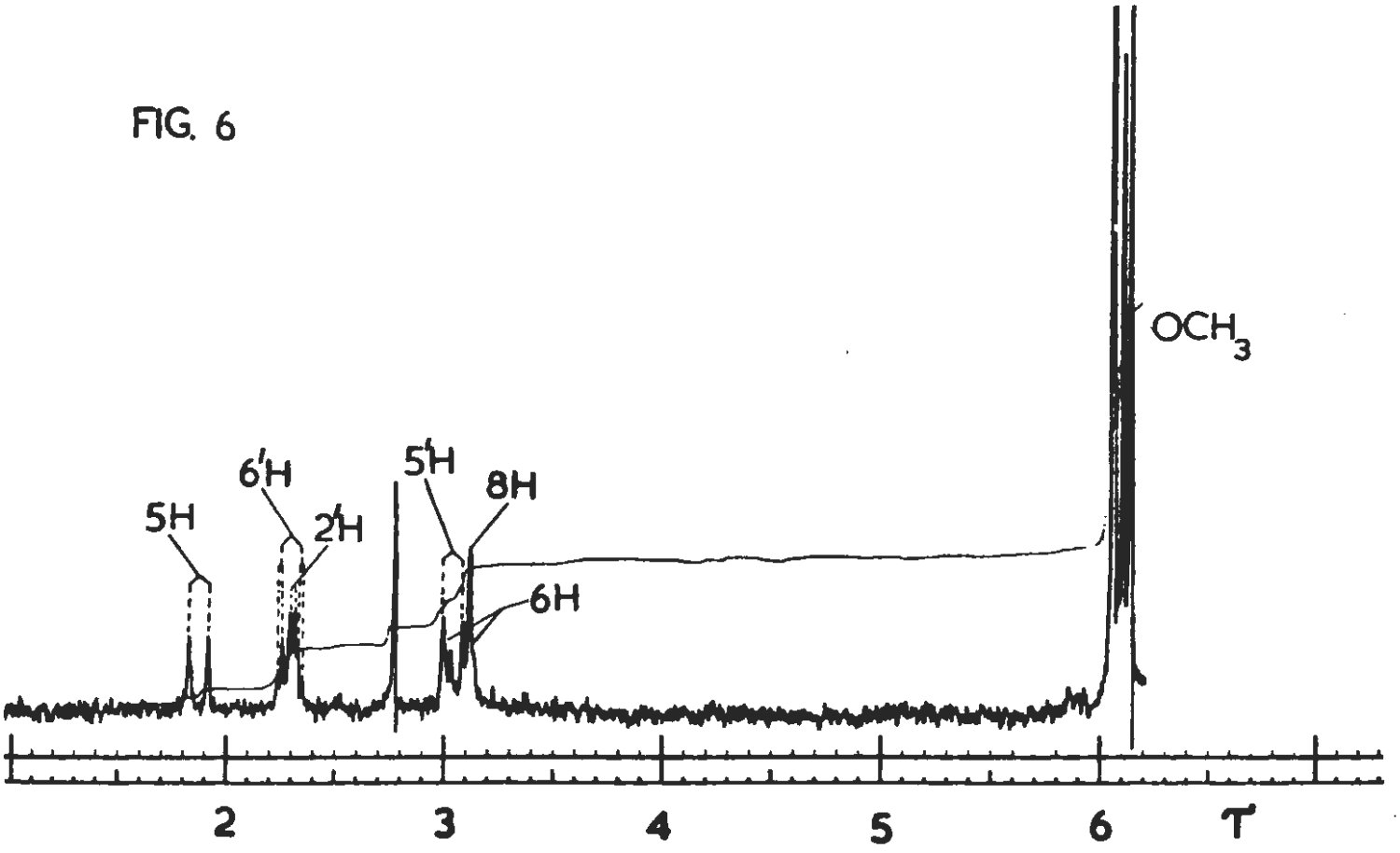


FIGURE 7. N.m.r. Spectrum of Tetramethoxyfisetin.

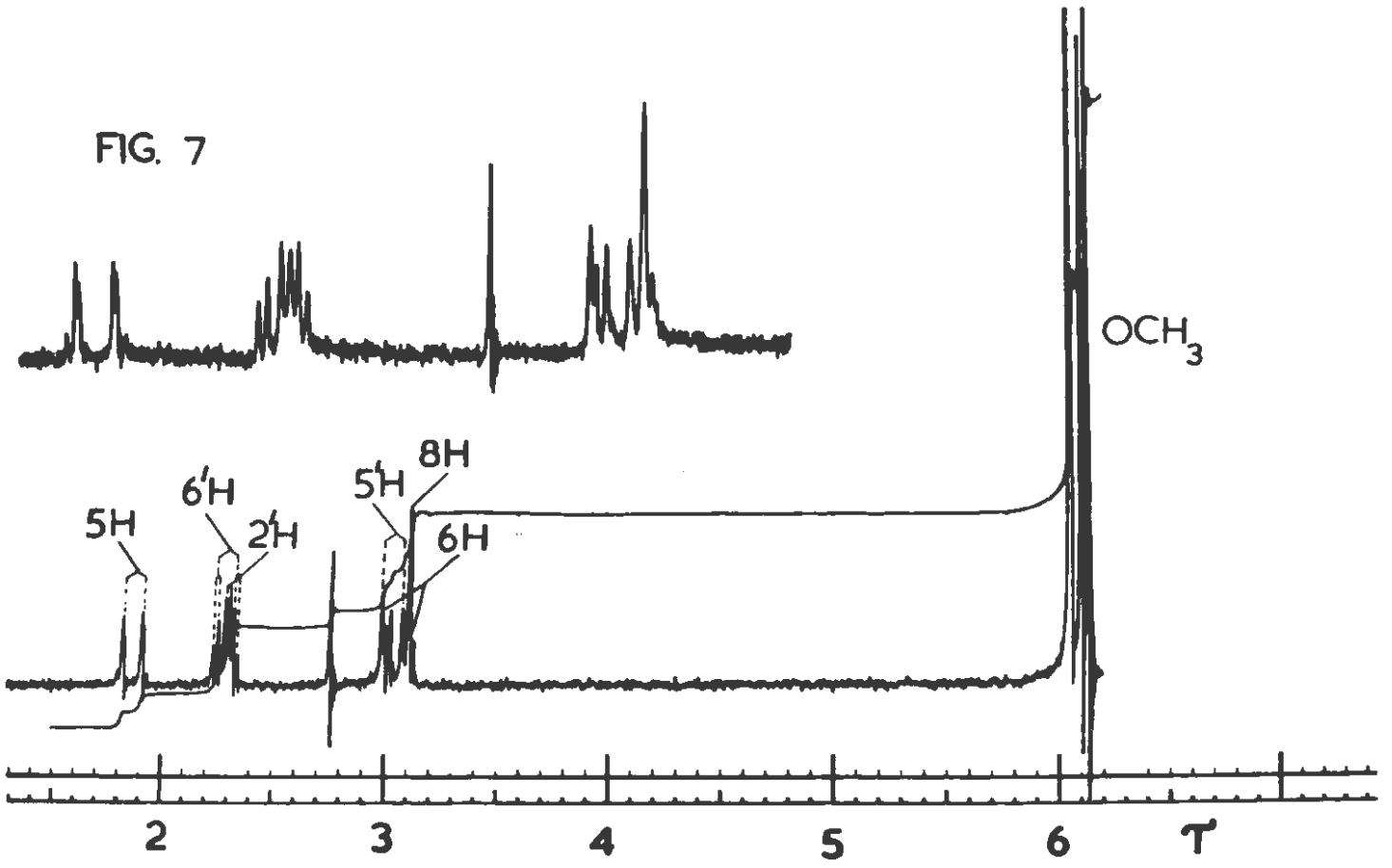


TABLE 1. N.m.r. spectra of derivatives of 3-methoxyfisetin

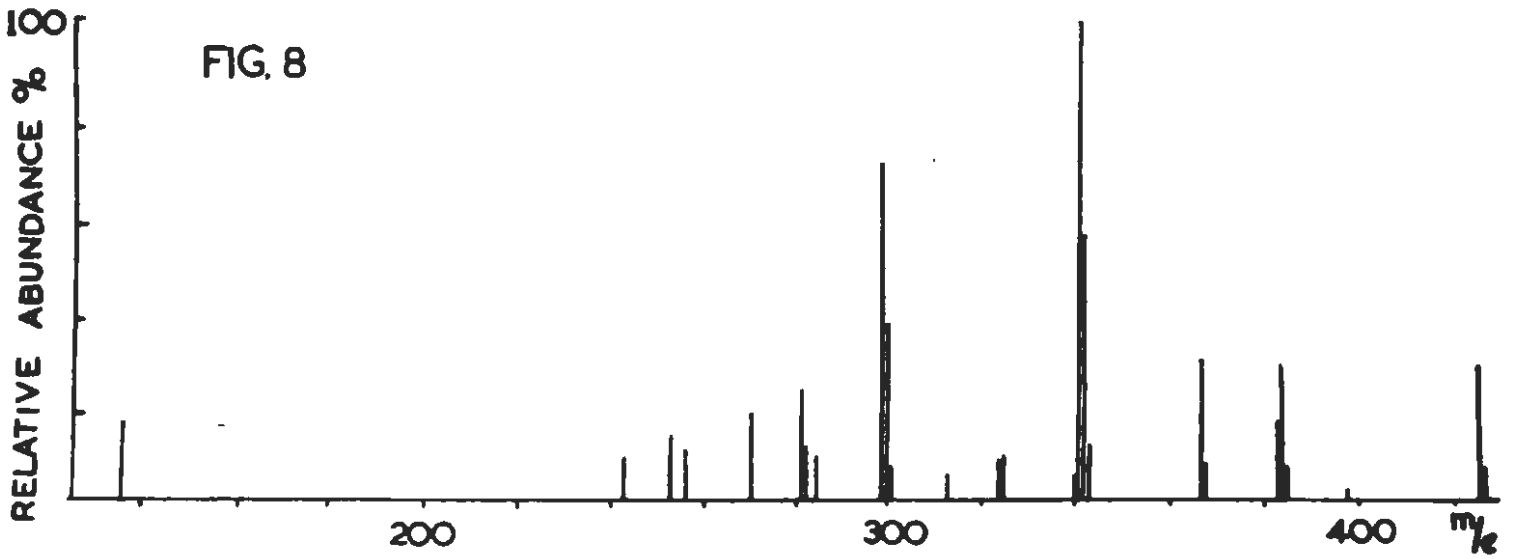
Chemical Shifts

	Methoxy			Acetoxy		Benzenoid					
	3	7	3'+4'	7	3'+4'	5	6	8	2'	5'	6'
Derivative											
Ac_3C	6.07			7.69	7.64	1.75	2.87	2.62	2.02	2.66	1.97
Me_3C	6.08	6.12	6.02			1.87	3.06	3.11	2.30	3.03	2.28
$Me_4Fis.$	6.10	6.13	6.05			1.86	3.05	3.11	2.29	3.03	2.28

Spin-spin Coupling Constants c./sec.

Derivative	$J_{5,6}$	$J_{6,8}$	$J_{5',6'}$	$J_{2',6'}$
Ac_3C	8.6	2.0	9	2.4
Me_3C	8.8		9.4	2.2
$Me_4Fis.$	8.6	2.2	9.3	2.2

FIGURE 8. Mass spectrum of the Triacetate of C - 7,3',4'-Triacetoxy-3-methoxyfisetin.



Mass spectrum of triacetate of C. The mass spectrum of the triacetate of C (c.f. Figure 8) shows a molecular ion at $^{m}/e$ 426 (relative abundance 27%) and prominent peaks at 398 (2%), 385 (6%), 384 (28%), 383 (16%), 368 (7%), 367 (29%), 356 (1%), 343 (11%), 342 (55%), 341 (100%), 340 (5%), 325 (9%), 324 (8%), 314 (2%), 313 (5%), 301 (6%), 300 (36%), 299 (70%), 285 (8%), 283 (10%), 282 (23%), 271 (17%), 257 (10%), 254 (13%), 244 (8%), and 137 (16%).

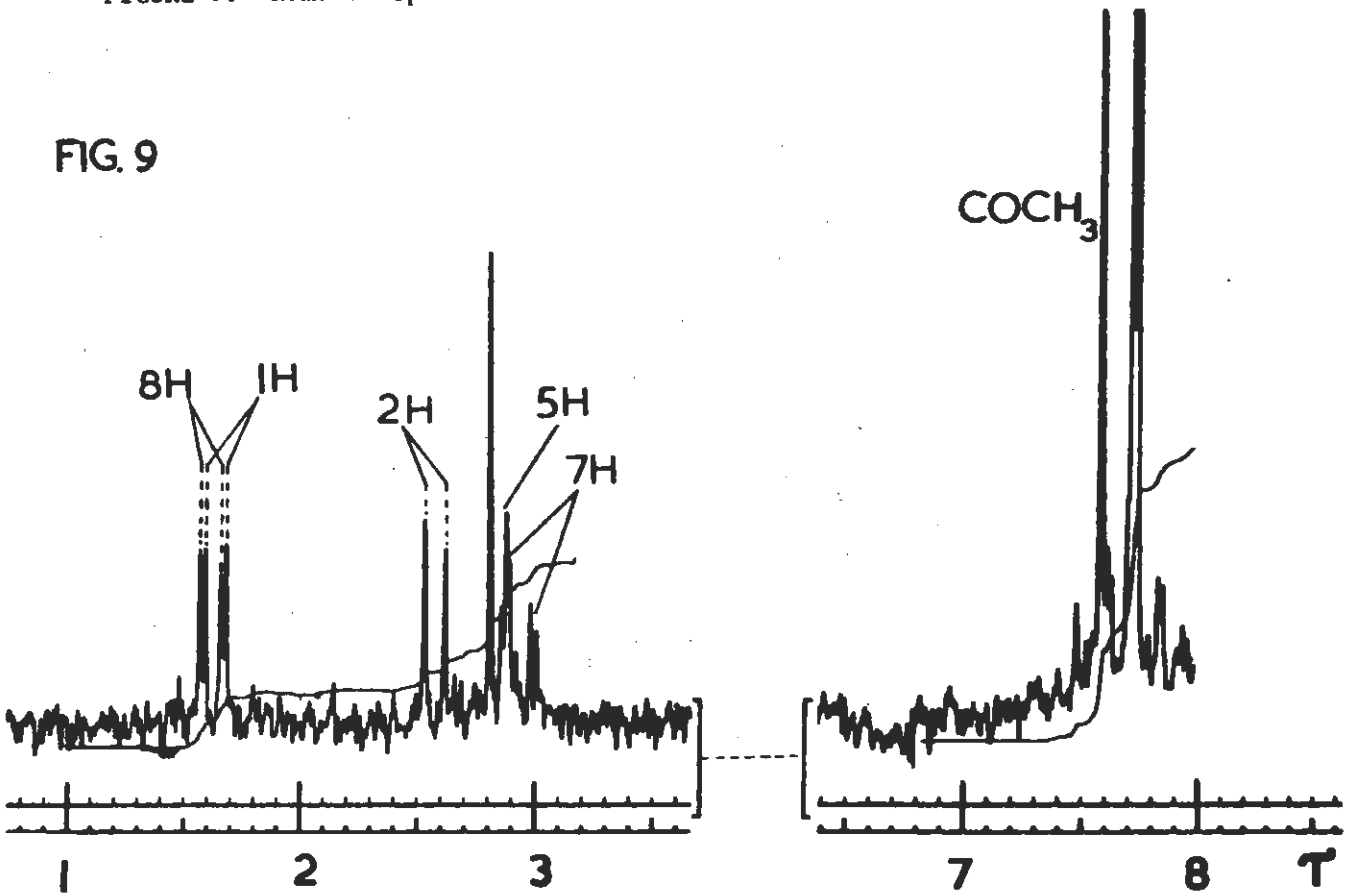
Isolation of D. From the chromatograms used for the isolation of C, the blue fluorescent band, immediately behind that for compound C and having R_F 0.27 (red-brown to ammoniacal silver nitrate) was cut out from each sheet. These were eluted with 90% ($^{v}/v$) ethanol and the combined eluates concentrated to yield a light brown solid (59 mg.) This still contained C as impurity. Purification was effected by crystallisation from ethanol yielding D as white rosettes of needles (32 mg.) m.p. 291°. M^+ 244 by mass spectrometry.

D gave an intense yellow colour with NaOH solution and a green colour with ferric chloride. KOH fusion under conditions described previously gave β-resorcylic acid, pyrogallol-4-carboxylic acid, and resorcinol. A large percentage of starting material remained in the phenolic fraction.

Ultraviolet spectra of D. The u.v. spectrum of D showed major peaks at 257 mμ and 336 mμ. Addition of boric acid - sodium acetate caused these peaks to shift to 262 mμ and 347 mμ respectively.

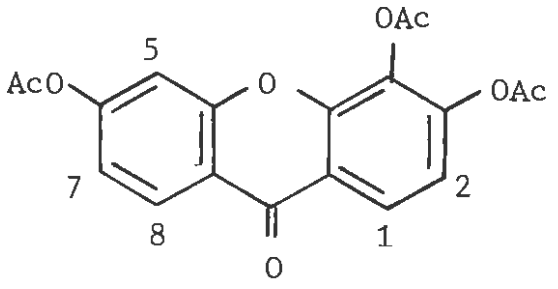
Triacetate of D. D (12 mg.) was treated with acetic anhydride (0.2 ml.) and pyridine (0.2 ml.) in a stoppered

FIGURE 9. N.m.r. Spectrum of the Triacetate of D - 3,4,6-Triacetoxyxanthone.



vessel at 30° for 17 hours. The white solid recovered from water crystallised from ethanol as white needles (10 mg.) m.p. 201° N.m.r. and mass spectra (M^+ 370) show the presence of three aromatic acetyl groups.

N.m.r. spectrum of the triacetate of D. An analysis of the n.m.r. spectrum of the triacetate of D (c.f. Figure 9) is given below. The proposed structure of the triacetate is given by (IV)



(IV)

Acetoxy proton resonance for 9 protons is at τ 7.56, τ 7.69, and τ 7.71. Benzenoid proton resonances are as follows: 1H, τ 1.63; 2H, τ 2.58; 5H, τ 2.88; 7H, τ 2.93; 8H, τ 1.61. Coupling constants are $J_{1,2}$ = 8.8, $J_{5,7}$ = 2.2, and $J_{7,8}$ = 8.8 c./sec.

Mass spectra. The mass spectrum of the triacetate of D has a molecular ion peak at $^m/e$ 370 (relative abundance 10%) and prominent peaks at $^m/e$ 328 (18%), 286 (60%), 244 (100%), and 202 (6%). Metastable peaks appear at $^m/e$ 290 (370→328), 249 (328→286), 208 (286→244), and 167.5 (244→202). The mass spectrum of the free phenolic form has a molecular ion peak at $^m/e$ 244 (relative abundance 100%)

and an M-42 peak at $^{m}/e$ 202 (46%). A metastable peak for this transition appears at $^{m}/e$ 167.5.

FIGURE 10. Two Dimensional Chromatogram of the Heartwood Extract of Acacia mearnsii. Solvents:Direction 1, water-saturated butan-2-ol. Direction 2, 2% (v/v) acetic acid.

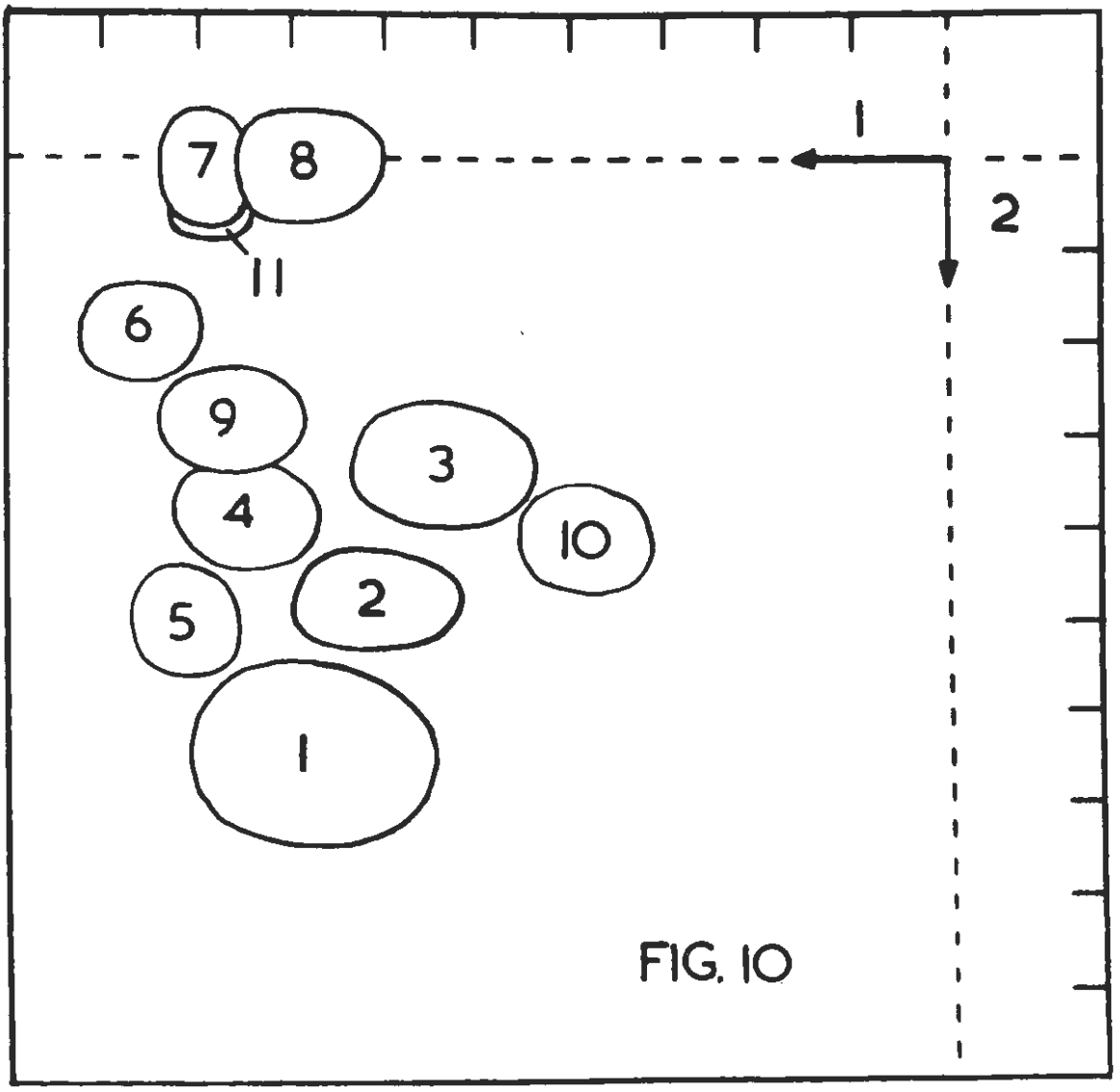


FIG. 10

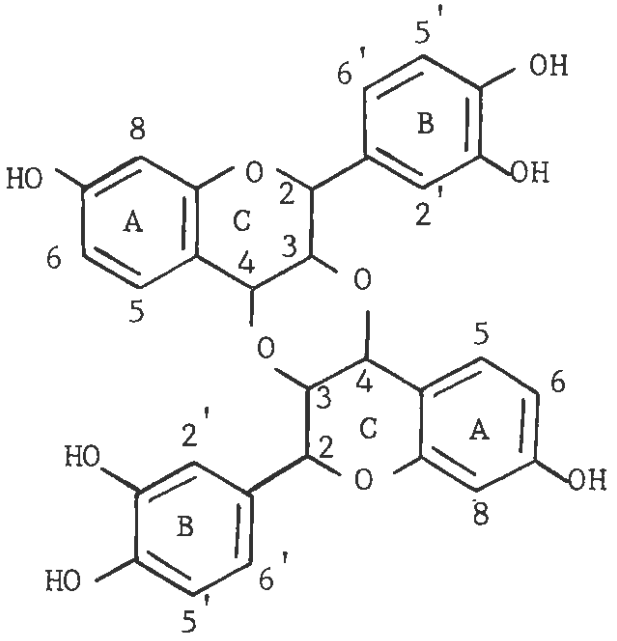
1. (+)-mollisacacidin
2. dimeric leucofisetinidins
3. trimeric leucofisetinidin
4. (+)-fustin
5. (-)-fisetinidol
6. (-)-butin
7. butein
8. fisetin
9. Compound A.
10. Compound B.
11. Compound C,D and E.

DISCUSSION.

Novel Components of Acacia Mearnsii Heartwood.

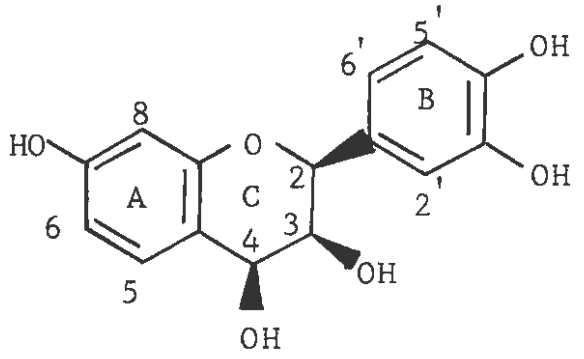
The extractives of Acacia mearnsii heartwood present an intricate pattern consisting of polymeric leucofisetinidin tannins, the main monomeric component (+)-mollisacacidin, and a series of interrelated flavonoids based on a similar pattern of hydroxy substitution. The two dimensional paper chromatogram of the heartwood extract, developed in water-saturated butan-2-ol and 2% ($^{v}/v$) acetic acid, is reproduced in Figure 10. Areas 1 to 8 represent the known components of the heartwood extract while areas 9 to 11 represent five new compounds. Four of these new compounds have been isolated and identified.

Compound A has been shown to be a dimeric proanthocyanidin based on leucofisetinidin and to be linked by a dioxane ring via C3 and C4 of one moiety to C4 and C3 respectively of the other [81] as shown by structure (1)



(I)

Compound B has been identified as (2S:3S:4S)-(+)-7,3',4'-trihydroxy-2,3-cis-flavan-3,4-cis-diol (II), a diastereoisomer of (+)-mollisacacidin.



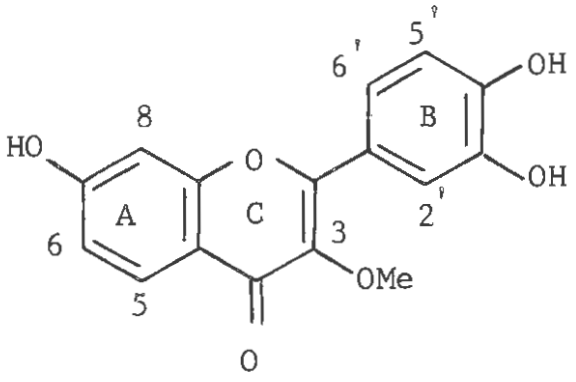
(II)

Re-examination of the water-insoluble baseline components, represented by areas 7,8, and 11 in Figure 10, reveals the presence of three blue-fluorescent compounds C,D, and E, which travel out from fisetin and butein when the chromatogram of the heartwood extract is developed in 25% (^{v}/v) acetic acid. These compounds partly underlie fisetin and are obscured by it when the chromatogram is developed in 2% (^{v}/v) acetic acid.

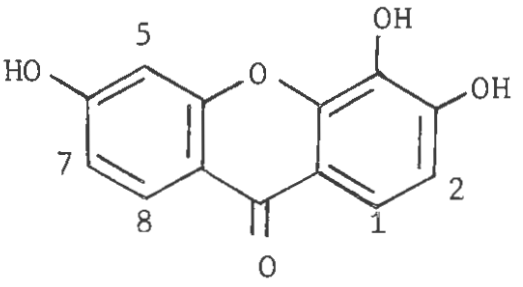
Compound C is 3-methoxyfisetin [82] (7,3',4'-trihydroxy-3-methoxyflavone) (III), the 3-methyl ether of the heartwood flavonol, fisetin.

Compound D is thought to be a trihydroxyxanthone and has tentatively been assigned the structure (IV).

Compound E is present in extremely low concentration, was very unstable and hence was not isloated.



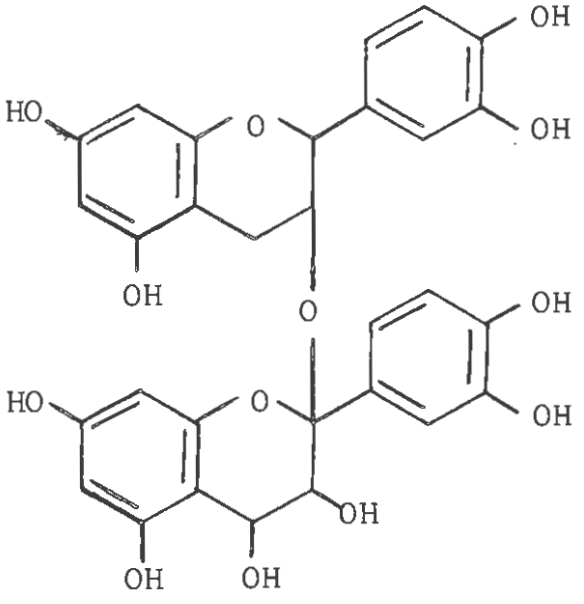
(III)



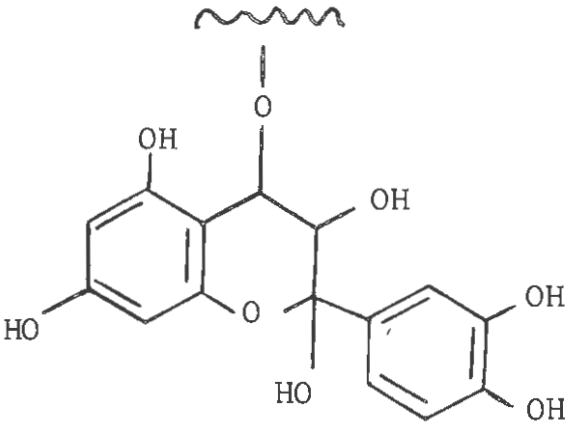
(IV)

Some naturally-occurring dimeric proanthocyanidins related to Compound A.

Various ether-linked dimeric proanthocyanidins isolated from natural sources have been reported. From Crataegus oxyacantha, Weinges [83] isolated a dimeric proanthocyanidin and postulated a C3 to C2 (V) or C3 to C4 (VI) ether link for the compound.

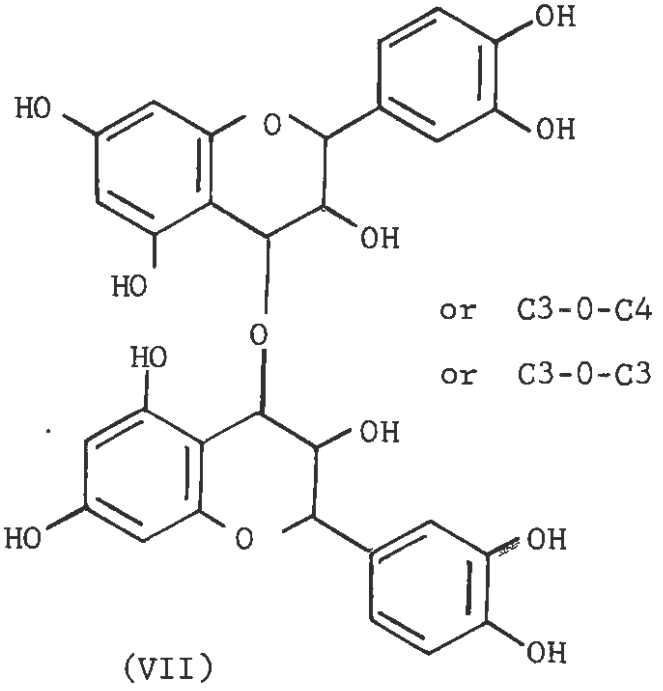


(V)



(VI)

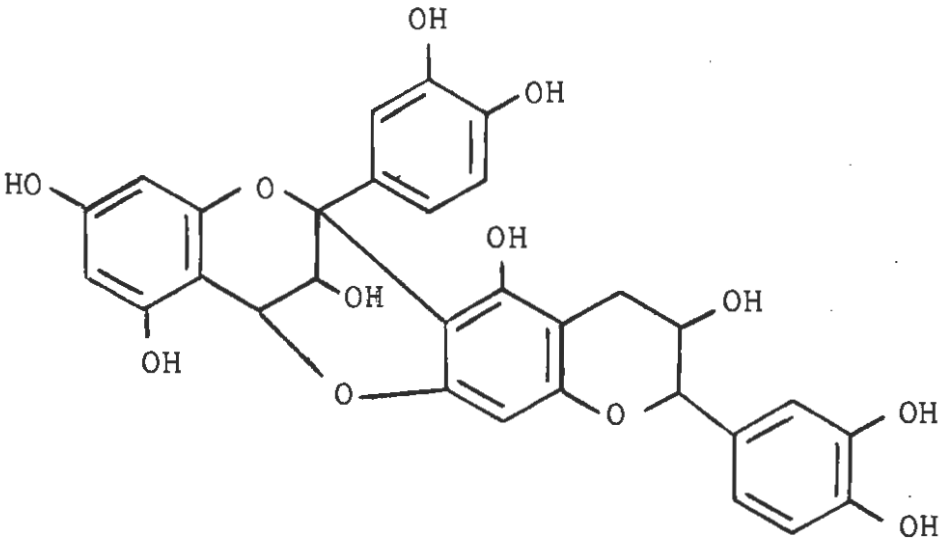
From the same plant source, Lewak [84] obtained a dimeric flavan-3,4-diol (VII) for which he proposed an ether-link between the heterocyclic alcohol groups.



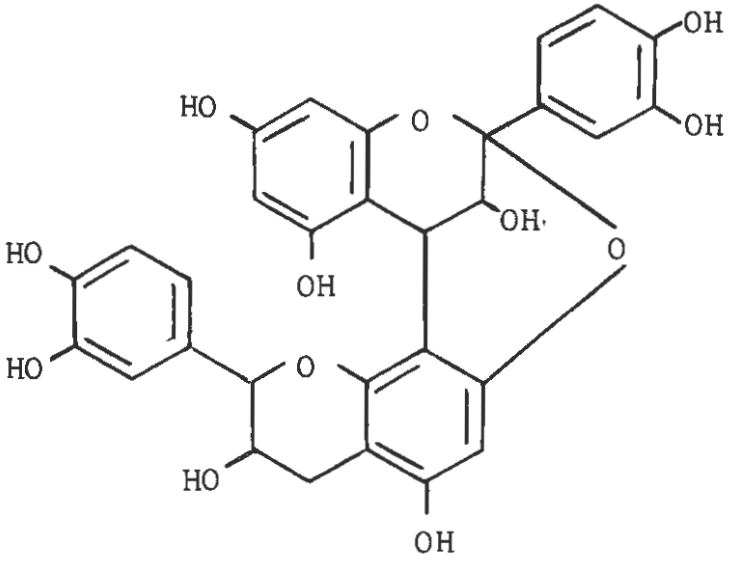
(VII)

From the unripe seed-pods and bark of Aesculus hippocastanum, Mayer and co-workers [85] isolated a double-linked dimeric proanthocyanidin (VIII) which was shown by n.m.r. spectral evidence to have a C2 to C6 (or C8)link and a C4 to C7 ether link.

Recently Weinges and co-workers [86] re-isolated this compound (VIII) as well as isomeric proanthocyanidins from Vaccinium vitus-idaea (cranberries) and Cola acuminata (cola nuts). These compounds, which yielded cyanidin and (-)-epicatechin on heating with alcoholic mineral acid, had the molecular formula $C_{30}H_{24}O_{12}$, and were classified as Group A compounds. Weinges postulated an alternative structure (IX) with a C4 to C6 (or C8) link and a C2 to C7



(VIII)

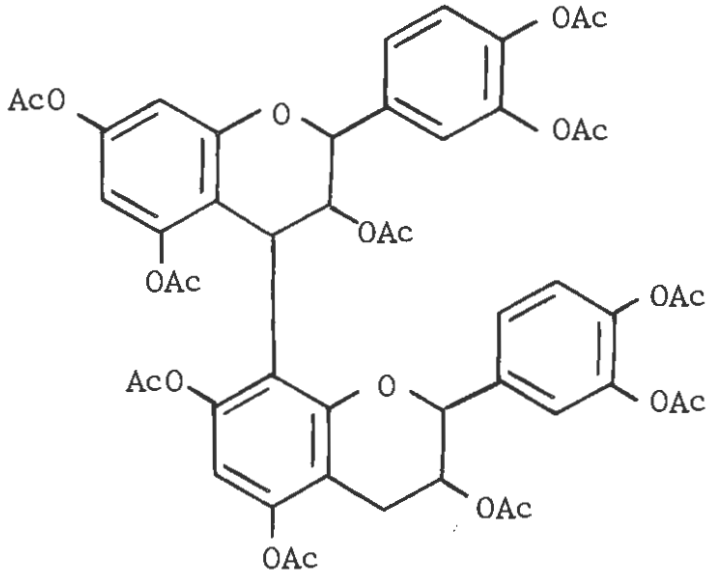


(IX)

ether link for the proanthocyanidins from cranberries and cola nuts but confirmed the structure for the proanthocyanidin from Aesculus hippocastanum as postulated by Mayer [85].

Other dimeric proanthocyanidins isolated from natural sources have all possessed a carbon-to-carbon, C4 to C6 (or C8), link between the two C15 flavan units. These include the proanthocyanidins from Myrica naga [87], from the Ouratea spp [88] and from the heartwood and bark extracts of Acacia mearnsii. [55,56,72,73].

Weinges [86] has classified the C4 to C6 (or C8) linked dimeric proanthocyanidins from Crataegus oxyacantha, Wistaria sinensis, Vaccinium vitis-idaea, Cola acuminata, and Gleditsia triacanthos which have the molecular formula $C_{30}H_{26}O_{12}$ as Group B. This group also includes the proanthocyanidins isolated from Persea gratissima by Geissman and Dittmar [89], and from Eucalyptus camaldulensis by Nisi and Panizzi [90].



(X)

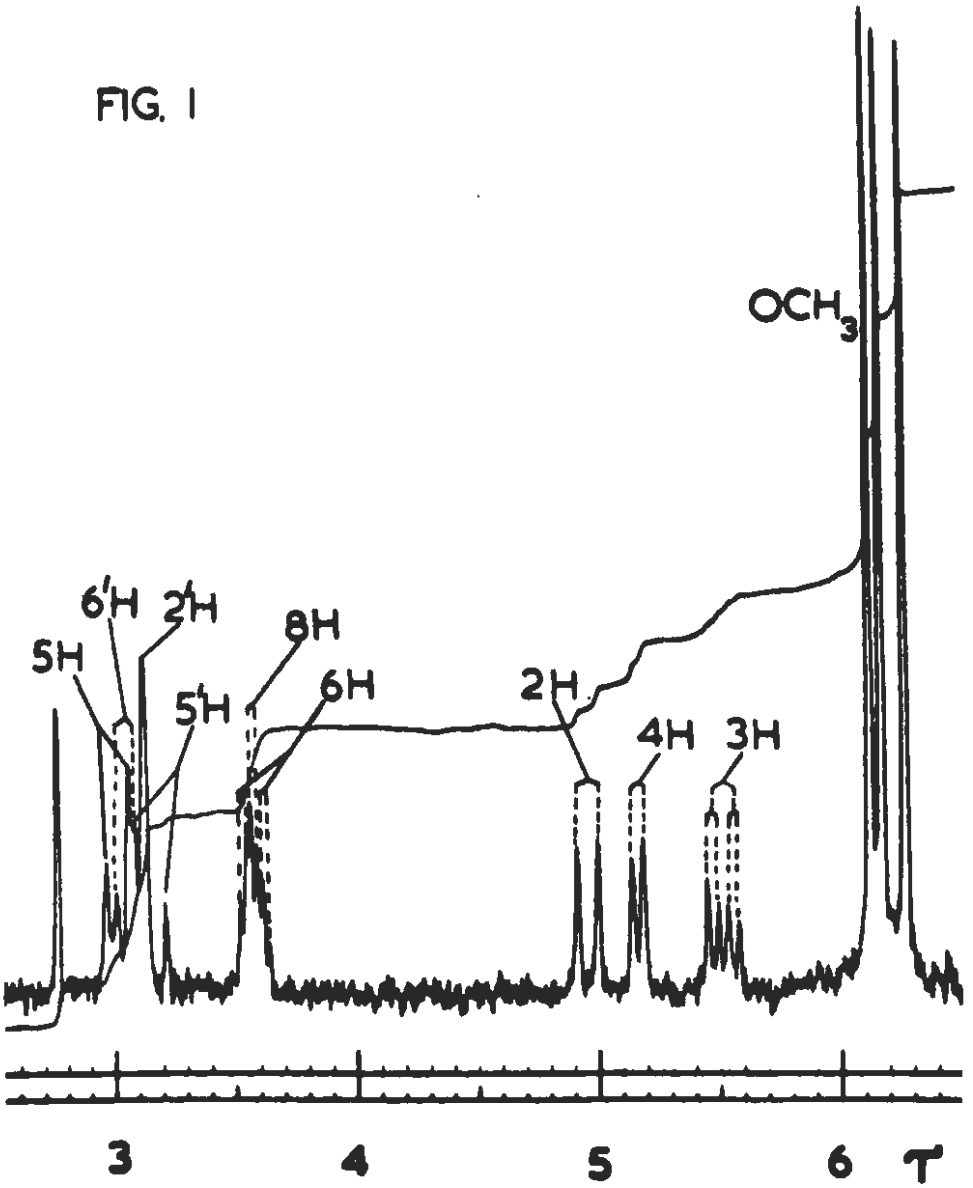
A recent publication by Weinges and co-workers [91] reports that the decaacetoxy derivatives of the proanthocyanidins from the above-mentioned fruits are four diastereoisomers (X) made up of units of (+)-catechin and (-)-epicatechin. The relative configurations of the diastereoisomers are given and the absolute configuration at C4 of the diastereoisomers is discussed.

Isolation and proof of structure of A.

Pure, non-crystalline A was obtained from Acacia mearnsii heartwood enriched initially by Craig counter-current separation followed by preparative paper chromatography. Treatment with mineral acid and heat resulted in the formation of a reddish-pink anthocyanidin which bore a close resemblance to fisetinidin chloride (7,3',4'-trihydroxy flavylium chloride). The chromophores of these two anthocyanidins had identical visible absorption maxima. The degradation products of A on alkali fusion with KOH were β-resorcylic acid and proto-catechuic acid which shows that A has similar phenolic hydroxy substitution to mollisacacidin. This preliminary evidence indicated that A was either an isomer or a polymer of leucofisetinidin. The eight possible stereoisomers of leucofisetinidin have already been identified [80] but the compound differed from these, thus indicating that A was probably a polymer of leucofisetinidin.

Methylation with diazomethane yielded a crystalline, optically active hexamethyl ether. The hexamethyl ether failed to form an acetate on heating with acetic anhydride/pyridine and showed no ionophoretic mobility in borate solution indicating the absence of a 3,4-cis-diol group and possibly the complete absence of any aliphatic hydroxy groups. Acetylation of A yielded an amorphous hexaacetate.

FIGURE 1. N.m.r. Spectrum of Hexamethyl Ether of A. Dioxane-linked Dimeric Leucofisetinidin.



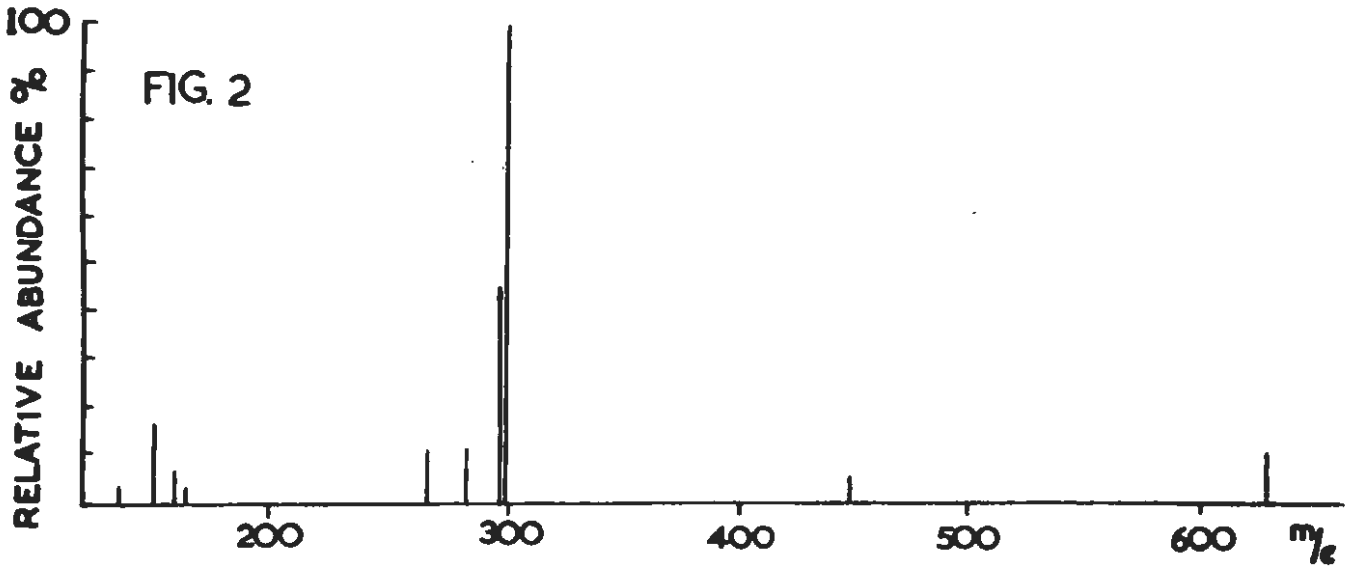
Evidence from n.m.r. and mass spectra of the hexamethyl ether (M^+ 628) and the hexaacetate (M^+ 796) is consistent with A being a dioxane-linked biflavanoid with a symmetrical structure around the two ether oxygens.

Compound A is the first reported [81] naturally occuring dimer of the dioxane type. Clark-Lewis and Williams [92] have suggested a similar structure for a synthetic dimer of teracacidin (7,8,4'-trihydroxyflavan-3,4-diol) obtained from ethanolysis of 7,8,4'-trihydroxy-2,3-trans-flavan-3,4-cis-diol. Maitte and co-workers [93] have synthesised a dioxane-linked dichroman by treating chroman-3,4-diol with toluene-p-sulphonic acid.

N.m.r. spectra.

The n.m.r. spectrum of the hexamethyl ether of the compound (Figure 1.) is essentially monomeric in character and bears a close resemblance to that of 7,3',4'-trimethoxy-2,3-trans-flavan-3,4-cis-diol, indicating that the corresponding protons of each C15 unit have identical magnetic environments and that each absorption band is due to two protons. A similar effect is clearly shown in the n.m.r. spectrum of the biflavonyl, AC_3, isolated from Araucaria cunninghamii by Rhahman and Bhatnagar [94]. The spectrum of the hexamethyl ether of A shows 12 benzenoid protons which excludes a link from an aromatic ring. Further, the six heterocyclic protons give rise to two superimposed first-order ABC systems which indicate that the mode of linkage must be via C3 and C4 of each moeity. The molecular weights of the hexamethyl ether and the hexaacetate of A are consistent with the mode of linkage being via two ether-links. A Dreiding model of A illustrates that only with two ether links from C3 and C4 of the one half of the molecule to C4 and C3 respectively

FIGURE 2. Mass Spectrum of Hexamethyl Ether of A - Dioxane-linked Dimeric Leucofisetinidin.



of the other half, will the corresponding protons of the two moeities have identical magnetic environments.

The chemical shifts of the heterocyclic protons (2H, τ = 4.95; 3H, τ = 5.30; 4H τ = 5.16) correspond with those of the teracacidin dimer synthesised by Clark-Lewis [95] (2H, τ = 5.12; 3H, τ = 5.56; 4H, τ = 5.15).

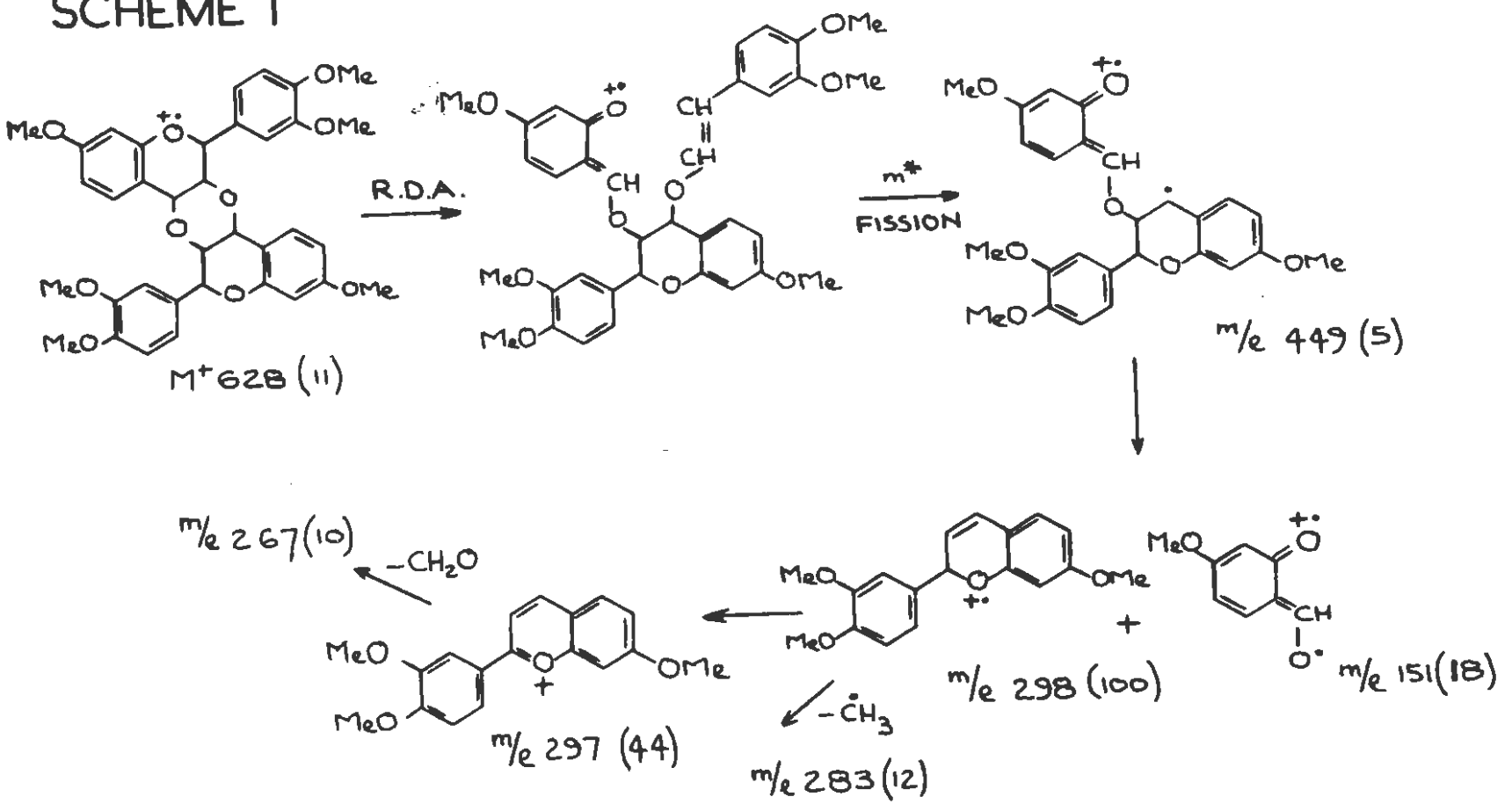
The n.m.r. spectrum of the hexaacetate resembles that of the hexamethyl ether of compound A with the chemical shifts of the heterocyclic protons corresponding. This can be anticipated because of the similar chemical environments of these protons in the two derivatives. Six acetyls can be counted from the integral of the singlet centred at τ, 7.72.

Mass spectra.

Accurate mass measurement on the molecular ion peak of the hexamethyl ether of A gave $^{m}/e$ 628.233269. This is in good agreement with the theoretical value of $^{m}/e$ 628.230850 for a molecular formula of $C_{36}H_{36}O_{10}$.

The mass spectrum of the hexamethyl ether of A (Figure 2) has a strong molecular ion peak at M^{+} 628 (Scheme 1). This ion, after an initial retro Diels-Alder rearrangement, undergoes homolytic fission across one of the ether links to give a fragment $^{m}/e$ 449. This transition is characterised by an intense metastable peak at the theoretical value of 321.0. Further fission across the remaining ether link yields the 7,3',4'-trimethoxyflav-3,4-ene ion $^{m}/e$ 298 and the ion-radicle $^{m}/e$ 151. Proton loss from the trimethoxyflavene ion gives an oxonium ion $^{m}/e$ 297 which loses CH_2O to give the fragment $^{m}/e$ 267.

SCHEME I


OMe
MeO
OMe
MeO
OMe
MeO
OMe
M+628 (11)
R.D.A.
CH
CH
CH
O
MeO
OMe
MeO
OMe
m*
FISSION
MeO
CH
O
MeO
OMe
MeO
m/e 449 (5)
m/e 267(10)
-CH2O
MeO
OMe
MeO
m/e 297 (44)
MeO
OMe
MeO
m/e 298 (100)
+
MeO
CH
O
m/e 151(18)
-CH3
m/e 283 (12)

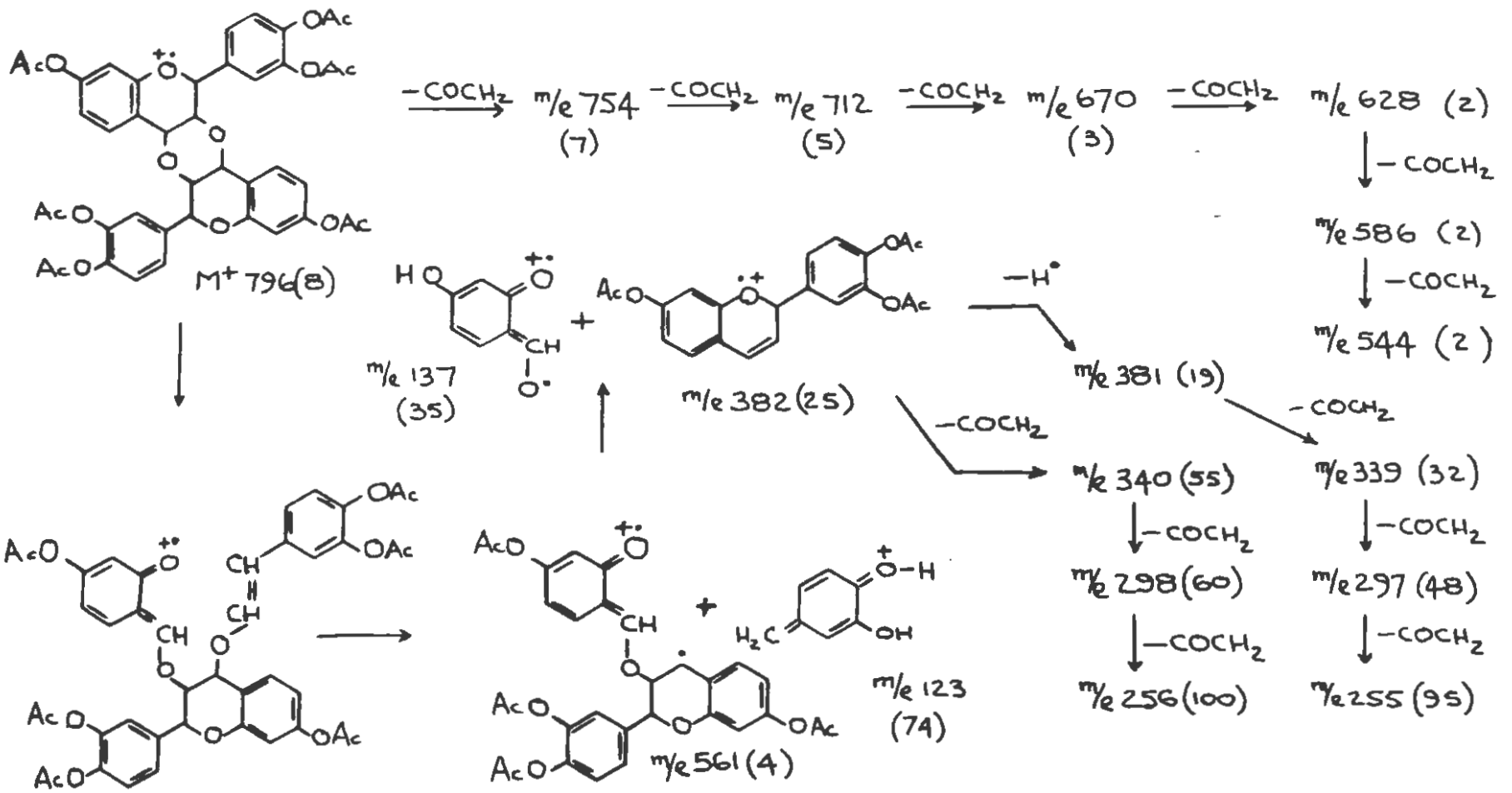
The mass spectrum of the hexacetate of A (scheme 2) shows the successive loss of six acetyls from the molecular ion $^{m}/e$ 796. The molecular ion also undergoes a retro Diels-Alder rearrangement with subsequent homolytic fission yielding the corresponding ion at $^{m}/e$ 561 and the fragment $^{m}/e$ 123. The fragment $^{m}/e$ 561 undergoes further fission to give the 7,3',4'-triacetoxyflav-3,4-ene ion $^{m}/e$ 382 and the ion at $^{m}/e$ 137. From the triacetoxyflavene ion and the ion at $^{m}/e$ 381 formed by proton elimination from the flavene ion, the successive loss of three acetyls can be followed in each case.

Stereochemistry of A.

The relative configuration of the assymetric centres can be discussed most conveniently by comparing the n.m.r. spectrum of the hexamethyl ether of A with those of the derivatives of stereoisomeric flavan-3,4-diols recorded by Drewes and Roux [80] and Clark-Lewis, Jackman, and Spotswood [69]. The coupling constants for the heterocyclic protons of A are $J_{2,3}$ = 8.6 c./sec. and $J_{3,4}$ = 4.6 c./sec. which suggest a 2,3-trans-3,4-cis-configuration. Clark-Lewis [95] records $J_{2,3}$ = 8.6 c./sec. and $J_{3,4}$ = 4.4 c./sec. for the synthetic teracacidin dimer.

The Dreiding model of A, with a 2,3-trans-3,4-cis-configuration and a presumed C2-equatorial phenyl-substituent, has C3-equatorial and C4-quasi-axial substituents. These bulky substituents act as conformational anchors and reduce conformational equilibrium (Roux [56,73]). Under these conditions the heterocyclic C-rings assume a 5-point coplanar conformation and the central dioxane-ring assumes a half-boat conformation with dihedral angles for C2, C3 and C3, C4 of 180° and 40° respectively. The calculated spin-

SCHEME 2


M+ 796 (8)
-COCH2
m/e 754 (7)
m/e 712 (5)
m/e 670 (3)
m/e 628 (2)
m/e 586 (2)
m/e 544 (2)
m/e 137 (35)
m/e 382 (25)
-H•
m/e 381 (19)
m/e 340 (55)
m/e 339 (32)
m/e 298 (60)
m/e 297 (48)
m/e 256 (100)
m/e 255 (95)
m/e 561 (4)
m/e 123 (74)

spin coupling constants using the Karplus relation [18] ($J_{2,3}$ = 10.0 c./sec. and $J_{3,4}$ = 4.6 c./sec.) agree with the observed values ($J_{2,3}$ = 8.6 c./sec. and $J_{3,4}$ = 4.6 c./sec.).

The absolute configuration of the assymetric centres is unknown at present. However, O.R.D. and C.D. experiments using cyclic carbonates and isopropylidene derivates of 7,3',4'-trimethoxy-2,3-trans-flavan-3,4-cis-diol as reference, may show whether the C-rings have the 2R:3R:4S or the alternate 2S:3S:4R-absolute configuration which are the two possibilities of a 2,3-trans-3,4-cis-dioxane-linked dimer with C2-equatorial phenyl substitution.

Compound B.

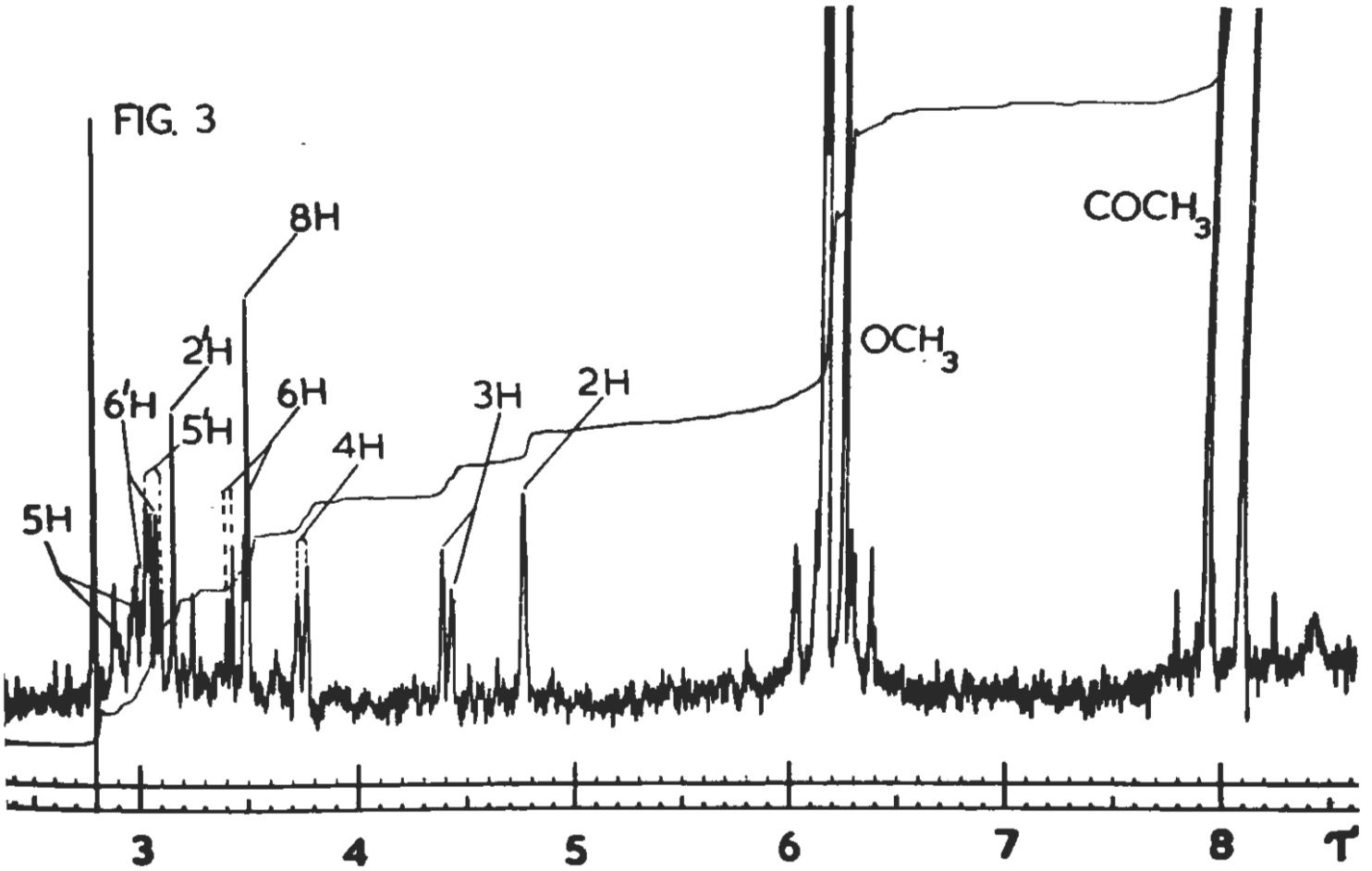
Isolation and identification.

A fraction of the Craig partition separation of enriched wax-free heartwood extract contained a mixture of compound B and dimeric and trimeric leucofisetinidins. The compound was separated from the mixture by preparative paper chromatography using both adsorptive and partition principles. White needles from aqueous alcohol were obtained. On a two-dimensional paper chromatogram developed in water-saturated butan-2-ol and 2% (v/v) acetic acid the compound had identical R_F's to synthetic (+)-7,3',4'-trihydroxy-2,3-cis-flavan-3,4-cis diol. Methylation of the compound yielded (+)-7,3',4'-trimethoxy-2,3-cis-flavan-3,4-cis-diol as white needles. A mixed m.p. with the synthetic reference compound gave no depression. The 3,4-diacetate of the methyl ether was obtained by acetylation in acetic acid/pyridine.

N.m.r. spectrum.

The 100 Mc. n.m.r. spectrum of the diacetate of the

FIGURE 3. N.m.r. Spectrum of the Diacetate of the Trimethyl Ether of B.
(+)-7,3',4'-Trimethoxy-2,3-cis-flavan-3,4-cis-diacetate.



trimethyl ether of B is reproduced in Figure 3 together with its interpretation. The spectrum, in comparison (see Table 1.) with synthetic optical isomers of 7,3',4'-trimethoxy-2,3-cis-flavan-3,4-cis-diacetates, (Drewes and Roux [79,80,96]) confirms its 2,3-cis-3,4-cis-configuration. The absolute configuration is established as 2S:3S:4S by its identity with the corresponding compound of known configuration obtained by epimerisation of (+)-mollisacacidin.

Mass spectrum.

The fragmentation of the diacetate of the methyl ether of B (c.f. Figure 4) in the mass spectrometer is typical of a 7,3',4'-trimethoxyflavan-3,4-diacetate (c.f. Drewes [97]) and is shown in Scheme 3. Initial elimination of acetic acid and the subsequent loss of an acetyl give the fragment $^{m}/e$ 297 which gives rise to the base peak in the spectrum. Prominent peaks are observed at $^{m}/e$ 222,180,165,137, and 151 resulting from an initial retro Diels-Alder fission of the molecular ion. The relative abundance of the M-60 peak is low in accordance with the observation for 3,4-cis-flavandiol diacetates by Drewes [97].

Occurrence of diastereoisomeric flavan-3,4-diols.

The presence of diastereoisomeric flavan-3,4-diols in the same tree is not uncommon. An analogous situation occurs in the heartwood of Guibourtia coleosperma [96] which contains three diastereoisomeric leucofisetinidins. These consist of (2R:3R:4R)-(-)-7,3',4'-trihydroxy-2,3-cis-flavan-3,4-cis-diol, (2R:3R:4S)-(-)-7,3',4'-trihydroxy-2,3-cis-flavan-3,4-trans-diol, and a 2,3-trans-3,4-cis-isomer. A similar pattern is present in Acacia auriculiformis [98,99] from which three diastereoisomers of teracacidin have been isolated. These are (2R:3R:4R)-(-)-7,8,4'-trihydroxy-2,3-cis-flavan-3,4-cis-

TABLE 1. N.m.r. Spectra of 7,3',4'-trimethoxy-2,3-cis-flavan-3,4-cis-diacetates

	Chemical Shifts							
	Acetoxy		Methoxy		H			
	3	4	7	3'+4'	2	3	4	5
Compound C	8.10	7.92	6.22	6.12	4.75	4.40	3.74	2.93
(+)-epimer*	8.05	7.88	6.19	6.09	4.69	4.33	3.67	2.93
(±)-epimer**	8.06	7.88	6.16	6.10	4.68	4.32	3.64	
(-)-isomer***	8.06	7.89	6.20	6.10	4.70	4.36	3.70	

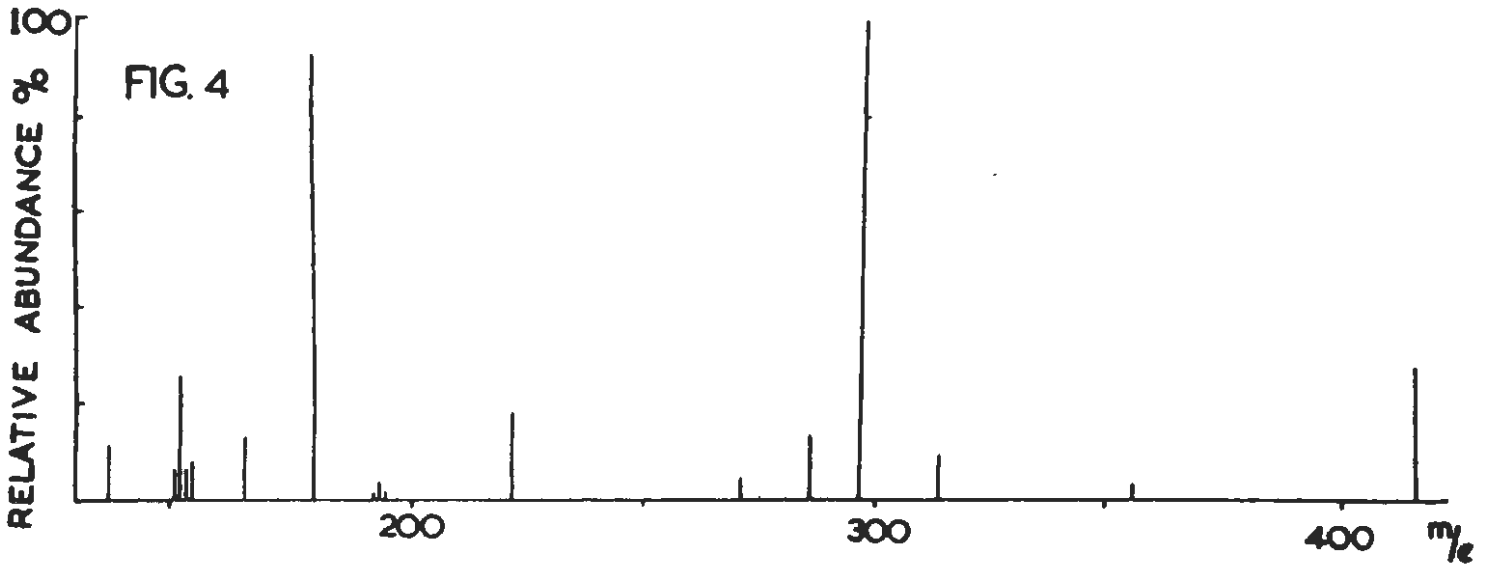
	Spin-spin Coupling Constants c./sec.	
	$J_{2,3}$	$J_{3,4}$
Compound C	1.0	4.3
(+)-epimer*	0.9	4.4
(±)-epimer**	1.0	4.0
(-)-isomer***	1	4

* By epimerization of (+)-mollisacacidin by Drewes and Roux[80]

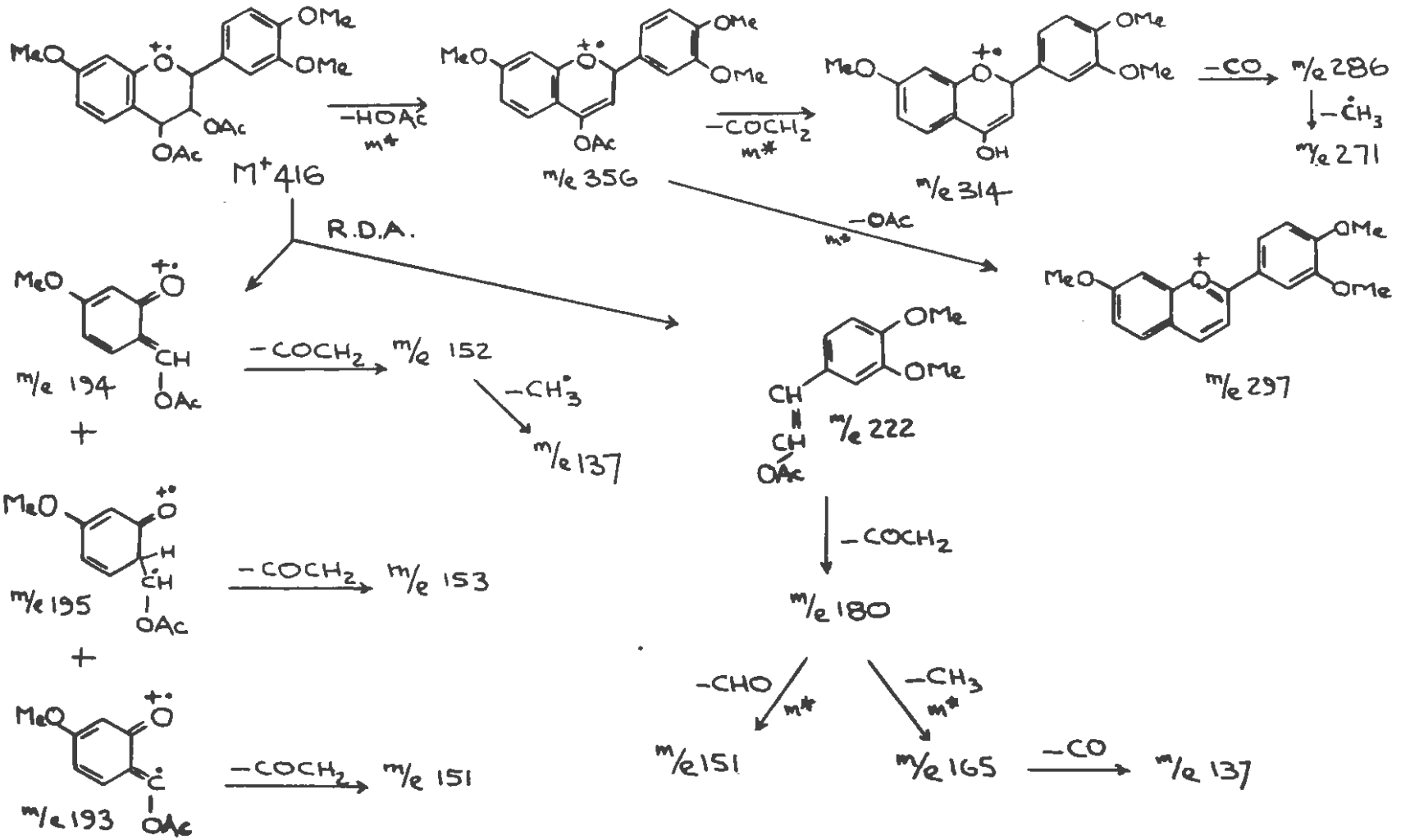
** By reduction of (±)-fustin from Rhus glabra[79]

*** The cis-cis isomer of (-)-mollisacacidin from Guibourtia coleosperma[96]

FIGURE 4. Mass Spectrum of the Diacetate of the Trimethyl Ether of B.
(+)-7,3',4'-Trimethoxy-2,3-cis-flavan-3,4-cis-diacetate.



SCHEME 3


MeO
OMe
OMe
OAc
OAc
M+ 416
-HOAc
m*
m/e 356
-COCH2
m*
OH
m/e 314
-CO
m/e 286
-CH3
m/e 271
R.D.A.
-OAc
m*
m/e 297
CH
OAc
m/e 194
-COCH2
m/e 152
-CH3
m/e 137
m/e 222
m/e 195
m/e 153
m/e 180
m/e 193
m/e 151
-CHO
m*
-CH3
m*
m/e 151
m/e 165
-CO
m/e 137

diol, (2R:3R:4S)-(-)-7,8,4'-trihydroxy-2,3-cis-flavan-3,4-trans-diol, and (2R:3S:4S)-(+)-7,8,4'-trihydroxy-2,3-trans-flavan-3,4-cis-diol.

Compound C.

3-methoxyfisetin was obtained from the concentrate of the contents of the forward-running fractions of the Craig partition separation of enriched wax-free heartwood extract. Repeated preparative paper chromatography using 25% (^{v}/v) acetic acid as developer gave the crystalline product.

Proof of structure.

Colour reactions.

The compound gives a green colour with ferric alum and a black colour with ammoniacal silver nitrate which is indicative of a catechol type B ring. Treatment with Mg/HCI affords a cherry-red colour[100] and fuming with ammonia gives a yellow complex, typical of flavonols.

The compound has a bright blue-fluorescence under u.v. light compared with the yellow-green fluorescence of fisetin and the chrome-yellow fluorescence of 4'-methoxyfisetin [28].

Alkali degradation.

Fusion with potassium hydroxide gave ℮-resorcylic acid and protocatechuic acid, showing a resorcinol A-ring and a catechol B-ring. Although demethylation may occur during the above reaction and thus lead to erroneous conclusions there is some evidence (e.g. 4-methoxygallic acid from mearnsitrin [76]) that this does not occur.

U.v. and i.r. spectra.

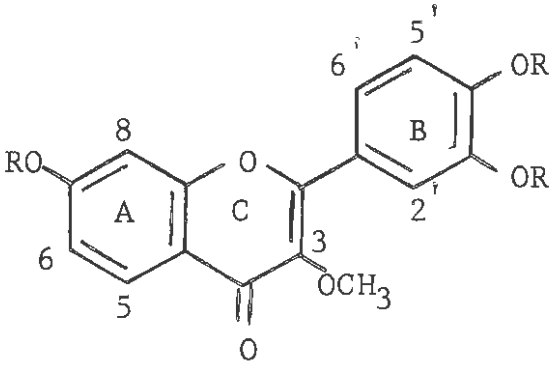
The i.r. spectrum of the compound showed carbonyl

absorption at 1607 cm^{-1}. The main u.v. absorption band is at 350 mμ. When the compound is complexed with boric acid-sodium borate, this band undergoes a bathochromic shift (13 mμ) due to the presence of the ortho-dihydroxy group in the B-ring.[101] However, the main u.v. absorption band was unaffected by addition of aluminium chloride due to the 3-O-methyl group which inhibits the formation of an aluminium chloride complex with the C3 and C4 oxygens.

Markham and Mabry[102] have shown that under anhydrous conditions flavonoids with ortho-dihydroxy groups form complexes with aluminium chloride which give rise to bathochromic shifts in the u.v. spectra. This reaction was completely reversed on the addition of dilute aqueous HCl. Thus the acidic conditions normally used for the detection of 3- or 5-hydroxy groups in flavonols prevents $AlCl_3$/ortho-dihydroxy group complex formation.

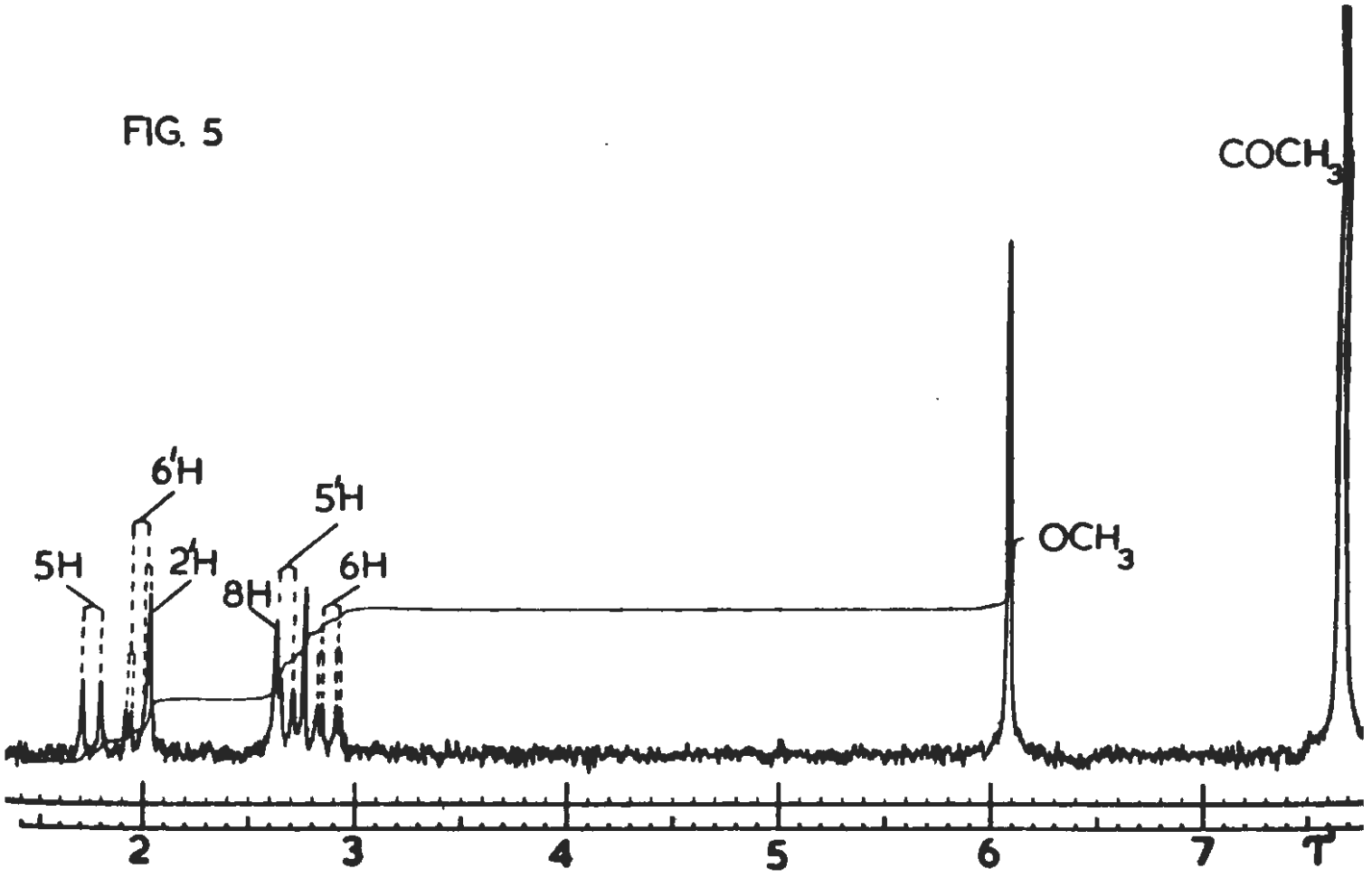
Triacetoxy-3-methoxyfisetin.

Acetylation with acetic anhydride/pyridine yielded a crystalline derivative which, by the above evidence and n.m.r. and mass spectral evidence has been shown to be 7,3',4'-triacetoxy-3-methoxyfisetin (XI, R = CH_3CO). This is consistent with C being 7,3',4'-trihydroxy-3-methoxyflavone (XI, R = H)



(XI)

FIGURE 5. N.m.r. Spectrum of the Triacetate of C - 7,3',4'-Triacetoxy-3-methoxyfisetin.



Tetramethoxyfisetin.

Methylation with diazomethane yielded crystalline tetramethoxyfisetin (XI, R = CH_3). For comparison purposes tetramethoxyfisetin was synthesised by methylating fisetin with diazomethane. A mixed m.p. of a mixture of these two derivatives gave no depression. The i.r. and n.m.r. spectra of these two derivatives showed complete identity.

Interpretation of n.m.r. spectra.

The 100 Mc. n.m.r. spectrum of 7,3',4'-triacetoxy-3-methoxyfisetin is reproduced in Figure 5. The spectrum shows proton absorptions for one methoxy group, three acetoxy groups, and six benzenoid protons. The benzenoid protons give rise to two simple first order ABX systems which partly overlie each other. The methoxy protons give rise to a singlet centred at τ, 6.07 and the acetoxy protons give rise to singlets at τ, 7.69 (3 protons) and τ, 7.64 (6 protons). The C4 carbonyl group causes an anticipated anisotropic shift of the 5H which appears as a downfield doublet (τ, 1.75). This proton is ortho-coupled ($J_{5,6}$ = 8.6 c./sec.) with the 6H which appears as a quartet centred at τ, 2.87. The meta-splitting of this quartet ($J_{6,8}$ = 2.0 c./sec.) is caused by the 8H which itself appears as a broadened singlet at τ, 2.62. The absorption pattern of the B-ring protons is a quartet, a doublet, and a broadened singlet. The doublet at τ, 2.66 is due to the 5'H, ortho-coupled ($J_{5'6'}$ = ~9 c./sec.) with the 6'H which appears as a quartet centred at τ, 1.97. The meta-splitting of the quartet ($J_{2'6'}$ = 2.4 c./sec.) is caused by the 2'H which appears as a broadened singlet at τ, 2.02.

The 100 Mc. n.m.r. spectra of tetramethoxy-fisetin from 3-methoxyfisetin and fisetin are reproduced in Figures 6 and 7 respectively. These two spectra show complete identity. Integration of the methoxy proton signals shows four methoxy groups. The benzenoid protons' absorption pattern of each of these spectra is similar to that of the benzenoid protons of triacetoxy-3-methoxy fisetin.

Interpretation of mass spectrum.

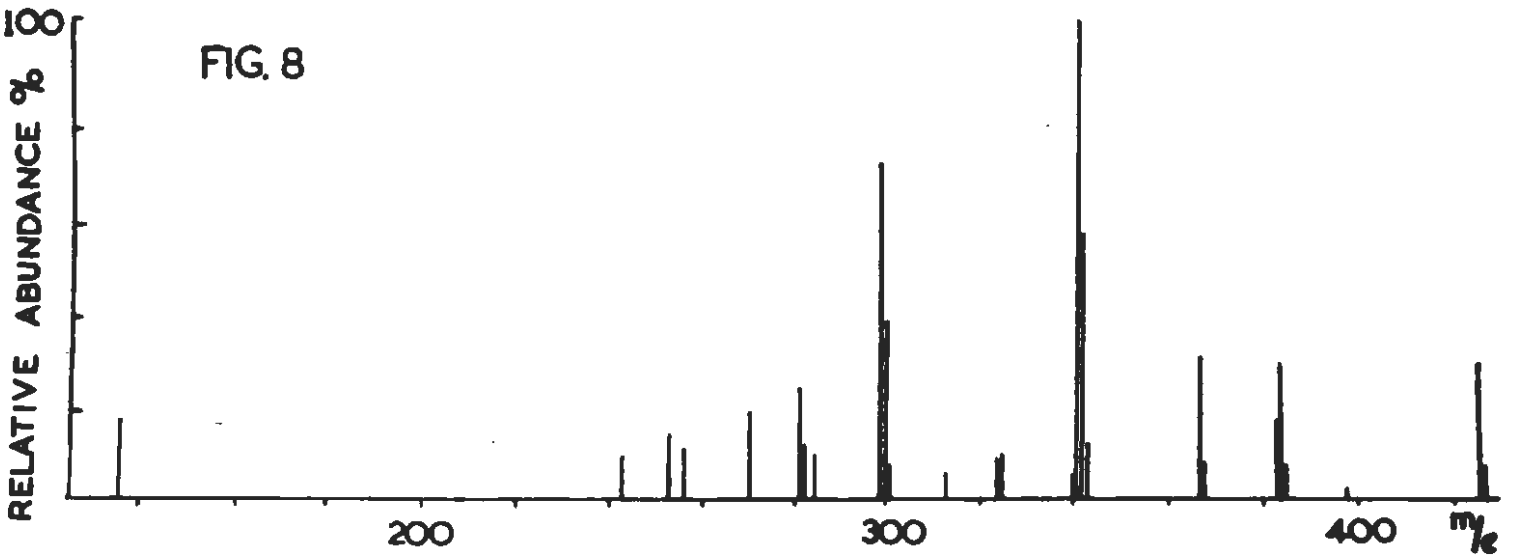
A suggested fragmentation pattern for triacetoxy-3-methoxyfisetin (c.f. Figure 8) is given in Scheme 4. The molecular ion peak at $^{m}/e$ 426 is in agreement with the molecular formula $C_{22}H_{18}O_9$. Three successive ketene losses from the molecular ion can be followed which confirms the presence of three phenolic hydroxy groups in the parent compound. The presumed loss of a methoxy radical to give a strong signal at $^{m}/e$ 367 provides evidence for the presence of a methoxy group. The presence of the ion at $^{m}/e$ 137 shows that the 7-position is not methoxylated.

Occurrence of methoxylated flavonols in Nature.

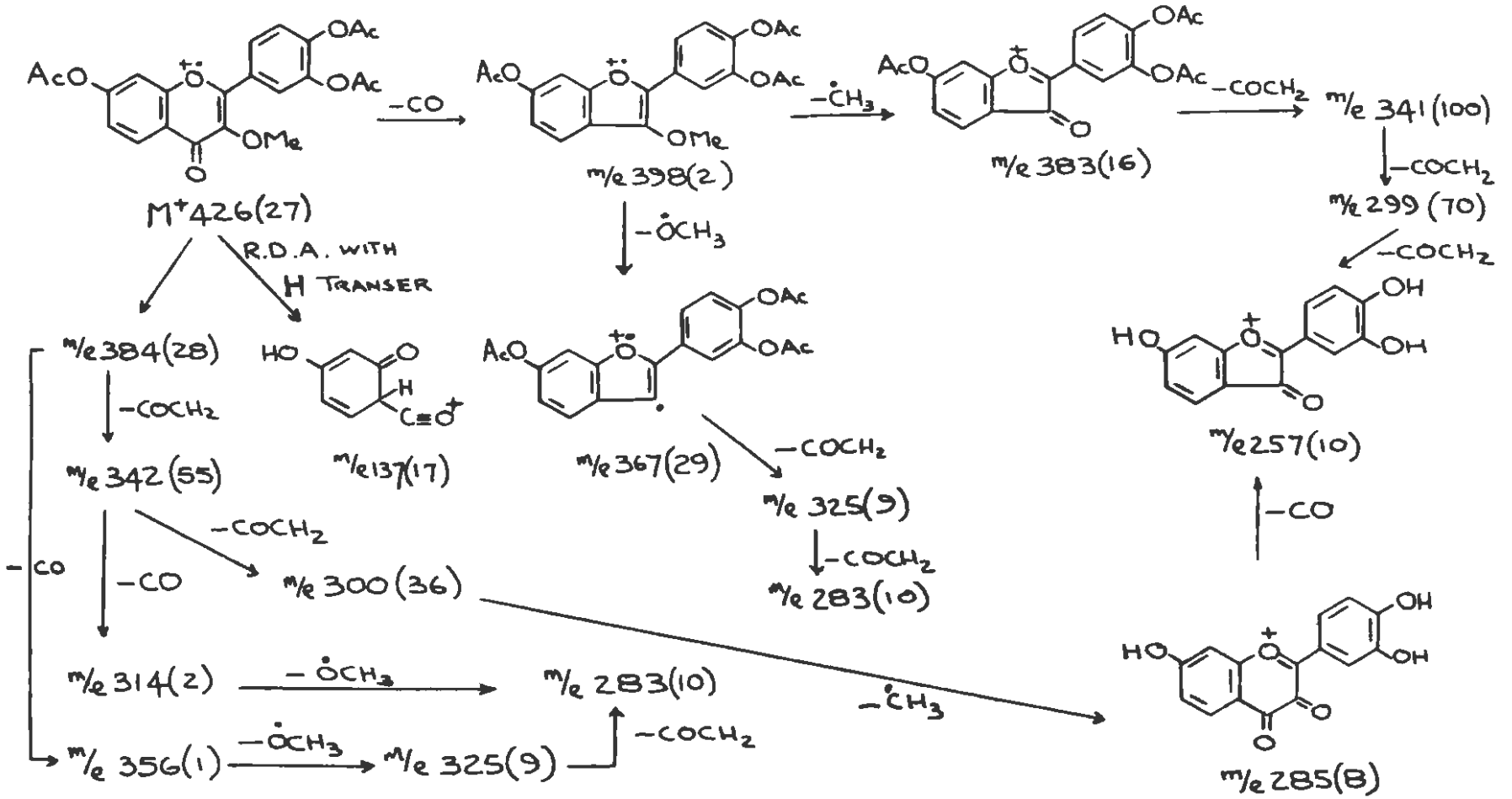
3-Methoxyfisetin is the first methoxylated flavonoid to be found in the heartwood or bark of *Acacia mearnsii*. The 4'-methoxyflavonol glycoside, mearnsitrin, isolated [76] from the leaves, is the only other methoxylated flavonoid that has been found in the tree.

Numerous methoxylated flavonols have been found in Nature. In particular, the occurrence of galangin (5,7-dihydroxyflavonol) together with its 3-O-methyl ether in galanga-root[103] (*Alpinia officinarum*) is of interest.

FIGURE 8. Mass spectrum of the Triacetate of C - 7,3',4'-Triacetoxy-3-methoxyfisetin.



SCHEME 4


M+426(27)
-CO
m/e 398(2)
-·CH3
m/e 383(16)
-COCH2
m/e 341(100)
-COCH2
m/e 299(70)
-COCH2
m/e257(10)
-CO
R.D.A. WITH H TRANSER
m/e384(28)
-COCH2
m/e 342(55)
-CO
-CO
-COCH2
m/e 300(36)
m/e137(17)
m/e 314(2)
-·OCH3
m/e 283(10)
m/e 356(1)
-·OCH3
m/e 325(9)
-COCH2
m/e 367(29)
-COCH2
m/e 325(9)
-COCH2
m/e 283(10)
-·CH3
m/e 285(8)

Compound D.

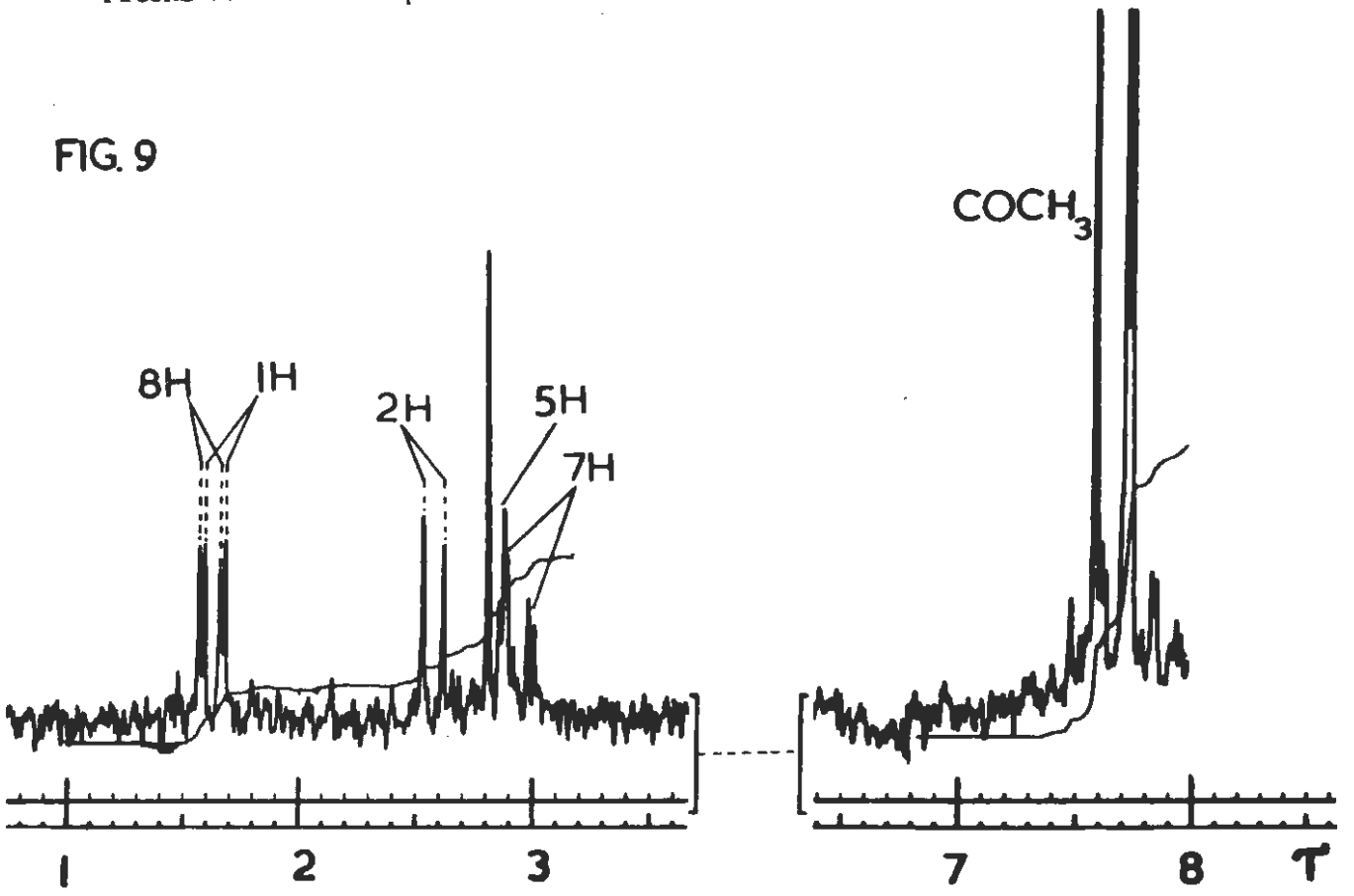
Isolation and identification

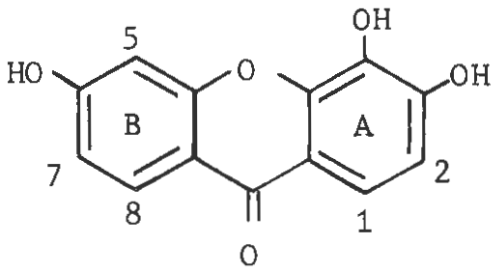
Compound D was isolated concurrently with 3-methoxyfisetin and was obtained pure by repeated crystallisation. D gave an intense yellow colour with NaOH. This reaction is typical of xanthones. KOH fusion gave the following degradation products: β-resorcylic acid, pyrogallol-4-carboxylic acid and resorcinol. A large percentage of starting material (green to ferric alum spray) was present in the phenolic fraction of fusion products indicating the stability of D.

The u.v. spectrum of D showed two major absorption bands at 257 mμ and 336 mμ. Addition of boric acid - sodium acetate caused a bathochromic shift from 336 to 347 mμ, showing the likely presence of an ortho-dihydroxy group. Mass spectrometry gives the molecular weight of D as M^+ 244. Compound D forms a crystalline triacetoxy derivative M^+ 370 (by mass spectrometry). The n.m.r. spectrum of the triacetate (Figure 9) shows acetoxy proton absorption for 9 protons (i.e. 3 acetoxy groups) and benzenoid proton absorption for 5 protons. The mass spectrum of the triacetate shows three successive ketene losses from the molecular ion to give an ion M^+ 244 which appears as the base peak.

The above spectrometric and other evidence has led to the postulation of the structure of D as a trihydroxy substituted xanthone. A detailed study of the n.m.r. spectrum of the triacetate gives the structure of D as (XII).

FIGURE 9. N.m.r. Spectrum of the Triacetate of D - 3,4,6-Triacetoxyxanthone.





(XII)

Analysis of n.m.r. spectrum.

Analysis of the n.m.r. spectrum of the triacetate has facilitated the placing of the hydroxy groups in the Compound D.

The downfield protons (c.f. Figure 9.) are assigned to the 1H and the 8H due to anisotropic shift caused by the keto-group. The 1H is coupled with the 2H (τ = 2.58) giving rise to a simple AB system with a large coupling constant ($J_{1,2}$ = 8.8 c./sec.) indicating that the protons are ortho to one another. This AB system shows that there are no further protons on the A ring; hence the ortho-dihydroxy group is at C3 and C4.

The 8H proton appears as part of an ABX system. The coupling constants of this system ($J_{7,8}$ = 8.8 c./sec. and $J_{5,7}$ = 2.2 c./sec.) indicate a 5,7,8-proton pattern for the B ring with the remaining hydroxy group at C6. Hence D is 3,4,6-trihydroxyxanthone.

Analysis of mass spectra.

The three ketene losses from the molecular ion of the triacetate are accompanied by their appropriate metastable peaks. The mass spectrum of the free phenolic form has the base peak at the molecular ion peak (M^+ 244). Further, the spectrum shows a strong $M^+ - 42$ peak which is accompanied by a metastable peak at $^m/e$ 167.5.

It has been suggested [104] that the fragment $^m/e$ 202 arises out of an initial ketonization of the C3 hydroxy group and subsequent elimination of ketene between C2 and C3.

Natural occurrence of xanthones.

Xanthones and their glycosides have been isolated from various natural sources. [105] Some from various parts of flowering plants; some from the metabolic products of the members of the lower fungi; one of lichen origin and one of animal origin.

Xanthones have not previously been found in wattle. 3,4,6-Trihydroxy-xanthone is thus the first reported xanthone to be isolated from this source.

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