SYNTHESIS OF FLAVAN-3,4-DIOLS, STEREOCHEMISTRY OF NOVEL BIFLAVANOLS AND NEW NON-TANNINS FROM ACACIA MEARNSII.

by

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FOREWORD.

This thesis is submitted in accordance with the regulations for the Degree of Doctor of Philosophy of Rhodes University. The work is wholly original, except where due reference is made in the text, and was carried out at the Leather Industries' Research Institute, Grahamstown. The thesis has not been submitted in whole, or in part, for any degree at any other University.

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SUMMARY.

The structural elucidation of condensed tannins, which are considered to consist of C_{15}-flavan units, presents certain difficulties. These tannins occur in extremely complex mixtures, and their isolation is complicated by their susceptibility to oxidative denaturation. Limitations in the formation of significant degradation products add to these problems.

Since condensed tannins of black wattle bark yield anthocyanidins on treatment with mineral acids, they are considered to be proanthocyanidin in character. For this reason the initial approach to the investigation of the structures of condensed tannins was by way of the synthesis of novel 7-hydroxyflavan-3,4-diols having a low degree of hydroxyl substitution. Submission of these to modern physical techniques such as nuclear magnetic resonance (n.m.r.) spectroscopy would yield valuable data regarding the chemical shifts and coupling constants of heterocyclic and benzenoid protons which may be used for subsequent work on more highly hydroxylated diols, biflavanols and finally the condensed tannins themselves.

Hence the four racemates of 7,4'-dimethoxyflavan-3,4-diol were synthesized - methyl ethers being chosen because of their greater stability than free-phenolic forms. Their preparation was abbreviated by synthesizing two racemates by stereospecific reduction methods and obtaining the remaining two from these by selective epimerization techniques.

The 2,3-trans-3,4-trans racemate was prepared from the corresponding chalcone dibromide via the 7,4'-dimethoxy-2,3-trans-dihydroflavonol by catalytic hydrogenation of the latter using Adams catalyst.

7,4'-Dimethoxyflavonol on reduction with copper chromite at high temperature and pressure gave a good yield of the 2,3-cis-3,4-cis racemate.

The 2,3-trans-3,4-cis racemate was obtained from the 2,3-trans-3,4-trans isomer by selective epimerization at C-4 using boron trifluoride and sodium boronhydride in diglyme.

Preparation of 2,3-cis-3,4-trans-7,4'-dimethoxyflavan-3,4-diol was more difficult due to 7-hydroxyl substitution which confers greater reactivity to the benzenoid A-ring, enhancing the possibility of inversion at the C-4 centre of the heterocyclic ring during the final stages of a difficult synthetic route. The diacetate of the desired 2,3-cis-3,4-trans isomer was obtained through epimerization by prolonged acetylation of the 2,3-cis-3,4-cis diol, followed by preparative thin-layer chromatography of the reaction products on silica gel.

Relative configurations of the four racemates of 7,4'-dimethoxyflavan-3,4-diol were established by n.m.r. spectroscopy and paper ionophoresis by comparison with results obtained for analogous compounds by earlier workers. The spin-spin coupling constants, obtained from the n.m.r. spectra of the isomers, were correlated with the measured dihedral angles using Dreiding models on the basis of the Karplus relation.

The n.m.r. spectral analysis of these racemates enabled assignment of the relative configurations of the natural guibourtacacidins (7,4'-dihydroxyflavan-3,4-diols) from Guibourtia coleosperma. Comparison of spectra showed the natural product to consist of a mixture of three diastereoisomers of which the 2,3-cis-3,4-trans isomer was predominant, with low concentrations of 2,3-trans-3,4-trans and 2,3-trans-3,4-cis diols.

Biflavanol components B ($C_{30}H_{26}O_{12}$) and D ($C_{30}H_{26}O_{13}$) from black wattle bark contain leucorobinetinidin nuclei. Epimerization of natural (+)-2,3-trans-3,4-trans-leucorobinetinidin was therefore attempted to enable chromatographic comparison with B and D. (+)-Leucorobinetinidin on autoclaving yielded the (+)-2,3-trans-3,4-cis, (+)-2,3-cis-3,4-trans, and (+)-2,3-cis-3,4-cis diastereoisomers. Paper chromatography of these epimerization products showed them to be distinct from the bark constituents B and D, and indicated the complexity of the latter.

The biflavanols B and D were isolated from the ethyl acetate extract of fresh black wattle bark. Due to the complex nature of the mixture and lack of adequate resolution from associated compounds during chromatography, even under optimum conditions, their isolation required a long succession of enrichment procedures. A large-scale manual countercurrent separation of the bark extract, followed by fractionation using an automatic Craig countercurrent machine yielded enriched fractions of B and D. Preparative paper chromatographic purification using both absorption and partition procedures gave these

products in chromatographically pure form.

Both B and D showed strong tendency to autoxidation in the free-phenolic forms and were consequently stabilized by methylation prior to their final purification by thin-layer chromatography on silica gel. Pure samples of the free-phenolic forms of B and D were obtained by preparative paper ionophoresis using a borate buffer solution.

Alkali fusion of B yielded the degradation products resorcinol, phloroglucinol, protocatechuic acid, gallic acid and β-resorcylic acid. Apart from protocatechuic acid, D gave the same products under similar conditions.

Both B and D on treatment with 3N hydrochloric acid and *iso*-propanol gave robinetinidin chloride and an orange pigment. These anthocyanidins were formed in relatively low yields (robinetinidin chloride, *ca*. 3-4%) compared with the higher yield from (+)-leuco-robinetinidin (*ca*. 25%) under corresponding conditions.

Acid-induced fission of B with 3N hydrochloric acid and ethanol gave, amongst others, (+)-catechin, the orange pigment, resorcinol and phloroglucinol. Similar fission of D yielded (+)-gallocatechin, resorcinol, phloroglucinol and the orange pigment.

The octa-methyl ether ($C_{38}H_{42}O_{12}$), $[\alpha]_D$ =-98.8°, octa-methyl ether diacetate ($C_{42}H_{46}O_{14}$), $[\alpha]_D$ =-71.9°, and deca-acetate ($C_{50}H_{46}O_{22}$), $[\alpha]_D$ = -51.3°, derivatives of P were prepared. Corresponding nona-methyl ether ($C_{39}H_{44}O_{13}$), $[\alpha]_D$ = -86.0°, nona-

methyl ether diacetate ($C_{43}H_{48}O_{15}$), $[\alpha]_D$ = -65.7°, and undeca-acetate ($C_{52}H_{48}O_{24}$), $[\alpha]_D$ = -42.7° derivatives of D were also prepared.

The relative configurations of B and D were determined by high-resolution n.m.r. spectroscopy of derivatives by analogy with spectra of monomeric flavan-3,4-diols. Spin-decoupling assisted in the assignment of proton coupling. The coupling constants of the 2-, 3- and 4-protons of the heterocyclic ring of the leucorobinetinidin units ($J_{2,3}$ = 9.5-10.0 c./sec. ; $J_{3,4}$ = 9.0-10.0 c./sec.) of B and D indicated a 2,3-trans-3,4-trans relative configuration of substituents for these portions of the molecules. The catechin moieties of the biflavanols reflected coupling constants ($J_{2,3}$ = ± 7.0 c./sec.), consistent with a 2,3-trans arrangement.

The leucorobinetinidin unit showed an ABX system of heterocyclic proton coupling, while the catechin portion gave a typical ABX_2 system. The acetyl signals of the methyl ether diacetates occurred upfield relative to the 4-acetyl of flavan-3,4-diols showing that both were at C-3 positions. The above served to confirm the hypothesis that the link between the two moieties occurs through the C-4 position of the heterocyclic ring of the leucorobinetinidin unit.

Mass spectrometry of the methyl ether diacetates of B and D reflected molecular weights of 774 and 804, respectively. The presence of prominent fragments (M = 210 and 180, in the case of the derivative of B, and M = 210, in D) was consistent with a linkage to either the C-6 or C-8 positions of the catechin moieties. This is supported by

the presence of resonance signals due to the uncoupled benzenoid protons (τ = 3.60 and 3.76 c./sec. for B and D, respectively) in the n.m.r. spectra of these derivatives.

Knowledge of the relative configurations of B and D, and their association in black wattle bark with monomeric flavonoid analogues of corresponding relative configuration and known absolute configuration, led to the assumption of a 2R, 3S, 4R absolute configuration for the leucorobinetinidin moiety and a 2R, 3S configuration for the catechin unit.

The biflavanols B and D therefore fall into a new class of compounds where "resorcinol" flavonoids are carbon-linked to members of the "phloroglucinol" series. They show limited tanning properties and are known as "phenolic half-tannins".

Degradation of B and D by treatment with mineral acid yields an orange pigment in both cases. A benzotropolone structure is proposed for this pigment by analogy with similar pigments previously obtained from the fermentation products of tea.

Apart from monomeric flavonoids and condensed tannins black wattle bark also contains a heterogeneous mixture of associated non-tannins.

The predominant carbohydrate components of the bark were sucrose and the cyclitol, (+)-pinitol, with lower concentrations of glucose and fructose. These carbohydrates were separated from the nitrogenous acids by cation-exchange chromatography. Sucrose was

obtained in crystalline form from the carbohydrate fraction on concentration of the aqueous solution. Hydrolysis of sucrose yielded fructose and glucose which were identified by paper chromatographic comparison with reference hexoses.

Crystalline (+)-pinitol was isolated from the mother liquor of sucrose. It was identified by preparation of the diisopropylidene derivative.

Preparative paper chromatography of the acidic fraction yielded the nitrogenous components which consisted mainly of the imino acids (-)-L-pipecolic acid, trans-4-hydroxy-(-)-L-pipecolic acid and (-)-L-proline. Accompanying these were lower proportions of the α-amino acids ~~α-alanine~~ alanine, arginine, aspartic acid, glutamic acid and serine. The imino acids were characterized by the preparation of derivatives and comparison with authentic samples of these acids, while the amino acids were detected by means of paper chromatography using reference compounds. Paper chromatography also showed the presence of trace amounts of shikimic and quinic acids. The nitrogenous acids together constitute approximately 3% of the bark extract.

After their isolation from the bark, the distributions of carbohydrate, amino acid and imino acid components in the leaves, twig bark, stem bark, root bark and heartwood of black wattle were compared by paper chromatography.

The petroleum ether-soluble portion of the bark (0.2% of dry bark weight) consisted of a number of constituents including alkanes. Of these only a novel long-chain β-diketone, stigmasterol

and β -sitosterol were present in sufficient concentration to warrant their isolation.

The β -diketone ($C_{32}H_{64}O_2$) formed a complex with cupric acetate and could be regenerated readily from this complex by acid treatment. N.m.r. spectroscopy of the β -diketone showed the presence of a vinyl proton, methylene, and terminal methyl protons, while infrared spectrometry indicated strong ketonic stretching vibrations and absorption due to O-H deformation. Mass spectrometry failed to give a molecular ion, possibly as a result of the instability of such long-chain products.

The two steroid alcohols could not be separated readily by chromatographic means. Mass spectrometric analysis of the mixture indicated that they were closely-related, probably differing only in the number of side-chain double bonds. From the fragmentation pattern of the mixture and its n.m.r. spectrum it was possible to deduce that the mixture was comprised of β -sitosterol and stigmasterol. Selective hydrogenation of the acetylated mixture converted the stigmasterol into β -sitosterol, giving a homogeneous product.

The presence of traces of shikimic and quinic acids in the non-tannin fraction of black wattle bark is of significance, since they are representative of a known pathway of biogenesis of flavonoids.

Monomeric flavonoid components of wattle bark may yield biflavanols by biogenesis, and these in turn may give rise to more highly condensed tannins.

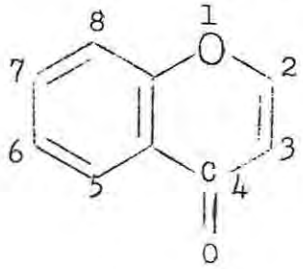
CONTENTS.

REVIEWS.

PART I

THE STEREOCHEMISTRY OF CATECHINS, DIHYDROFLAVONOLS AND LEUCOANTHOCYANIDINS.

The flavonoids are derived from the chromone nucleus I which is planar in structure and stereochemically inert. In chromones which are substituted at carbon atoms 2 and 3, such as flavanones (flavan-4-ones) II, dihydroflavonols (flavan-3-ol-4-ones) III, and in catechins (flavan-3-ols) IV and leucoanthocyanidins (flavan-3,4-diols) V, centres of assymmetry are introduced and stereoisomers may thus occur.



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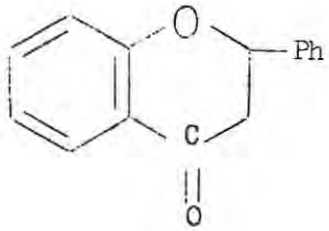

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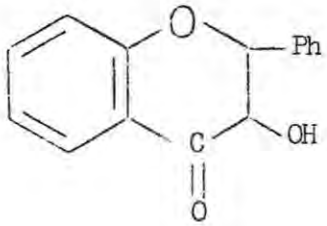

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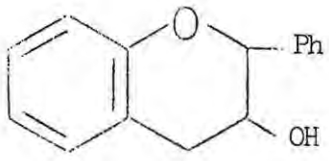

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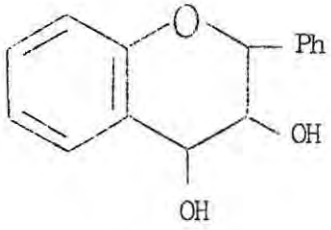


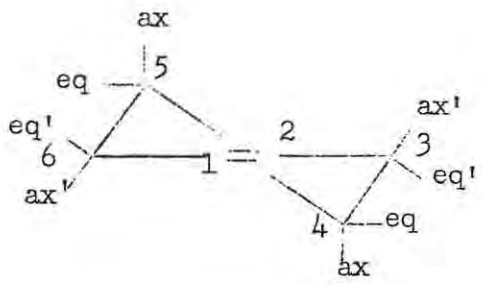
(V)

The Catechins.

The flavan-3-ols, generally termed "catechins", were the first flavonoid compounds to have their stereochemistry investigated. The relative and absolute configurations of flavonoids, such as dihydro-

flavonols and flavan-3,4-diols, may be established readily by converting them into their corresponding flavan-3-ols. The configurations of the resulting catechins may then be determined by methods analogous to those used in the case of catechin and epicatechin. The stereochemistry of the catechins is therefore of great significance in studying the stereochemistry of the flavan derivatives in general, and of the flavan-3,4-diols in particular.

The planar γ-pyrone ring of the chromone nucleus I becomes distorted on reduction in order to give derivatives of the types II to V. Barton, Cookson, Klyne ahd Shoppee[1] showed that the geometry of the double bond in cyclohexene required carbon atoms 1, 2, 3 and 6 to be coplanar and hence the puckered or "half-chair" conformation VI was proposed.



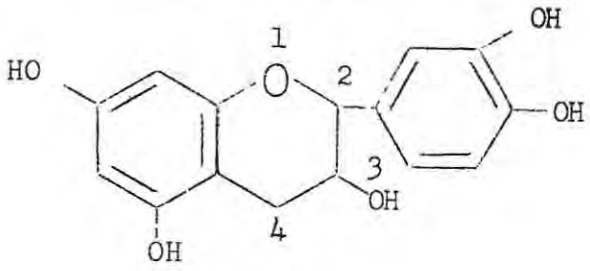
ax = axial
eq = equatorial

(VI)

The C-H bonds at C-3 and C-6 are not genuinely equatorial or axial in character but approximate to these positions. They suggested that such bonds be designated quasi-axial (ax') and quasi-equatorial (eq').

The conformation of the heterocyclic ring in catechin VII was considered by Roberts[2] to resemble that of cyclohexene. He visualized the oxygen ring as having a half-chair shape VIII, with the carbon atoms

between C-4 and the heterocyclic oxygen coplanar. The benzene ring which is attached at C-2 was expected to be in the more stable equatorial position, while in the two epimeric forms of catechin the hydroxyl group attached at C-3 would be in the axial and equatorial forms, respectively.



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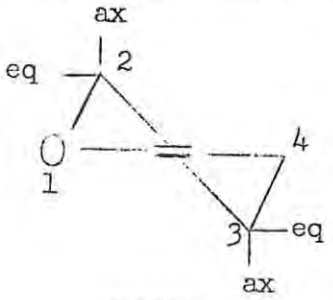

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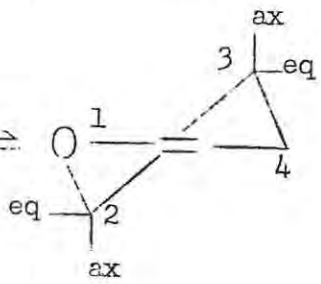
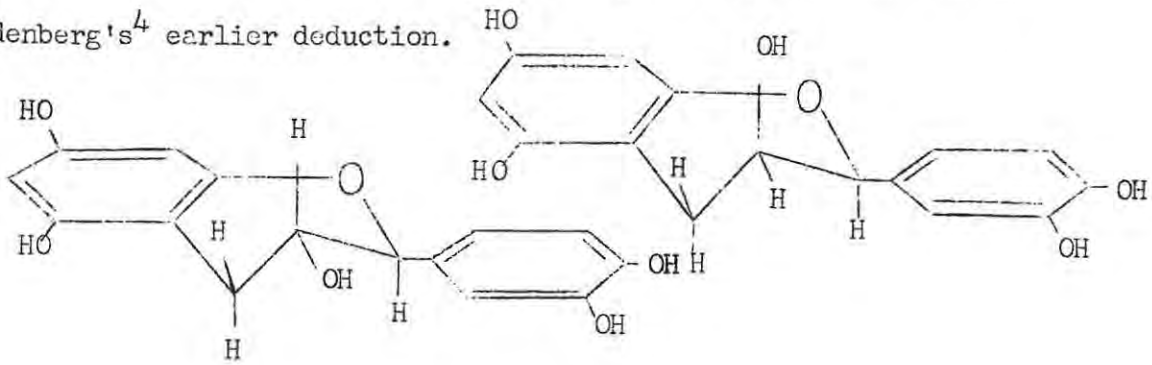


(IX)

The energy barrier between the two half-chair conformations VIII and IX is small and when conformational inversion occurs groups which are 2 (ax) and 3 (ax) in VIII become 2 (eq) and 3 (eq) in IX, and vice versa.

By means of molecular models King, Clark-Lewis and Forbes[3] demonstrated that the heterocyclic nucleus in the flavans was puckered, and hence represented catechin and epicatechin by X and XI respectively. They also showed that catechin is the trans-isomer (as will be described later), while epicatechin has the cis-configuration, thus confirming Freudenberg's[4] earlier deduction.

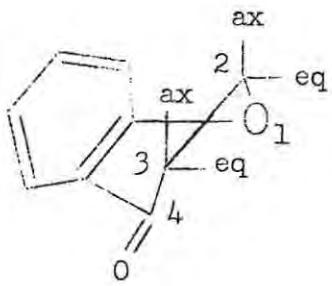


(X)

(XI)

The half-chair conformation for the catechins was also favoured by Mahesh and Seshadri[5], while Joshi and Kulkarni[6] implied this mode of representation in their synthetic work on the 6-methyl-4'-methoxy-flavan-3,4-diols and related catechins.

An examination of molecular models by Whalley[7] made him aware that chroman and chromanone systems could be represented by an alternative "boat" conformation XII. This was more strained than the half-chair form and was therefore less likely. When the 2-hydroxyisoflavones XIII and flavanones II were represented by the boat form the plane of the carbonyl group was situated at an angle of approximately 35° to that of the benzene ring. This condition would effectively destroy the conjugation between the two systems. Shaw and Simpson's[8] infrared data on the



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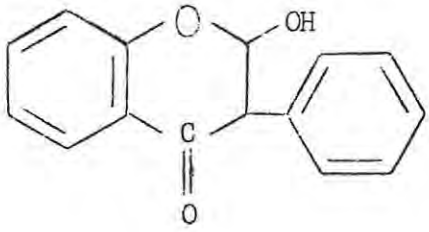
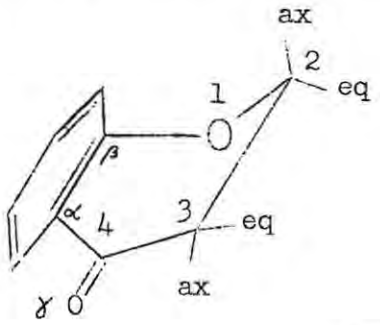


(XIII)

flavanones clearly indicated that the carbonyl group and the benzene ring were conjugated and thus the half-chair representation for chromanones was proved valid.

After consideration of the infrared absorption data of

Shaw and Simpson[8], Philbin and Wheeler[9] concluded that in the flavanones such as XIV the atoms 1, β, α, 4, γ and 3 were coplanar and C-2 was the only out-of-plane atom. This led them to their proposed "sofa" conformation XIV. From a study of bond angles of the sofa form, by



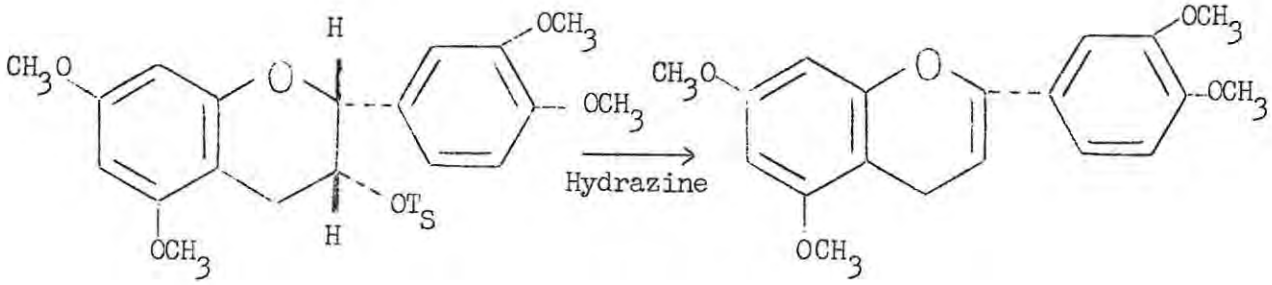
(XIV)

means of molecular models, they found that no steric hindrance would arise in XIV if C-3 and C-4 carried hydroxyl groups in any axial-equatorial combination. The model would readily "flip" to the opposite conformation in which the axial and equatorial bonds at C-2 and C-3 were interchanged.

Freudenberg et al.[10] investigated the catechins since the early nineteen twenties and concluded that catechin and epicatechin were epimers. Epicatechin was synthesized by Geissman and Lischner[11] who proposed the 2,3-cis configuration for it. Huckel et al.[12], on the other hand, presented evidence supporting a 2,3-cis structure for catechin.

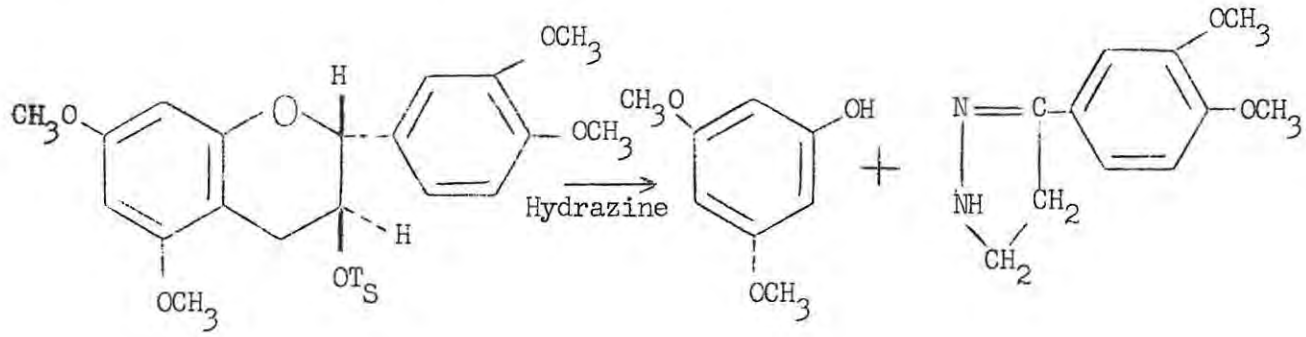
King[3], Clark-Lewis[13] and Whalley[7] eventually proved that catechin is the 2,3-trans compound, while epicatechin is the 2,3-cis diastereoisomer. Freudenberg et al.[4] indicated the possible 2,3-cis configuration of epicatechin by the elimination reaction occurring when

(-)-epicatechin tetramethyl ether 3-toluene-p-sulphonate XV was heated with hydrazine to yield the flav-2-ene **XVI.** A similar elimination with synthetic (±)-2,3-cis-7,8,3',4'-tetramethoxyflavan-3-toluene-p-sulphonate gave the corresponding 7,8,3',4'-tetramethoxyflav-2-ene[14].



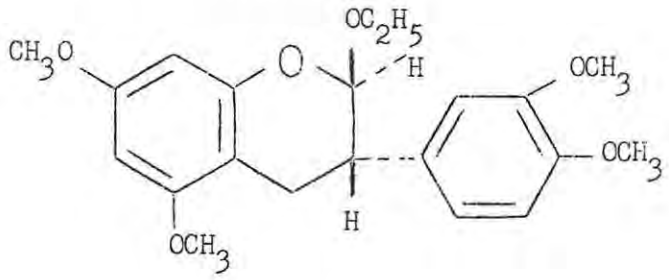
(XV) (XVI)

The reactions above were considered to be trans eliminations (E_2) which probably occurred in conformations with 2(ax)-H and 3(ax)-OTs permitting the four centres taking part in the reaction to be coplanar. Contrary to the (-)-epicatechin derivative, (+)-catechin tetramethyl ether-3-toluene-p-sulphonate XVII did not undergo the above reaction, but instead resulted in a fission to phloroglucinol dimethyl ether and 3-(3,4-dimethoxyphenyl)-pyrazoline XVIII.



(XVII) (XVIII)

(+)-Catechin was therefore considered to be the 2,3-trans compound while epicatechin was the 2,3-cis isomer. Support for these conclusions was given by the two molecular rearrangements which (+)-catechin tetramethyl ether undergoes. These were interpreted as 1,2- rearrangements of trans groups. The first of these occurred when (+)-catechin tetramethyl ether 3-toluene-p-sulphonate XVII was heated with potassium acetate in ethanol at elevated temperature to yield the 2-ethoxy-isoflavan XIX[15]. This rearrangement is of interest because it proceeds with retention of optical activity, and clearly indicates that inversion occurs at C-3 due to neighbouring-group participation[7]. The product XIX was regarded as the 2,3-trans-compound formed by inversion at both C-2 and C-3 centres such as occurs in certain cases of the Wagner-Meerwein transformation[16].

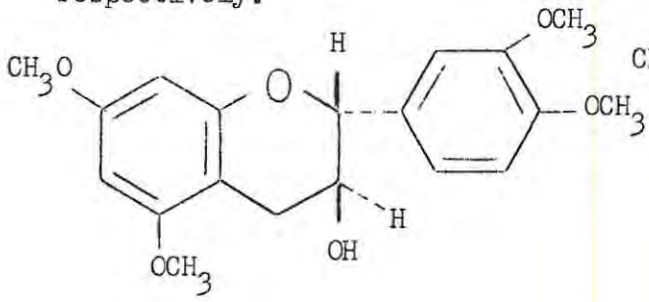


(XIX)

The second rearrangement occurred when (+)-catechin tetramethyl ether was treated with phosphorus pentachloride[14,17] yielding a reactive 2-chloro-isoflavan which in turn reacted with ethanol to give the same optically active 2-ethoxyisoflavan XIX. Epicatechin derivatives did not undergo these rearrangements.

(+)-Catechin and (-)-epicatechin tetramethyl ethers were therefore assigned the 2,3-trans and 2,3-cis structures XX and XXI, and

were at that time represented by conformations XXII and XXIII, respectively.



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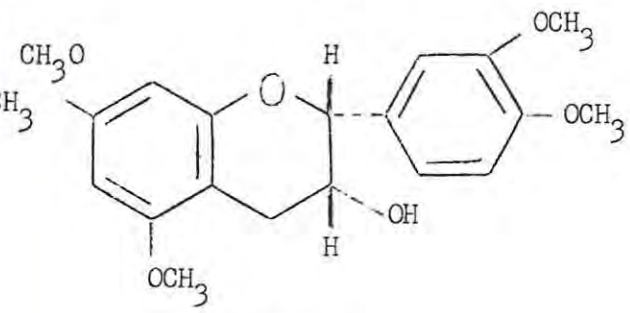

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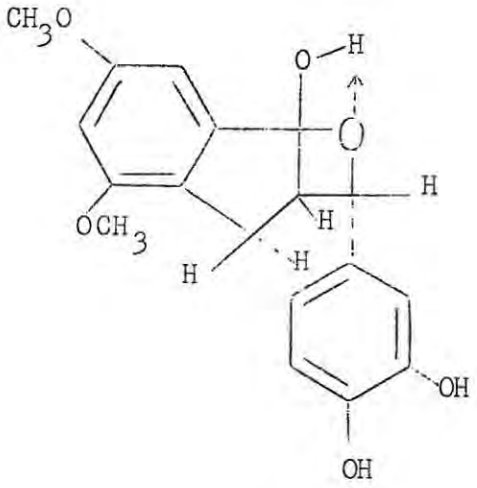

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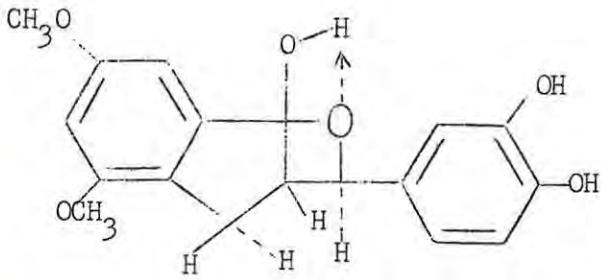


(XXIII)

The observed higher R_F value on cellulose sheets of (+)-catechin compared with (-)-epicatechin was ascribed to a measurable difference in molecular size and shape, and Roberts[18] thought that this was due to (-)-epicatechin possessing a more compact molecule than (+)-catechin.

The conformations XXII and XXIII have subsequently been shown

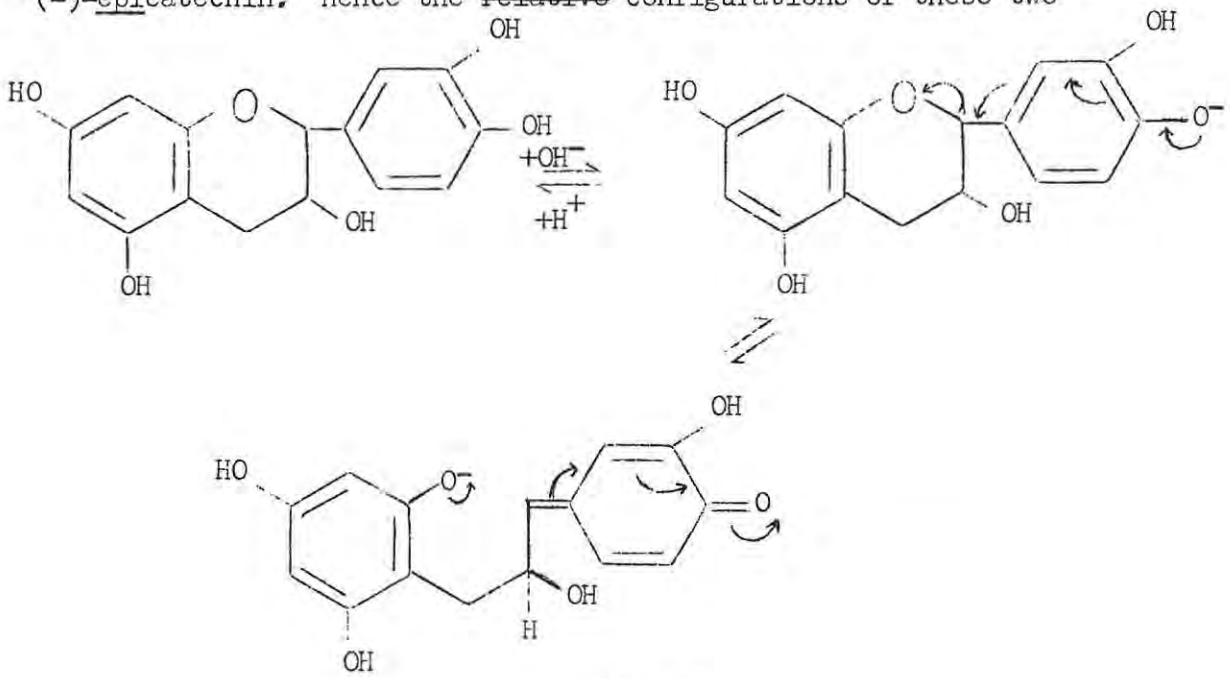
to be inaccurate when compared with the absolute configurations of the flavan-3,4-diols. Recent chemical evidence[19], confirmed by nuclear-magnetic-resonance studies, has shown that the 2-phenyl group in flavan-3,4-diols assumes the more stable ~~equational~~ equatorial position due to its greater bulk, compared with other substituent groups.

Infrared spectra of flavan-3,4-diols by Philbin et al.[20] showed that in the case of a 2,3-trans-3,4-trans-diol a ~~sharp~~ strong absorption ~~signal~~ at 3608 $cm.^{-1}$ resulted from two different O-H stretching frequencies. One involved the π-bonding of the hydrogen atom of the 3-hydroxyl group to the phenyl ring, while the other showed the weak bonding of the hydrogen atom of the 4-hydroxyl group to the oxygen atom at C-3.

The catechins undergo epimerization and racemization in hot aqueous solutions[21]. Thus (+)-catechin may be epimerized to (+)-epi-catechin and (-)-epicatechin to (-)-catechin. This reaction could proceed via inversion of the 2-aryl group and might conceivably involve the formation of an intermediate quinone XXIV[22].

Birch, Clark-Lewis and Robertson[23] proved that the inversion involved the 2-aryl group, leaving the 3-hydroxyl group intact. This was done by reducing (+)-catechin and (-)-epicatechin tetramethyl ethers with sodium in liquid ammonia. Ring opening, through the splitting of a benzyl ether linkage, occurred to yield diphenyl propanols which were methylated. (+)-Catechin gave the pentamethoxy 1,3-diaryl-propane-2-ol XXV with an excess of the (-)-enantiomer and (-)-epicatechin yielded 1-(3,4-dimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-propane-2-ol XXVI, with an excess of the (+)-enantiomer. These reductions established that

the 3-hydroxyl was of opposite configuration in (+)-catechin and (-)-epicatechin. Hence the ~~relative~~ absolute configurations of these two



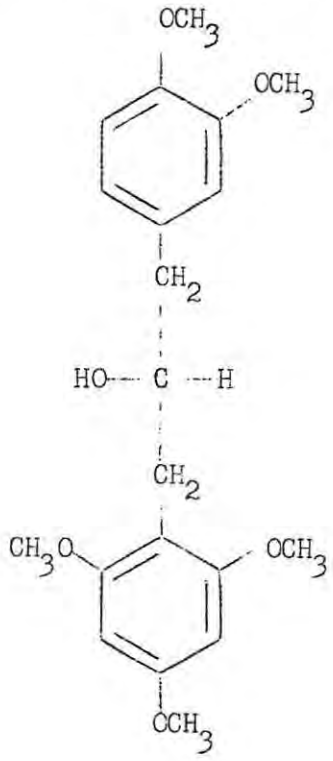
(XXIV)

compounds could be deduced.

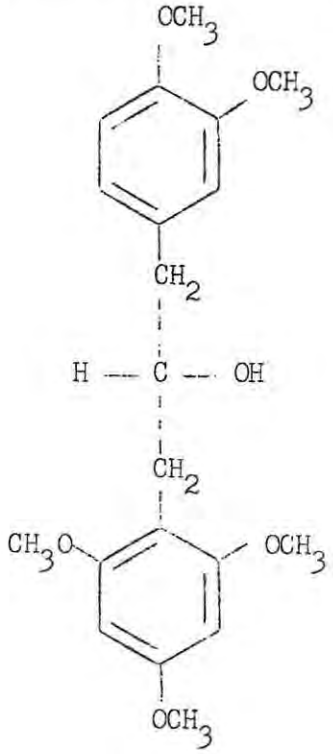
The absolute configurations of (+)-catechin and (-)-epicatechin were initially proposed by Freudenberg[24] who had assumed that epimerization involved the 2-aryl group. This assumption has subsequently been proved correct. (+)-Catechin which has a low rotation in ethanol, formed a strongly dextro-rotatory (+)-epicatechin on epimerization.

Proof of the absolute configurations of (+)-catechin and (-)-epicatechin was provided[23] by the application of Prelog's[25] atrolactic acid method to the 5,7,3',4'-tetramethyl ether of (-)-epicatechin. This consisted of treating the 3-phenyl glyoxylate XXVII with methyl magnesium iodide and hydrolising the resultant atrolactic ester XXVIII to give atrolactic acid with an excess of the (-)-isomer

XXIX. This indicated that (-)-epicatechin had configuration XXX,

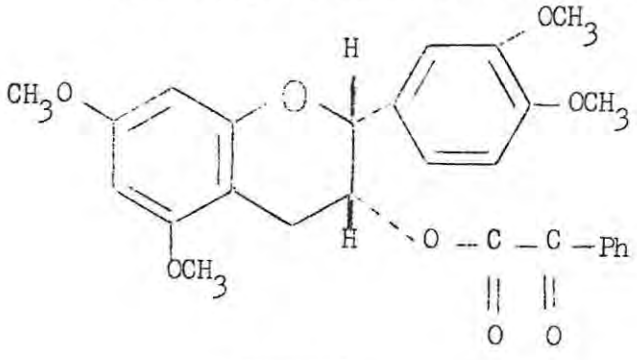


(XXV)

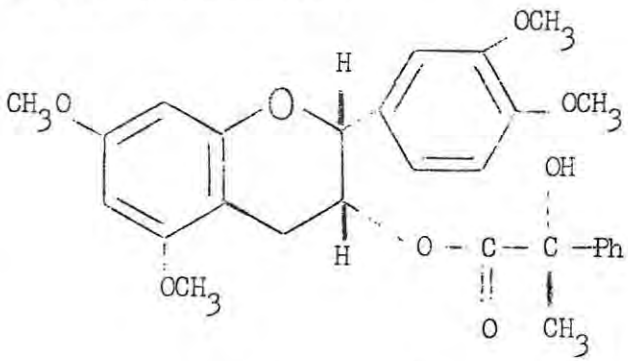


(XXVI)

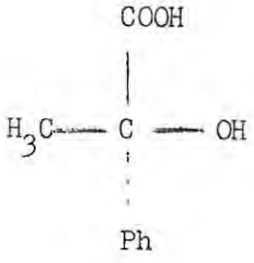
while (+)-catechin would then have the configuration XXXI.



(XXVII)



(XXVIII)



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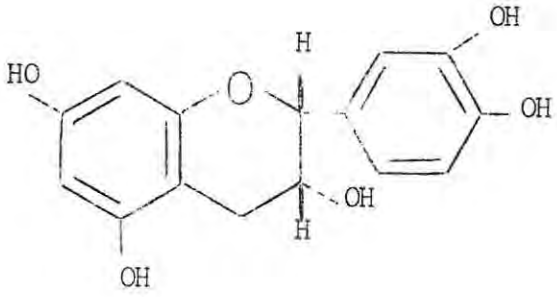

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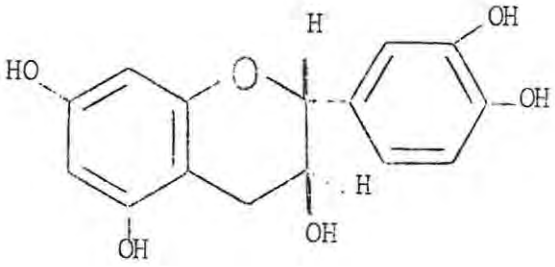
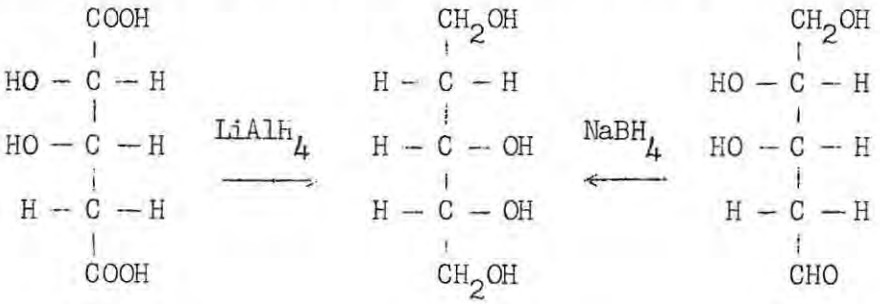


(XXXI)

The absolute configuration of (+)-catechin was confirmed independently by Hardegger, Gempeler and Zust[26] who proved that exhaustive ozonolysis of (+)-catechin gave the dicarboxylic acid XXXII. This acid was esterified and then reduced with lithium aluminium hydride. The resultant tetrahydric alcohol XXXIII was characterized as tetraphenylurethane, identical with an authentic compound prepared from 2-desoxy-D-ribose XXXIV by reduction with sodium boronhydride.



(XXXII) (XXXIII) (XXXIV)

The configuration assigned to (-)-epicatechin by Freudenberg[24] has been confirmed by Zust, Lohse and Hardegger[27] by exhaustive ozonolysis similar to that used for (+)-catechin. Hence the configurations of

the assymmetric centres C-2 and C-3 were established for (+)-catechin and (-)-epicatechin, and their absolute configurations proved.

By using the R and S nomenclature of Cahn, Ingold and Prelog[28] it follows that (+)-catechin is (2R, 3S)-5,7,3',4'-tetrahydroxyflavan-3-ol, while (-)-epicatechin is (2R, 3R)-5,7,3',4'-tetrahydroxyflavan-3-ol.

In recent years the geometrical configurations of flavan derivatives have been established with certainty, mainly as a result of the application of physical methods, notably nuclear-magnetic-resonance spectroscopy.

The cis and trans stereochemistry at C-2 and C-3 of the catechins is clearly correlated with the low spin-spin coupling constants ($J_{2,3}$= 0 - 1 c./sec.) for the 2,3-cis compounds and the much higher value ($J_{2,3}$= 6.5 c./sec.) for the 2,3-trans isomers[29,30]. Thus, assuming a half-chair conformation, the large 2-aryl group is equatorial in both instances, while the hydroxyl group at C-3 is axial in (-)-epicatechin, i.e. (2,3-cis)-type compounds, and equatorial in (+)-catechin, i.e. (2,3-trans)-types.

Recently Clark-Lewis et al.[31] measured the dihedral angles for the half-chair and sofa conformations by means of Dreiding models, and compared the coupling constants for 2,3-trans -and 2,3-cis-flavan-3-ols with those calculated from the Karplus relation[32]. Unfortunately the approximate nature of the Karplus equation did not allow them to distinguish between the alternative conformations.

For the 2,3-trans compounds it was shown that conformations where the 2-aryl group was equatorial, was favoured. In the case of

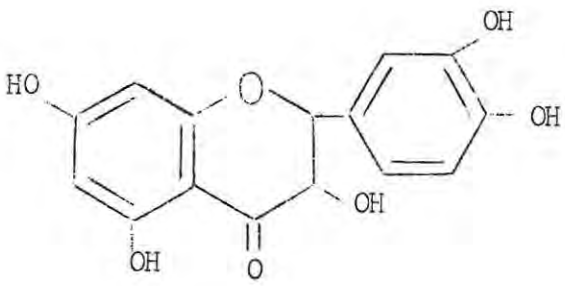
2,3-cis compounds it was found that the agreement between the coupling constants and the observed values was poor, and it was thought that this was due to distortion of the conformations in which the 2-aryl group was equitorial, or a high percentage population of conformations where this group was axial. If conformational inversion is considered, the situation arises where it becomes difficult to decide which of the four conformations (2 equivalent half-chair and 2 equivalent sofa) is preferred.

The Dihydroflavonols.

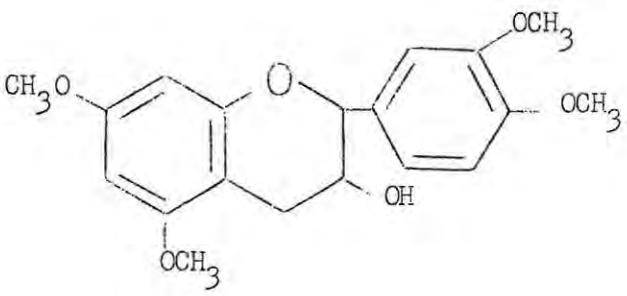
The 3-hydroxyflavanones III, commonly termed the "dihydroflavonols", occur naturally and several synthetic representatives have also been prepared. These compounds may be successively reduced to flavan-3,4-diols and catechins. Theoretically the dihydroflavonols could exist, like the catechins, in cis- and trans- forms. However, the natural dihydroflavonols which have been investigated so far all have the stable trans structure.

(+)-Dihydroquercetin XXXV on treatment with acids[33] or bases[34] did not yield the expected cis-trans mixture but gave instead a single racemate. Hydrogenolysis of the tetramethyl ether of this racemate gave (±)-catechin tetramethyl ether XXXVI[15]. This proved the trans configuration of (±)-dihydroquercetin. Similarly, hydrogenation of (+)-dihydrokaempferol trimethyl ether XXXVII yielded (+)-afzelechin trimethyl ether XXXVIII.

These results were in agreement with Mahesh and Seshadri's[5] conclusions that the natural dihydroflavonols all belonged to the trans

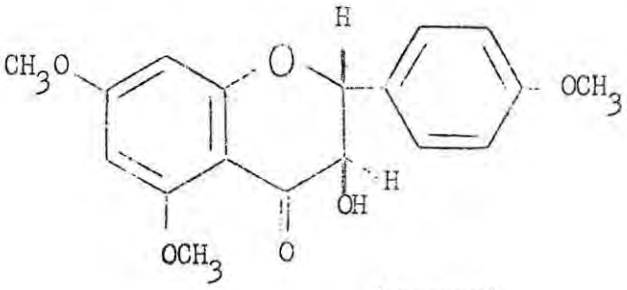


(XXXV)

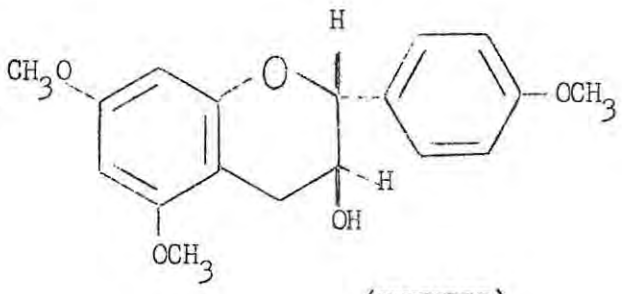


(XXXVI)

series because they may be easily dehydrogenated, but not readily dehydrated. Certain synthetic dihydroflavonols such as naringenin



(XXXVII)



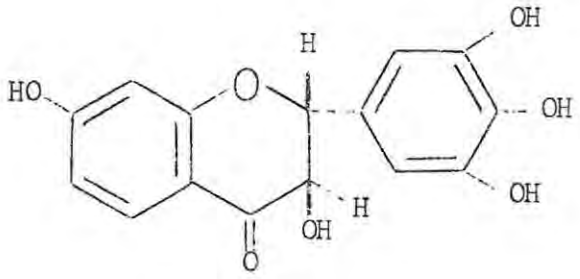
(XXXVIII)

(5,7,4'-trihydroxyflavan-3-ol-4-one), however, belong to the <u>cis</u> series[5]. These are readily dehydrated to flavones, but not easily converted to the corresponding flavonols.

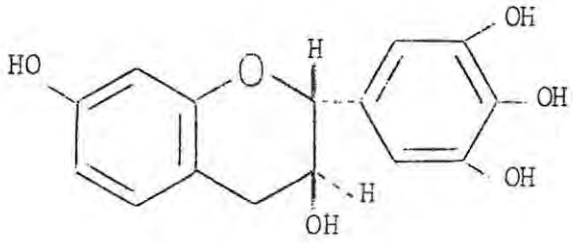
Catalytic hydrogenation of (+)-dihydrorobinetin[35] XXXIX with platinum oxide to the flavan-3,4-diol and subsequently with palladium, yielded (-)-robinetinidol XL which is an analogue of (+)-catechin.

From the experimental results above the absolute configurations of the dihydroflavenols were deduced. Thus (+)-dihydroquercetin, (+)-

dihydrokaempferol, (+)-dihydrorobinetin and (+)-fustin all have the 2R, 3R configuration, while (-) fustin was found to be 2S, 3S. With



(XXXIX)



(XL)

the exception of (-)-fustin, obtained by Freudenberg and Weinges[36] from the wood of Cotinus coggrygria, all the naturally occurring dihydroflavanols are dextrorotatory in most solvents. Hence it may be stated that all, except (-)-fustin, have the absolute configuration similar to (+)-catechin, while (-)-fustin is similar to (-)-catechin. As such (-)-fustin is therefore an optical antipode of the (+)-dihydroflavonols and with (+)-fustin it forms an enantiomeric pair of dihydroflavonols.

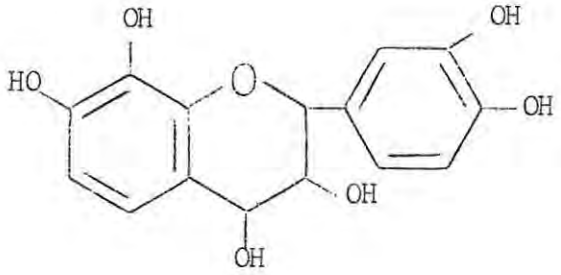
Reduction of dihydroflavonols with zinc and hydrochloric acid yield flavanones through the hydrogenation of the 3-hydroxy group.

The Leucoanthocyanidins.

The flavan-3,4-diols V, representing one class of "leuco- or pro-anthocyanidins", contain three centres of assymmetry in comparison with two such centres in the flavan-3-ols. Each flavan-3,4-diol may therefore yield four racemates and hence eight optically active forms.

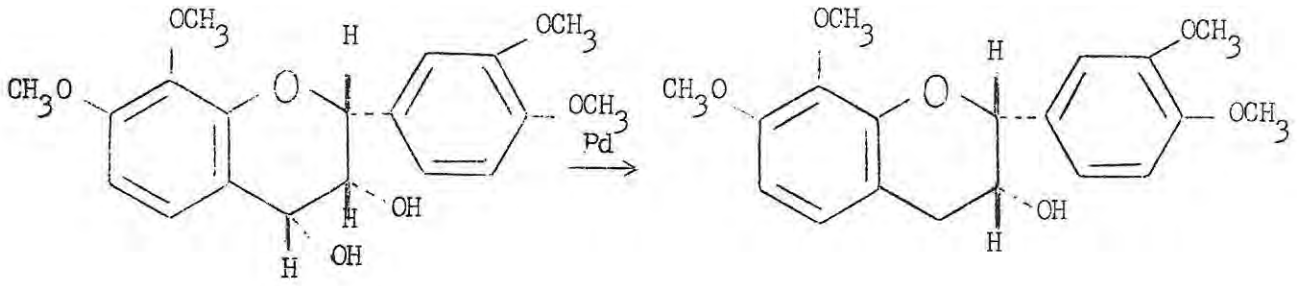
Mozingo and Adkins[37] obtained the first leucoanthocyanidin, (±)-flavan-3,4-diol V, by reduction of (±)-3-hydroxyflavanone with copper chromite at high temperature and pressure.

King and Bottomley[38] isolated melacacidin XLI, the first flavan-3,4-diol to be obtained from natural sources, and also synthesized one of the four possible racemates of its tetramethyl ether by hydrogenation of the corresponding flavonol with Raney nickel. Catalytic reduction of a flavonol would give 2,3-cis addition, hence melacacidin was expected to have 2,3-cis configuration.



(XLI)

The reduction product formed a high yield of a cyclic carbonate and an isopropylidene derivative, indicating that the hydroxyl groups at C-3 and C-4 were probably cis. Hence melacacidin was regarded as the 2,3-cis-3,4-cis compound, and this has subsequently been confirmed from nuclear-magnetic-resonance data[39]. Reduction of (-)-melacacidin tetramethyl ether XLII over a palladium catalyst produced (-)-7,8,3',4'-tetramethoxyflavan-3-ol XLIII which is an analogue of (-)-epicatechin. Therefore (-)-melacacidin has the 2R, 3R, 4R configuration[40].



(XLII) (XLIII)

(-)-Teracacidin (7,8,4'-trihydroxyflavan-3,4-diol) was shown by Clark-Lewis and Katekar[41] to be stereochemically and structurally similar to (-)-melacacidin. Hence (-)-teracacidin was also assigned the 2R, 3R, 4R configuration.

Weinges[42] showed that (-)-leucofisetinidin [(-)-7,3',4'-trihydroxyflavan-3,4-diol] was an analogue of (-)-catechin and therefore had the 2,3-trans configuration. He assigned the absolute configuration 2R, 3R, 4R to this compound. This has subsequently been shown to be inaccurate and has been revised to 2S, 3R, 4S[43].

(+)-Mollisacacidin is the enantiomorph of (-)-leucofisetinidin. Hydrolysis of the trimethyl ether of the former compound gave (-)-fisetinidol trimethyl ether, an analogue of (+)-catechin tetramethyl ether. Hence Clark-Lewis and Katekar[41] proposed the 2R, 3S, 4S configuration for (+)-mollisacacidin. Synthetic (+)-leucorobinetinidin [(+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol][43] is an analogue of (+)-mollisacacidin and was thought to have the same 2R, 3S, 4S configuration.

These assignments of absolute configurations of flavan-3,4-

diols[44] depended on two factors - firstly, the established absolute configurations of (+)-catechin and (-)-epicatechin which both possess the 2R configuration as shown before, and secondly, the assumption that certain 3,4-diols were cis-glycols because they readily formed iso-propylidene derivatives. This assumption has been found to be rather unreliable[45], although generally accepted at that time.

As has already been indicated, the stereochemistry of flavan-3,4-diols at C-2 and C-3 may be determined fairly readily by the conversion of the diols to their corresponding flavan-3-ols. Investigation of the molecular rotations of these flavan-3-ols and their derivatives enabled Weinges[35] to determine whether the compound belonged to the (+)-catechin (2,3-trans) or (-)-epicatechin (2,3-cis) series. Since the absolute configurations of these two catechins were known, it was possible to assign C-2 and C-3 configurations to the flavan-3-ol formed, and hence to the original flavan-3,4-diol.

The 3,4-cis or -trans nature of the diol groups of flavan-3,4-diols may be established with some certainty by observing the cleavage rate of the 1,2-glycol unit with either periodic acid[46,47], or with lead tetra-acetate[47,48,49]; fission of a 3,4-cis diol group being faster than that of a 3,4-trans diol grouping.

Brown and co-workers[46] showed that 3,4-cis diols usually formed higher yields of isopropylidene derivatives than 3,4-trans diols. Drewes and Roux[45], on the other hand, found that the yields of iso-propylidene derivatives formed by methylated flavan-3,4-diols did not clearly distinguish between the 3,4-cis and 3,4-trans diols.

Formation of high yields of cyclic carbonates by 3,4-cis diols appears to be more diagnostic in differentiating between 3,4-cis and 3,4-trans diols[50], but cannot be regarded as absolute proof since cyclic carbonates of 3,4-trans compounds have been synthesized by Bokadia et al.[48], Corey, Philbin and Wheeler[50] and Fujise, Hishida et al.[51].

Reaction with 2,2-dimethoxypropane was used by Brown and MacBride[52] to assign relative stereochemistry to five pairs of 2,3-trans-flavan-3,4-diols. They reacted (+)-2,3-trans-flavan-3,4-diols with 2,2-dimethoxypropane in the presence of toluene-p-sulphonic acid as catalyst to give high yields of isopropylidene derivatives from cis-isomers and no yield, ~~or~~ only trace quantities, from the trans isomers. The same diol pairs were examined by thin-layer chromatography on silica gel ; cis diols having higher R_F values than the trans diols. This effect was interpreted as being due to the formation of stronger hydrogen bonds with the stationary phase by trans diols than by the cis diols.

In recent years a study of the configurations of flavan-3,4-diols has been made with the aid of nuclear-magnetic-resonance spectroscopy by workers such as Corey, Philbin and Wheeler[50], Clark-Lewis et al.[30,39], Roux and et al.[47,53] and Vickars[54].

Conroy[55] used the valence bond calculation of Karplus to correlate the dihedral angles of neighbouring protons and their coupling constants, and showed that coupling is highest at dihedral angles of 0° (J = 8.0 c./sec.) and 180° (J = 9.2 c./sec.), and lowest at an angle

of approximately 90° (J = 0 c./sec., or less).

By measuring the spin-spin coupling constants of the C-2, C-3 and C-4 protons of fully substituted flavan-3,4-diols it therefore becomes possible, in most cases, to interpret the nuclear-magnetic-resonance spectra of such compounds and to assign their stereochemical configurations.

Roux et al.[53,56] examined the configuration of diol groups in flavan-3,4-diols by means of paper chromatography and paper ionophoresis. Correlation of paper chromatographic and paper ionophoretic behaviour of methylated flavan-3,4-diols with their stereochemistry, as determined from n.m.r. data, offered simple but fairly reliable criteria for differentiating between certain cis and trans diol configurations.

On paper impregnated with aqueous sodium borate buffers the isomers of 3,4-cis diol configuration exhibited lower R_F values than those with 3,4-trans arrangement. Only the former groups showed positive ionophoretic mobility. These simple methods have been found useful for determining the purity of geometrical isomers, and also for differentiating between 3,4-cis and 3,4-trans glycol arrangements.

Paper ionophoresis has been used on a preparative scale by Drewes and Roux[57] for the separation of the methyl ethers of isomeric flavan-3,4-diol components extracted from Acacia auriculiformis heartwood. The mixture of methylated isomers was resolved on Whatman no.3 paper which had been previously impregnated with sodium borate-boric acid buffer. By applying a potential across the electrodes the 2,3-trans-

3,4-cis isomer gave a large positive mobility, while the 2,3-cis-3,4-trans racemate resulted in a lower negative migration. Elution of these bands with 50% aqueous ethanol yielded pure isomers.

Stereospecific Syntheses of Flavan-3,4-diols.

Comparison of physical and chemical properties of flavan-3,4-diols, synthesized by stereospecific or epimeric methods, with their naturally-occurring counterparts greatly facilitates the elucidation of the stereochemistry of these compounds. This is of even greater significance in the case of free-phenolic forms of flavan-3,4-diols where direct chromatographic comparison with the natural products may be made.

Nuclear-magnetic-resonance spectrometric studies of flavonoid compounds showed the potential value of this technique for the determination of the geometrical configurations of flavan-3,4-diols. In order to achieve this a complete set of four racemates of a synthetic flavan-3,4-diol is necessary to enable interpretation of the spectra of analogous naturally-occurring compounds.

(a) Flavan-3,4-diols from Flavonols.

In 1938 Mozingo and Adkins[37] synthesized the first leucoanthocyanidin, (±)-flavan-3,4-diol, by catalytic hydrogenation of (±)-3-hydroxyflavanone.

King and Clark-Lewis[58] prepared the first crystalline synthetic leucoanthocyanidin, 7,8,3',4'-tetramethoxyflavan-3,4-diol, by catalytic reduction of 7,8,3',4'-tetramethoxyflavonol in ethanol over

Raney nickel catalyst at 100° and 100 atmospheres of pressure. This diol was thought to be the 2,3-cis-3,4-cis compound because it formed an isopropylidene derivative and gave a high yield of a cyclic carbonate. Also, it was known that catalytic reduction of ethylenic double bonds of planar flavonoids leads to cis addition. The synthetic diol was therefore regarded as the cis-cis racemate and this had subsequently been confirmed by n.m.r. spectroscopy[39].

More recently Fujise, Fujise and Hishida[59], Clark-Lewis, Jackman and Williams[30], and Roux et al.[47,53] used this method for the synthesis of 2,3-cis-3,4-cis-flavan-3,4-diols.

(b) Flavan-3,4-diols from Dihydroflavonols.

Isolation and syntheses of the dihydroflavonols occurred long after those of flavones and flavonols. In 1934 Oyamada[60] found fustin (7,3',4'-trihydroxyflavan-3-ol-4-one) occurring with fisetin (7,3',4'-trihydroxyflavone-3-ol) in Rhus ssp. He found fustin to be 2,3-dihydrofisetin. All the known natural dihydroflavonols which have been isolated since are regarded to have the C-2 phenyl and C-3 hydroxyl groups equatorial and trans to one another, and hence they belong to the 2,3-trans catechin series. Flavan-3,4-diols obtained by reduction of these dihydroflavonols will therefore have the 2,3-trans configuration. Mahesh and Seshadri[5] and Kulkarni and Joshi[6] made a detailed study of the stereochemistry of the dihydroflavonols and found that they were useful starting materials for the synthesis of flavan-3,4-diols, due to the fact that they are more readily reduced than the flavonols.

(i) Catalytic Hydrogenation of Dihydroflavonols.

The first successful catalytic reduction of a free-phenolic dihydroflavonol was achieved by Roux and Freudenberg[61] who hydrogenated dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one) with Adams catalyst to give 7,3',4',5'-tetrahydroxy-flavan-3,4-diol (leuco-robinetinidin) in a crystalline form. Initially this compound was thought to have the 2,3-trans-3,4-cis configuration[62].

Bognar and Rakosi[63] synthesized one of the stereoisomeric racemates of flavan-3,4-diol in high (70-80%) yield by the reduction of flavanone-3-ol (a) by palladium-charcoal in ethanol or acetic acid solution, (b) by treatment with sodium boronhydride in methanolic solution, and (c) by the reaction of an ethereal solution of the dihydroflavonol with lithium aluminium hydride. From considerations of Barton's rules[64] for the reduction of ketones it was inferred that the resulting unsubstituted flavan-3,4-diol had the 2,3-trans-3,4-trans configuration.

Hydrogenation of 7,8,3',4'-tetramethoxy-trans-flavanonol by Kulkarni and Joshi[65] over a platinum catalyst in acetic acid yielded a flavan-3,4-diol which was regarded as identical with the tetramethyl ether of (±)-melacacidin (2,3-cis-3,4-cis)[58]. This compound has subsequently been shown to be the ~~2,3-cis-3,4-trans~~ 2,3-trans-3,4-cis racemate[59].

Keppler[66], and also Roux and Freudenberg[61], reduced (±)-fustin obtained from Rhus glabra and Rhus succedanea with Adams catalyst in methanol to give (±)-leucofisetinidin (7,3',4'-trihydroxyflavan-3,4-diol).

Similarly, (+)-fustin from Acacia mearnsii[67] and (-)-fustin from Rhus cotinus[36] on reduction with platinum oxide gave (+)-leucofisetinidin and (-)-leucofisetinidin, respectively. Due to the fact that these compounds all formed isopropylidene derivatives in fair yield they were originally regarded as having 2,3-trans-3,4-cis configurations.

The relative configurations of (+)-mollisacacidin, (-)-leucofisetinidin and (+)-leucorobinetinidin were subsequently revised by Drewes and Roux[47] to the 2,3-trans-3,4-trans arrangements. This was done by comparing the oxidation rates and n.m.r. spectra of these compounds with synthetic 2,3-trans-3,4-trans and 2,3-trans-3,4-cis analogues.

Hydrogenation of 7,4'-dimethoxy-2,3-trans-dihydroflavonol over Adams catalyst afforded the 7,4'-dimethoxy-2,3-trans-flavan-3,4-trans-diol[53]. Fujise et al.[68,69,70] used the catalytic hydrogenation of 3-hydroxyflavanones to synthesize trans, trans isomers of 5,7,3',4'-tetramethoxyflavan-3,4-diol, 5,7,3',4',5'-pentamethoxyflavan-3,4-diol, 7,3',4'-trimethoxyflavan-3,4-diol, 4'-methoxyflavan-3,4-diol and 7-methoxyflavan-3,4-diol.

Catalytic hydrogenation of dihydroflavonols with Adams catalyst is stereospecific and affords flavan-3,4-diols with the 2,3-trans-3,4-trans configuration.

(ii) Reduction of Dihydroflavonols with Metal Hydrides.

In 1950 Mirza and Robinson[71] reduced kaempferol (3,5,7,4'-tetrahydroxyflavone) to leuco-pelargonidin (5,7,4'-trihydroxyflavan-

3,4-diol) using $LiAlH_4$. Birch et al.[72] used the same catalyst to reduce the acetate of quercetin (3,5,7,3',4'-pentahydroxyflavone) to leucocyanidin. These workers regarded the action of lithium aluminium hydride, through the donation of hydride ions, to be closely allied to the mechanism of reducing coenzymes.

Swain[73] hydrogenated taxifolin (5,7,3',4'-tetrahydroxyflavan-3-ol-4-one) from Douglas fir bark with sodium boronhydride to yield non-crystalline 5,7,3',4'-tetrahydroxyflavan-3,4-diol. The product was found to be very labile and on treatment with 2N hydrochloric acid it gave a white amorphous polymer. (±)-4'-Methoxy-6-methylflavanonol, (±)-7,8,3',4'-tetramethoxyflavanonol and (±)-7,3',4'-trimethoxyflavanonol have been reduced by Kulkarni et al.[6,65] with lithium aluminium hydride to give mixtures of 3,4-cis and 3,4-trans diols. From a consideration of the von Anwers-Skita rule[74], which postulates that the lower melting isomer should be the 3,4-cis diol, they regarded the higher melting diol obtained from 4'-methoxy-6-methylflavanonol as the 2,3-trans-3,4-trans racemate, while the lower melting diol would then have the 2,3-trans-3,4-cis configuration.

These diols were re-examined by Brown, Bokadia et al.[48]. From the formation of cyclic derivatives, their rates of reaction with lead tetra-acetate and their n.m.r. spectra they concluded that the above assignments should be reversed.

Fujise et al.[68,69,70] formed mixtures of 2,3-trans-3,4-cis and 2,3-trans-3,4-trans-flavan-diols, methylated at 7 ; 4' ; 7,3',4';5,7,3',4' and 5,7,3',4',5' positions, from the corresponding trans-dihydroflavonols

by the reduction with lithium aluminium hydride. No indication was given regarding the separation of the mixture of stereoisomers.

trans-Dihydroflavonols, possessing the 2(eq), 3(eq) conformation, usually yield mixtures of epimeric diols with 4(eq)-OH and 4(ax)-OH on reduction with complex hydrides.

(iii) Reduction of Dihydroflavonols with $LiAlH_4$ + $AlCl_3$ Reagent.

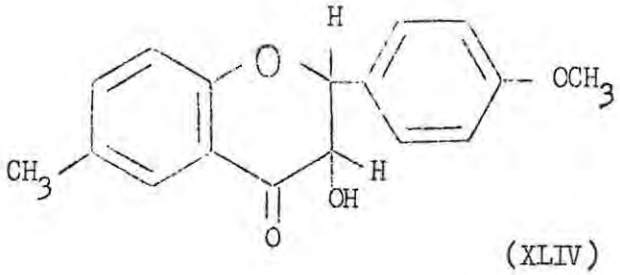
During 1961 Bokadia, Brown et al.[48] hydrogenated 3-hydroxy-flavanone with a mixture of $LiAlH_4$ and $AlCl_3$ to yield a flavan-3,4-diol identical with that obtained by Bognar et al.[19] from 3-hydroxyflavanone oxime via the 4-amino-flavan-3-ol. The former workers investigated the stereochemistry of the diol. From considerations of the formation of a cyclic carbonate, the yield of isopropylidene derivative and the rate of reaction with lead tetra-acetate, they concluded that the diol possessed the 2,3-trans-3,4-cis configuration. An examination of the n.m.r. spectrum of the methylated diacetate of the flavan-3,4-diol confirmed this arrangement.

Clark-Lewis, Jackman and Williams[75] synthesized pairs of 2,3-trans-flavan-3,4-diols from racemic trans-dihydroflavonols. The 4'-methoxy-6-methyl-2,3-trans-flavan-3,4-trans-diol was obtained by reduction of 4'-methoxy-6-methyl-2,3-trans-dihydroflavanol in acetic acid over platinum-charcoal, and by its reduction with sodium boron-hydride or with the lithium aluminium hydride. The 4'-methoxy-6-methyl-2,3-trans-flavan-3,4-cis-diol was initially found as a by-product in the reduction of the above dihydroflavonol, but was more readily synthesized

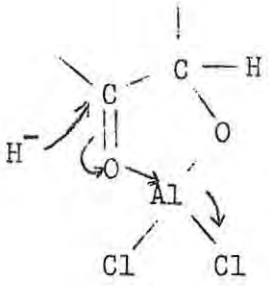
by hydrogenation with $LiAlH_4$ + $AlCl_3$ reagent.

The reduction with the "mixed reagent", $LiAlH_4$ + $AlCl_3$, was successfully applied by Drewes and Roux[76] in the synthesis of (±)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-cis-diol from (±)-7,3',4'-trimethoxy-2,3-trans-dihydroflavonol, and by Lillya, Drewes and Roux[77] in the preparation of (+)-5,3',4',5'-tetramethoxy-2,3-trans-flavan-3,4-cis-diol from (+)-5,3',4',5'-tetramethoxy-2,3-trans-dihydroflavonol.

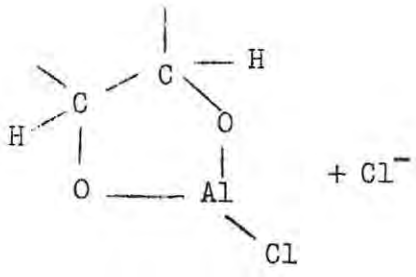
A possible explanation of this reduction mechanism with the "mixed reagent" was advanced by Clark-Lewis et al.[75] They considered that the co-ordination of aluminium with the 3-ol-4-one XLIV could lead to the formation of 3,4-cis-diols with $LiAlH_4$ + $AlCl_3$, just as a hydride ion attack on a keto-alcohol system XLV ought to yield the cis-glycol complex XLVI.



(XLIV)

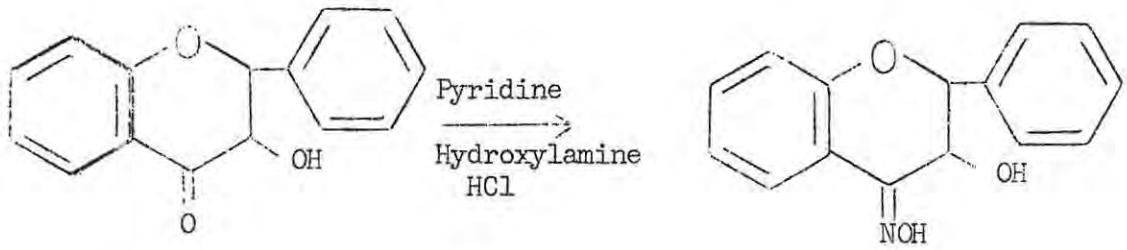


(XLV)

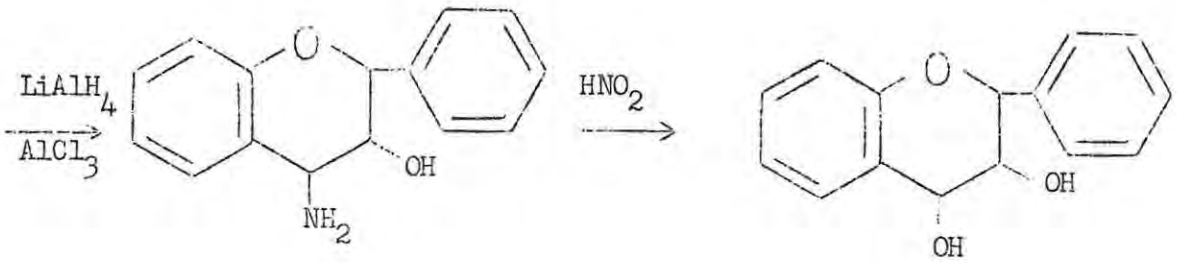


(XLVI)

2,3-trans-3,4-cis-Flavandiols may also be prepared by the method of Bognar, Rakosi, Fletcher, Philbin and Wheeler[19,78], who converted 3-hydroxyflavanone XLVII to the oxime XLVIII which was reduced to the amine XLIX, which in turn yielded the 2,3-trans-flavan-3,4-cis-diol L on reaction with nitrous acid.



(XLVII) (XLVIII)



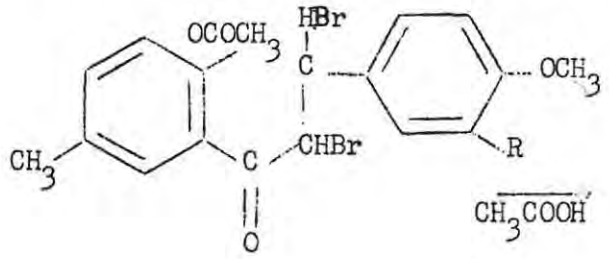
(XLIX) (L)

The above method is, however, more laborious than the more direct "mixed reagent" synthesis of 2,3-trans-3,4-cis-diols.

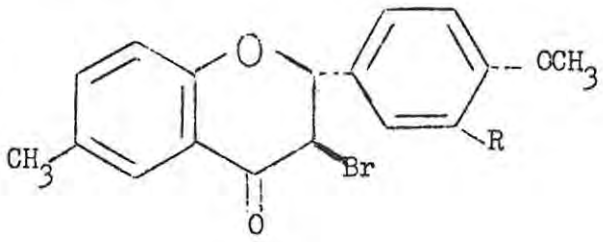
(c) Flavan-3,4-diols from 3-Bromoflavanones.

The preparation of flavan-3,4-diols via the 3-bromoflavanones provides the only synthetic course to diols with the 2,3-cis-3,4-trans

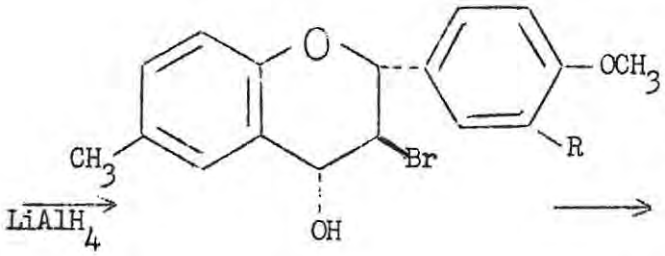
configuration. These diols are the most difficult of the four racemates to synthesize, and the only alternative synthesis is by epimerization of the 2,3-cis-3,4-cis isomer to the diacetate of the 2,3-cis-3,4-trans racemate (see later). The synthetic method has been applied by Kulkarni and Joshi[79] to the preparation of the third racemate of 4'-methoxy-6-methylflavan-3,4-diol. They cyclized the chalcone dibromide LI with acetic acid to give the 3-bromoflavanone LII (R=H) which was reduced with $LiAlH_4$ to the 3-bromoflavan-4-ol LIII (R=H). This was in turn acetylated with acetic anhydride and potassium acetate to yield 4'-methoxy-6-methylflavan-2,3-cis-3,4-trans-diol diacetate LV (R=H).



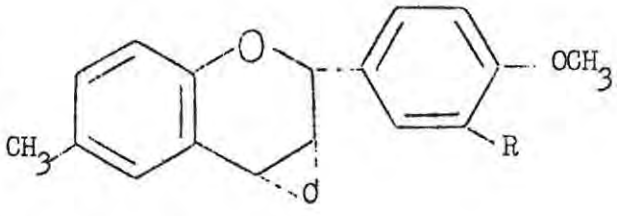
(LI)



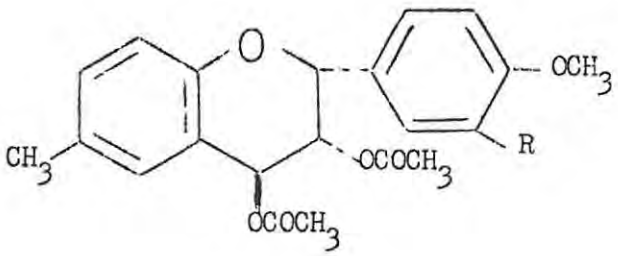
(LII)



(LIII)



(LIV)



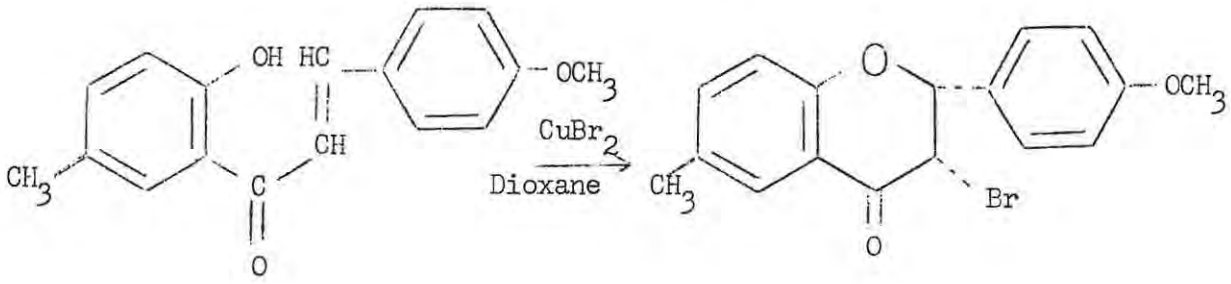
(LV)

The same method was used by Clark-Lewis, Jackman and Williams[75], with some difficulty, for the synthesis of 6-methyl-3',4'-dimethoxy-2,3-cis-flavan-3,4-trans-diol-diacetate. The preparation was based on the reduction of the lower-melting diastereoisomer of the 3-bromoflavanone with lithium aluminium hydride. This 3-bromoflavanone was found by Clark-Lewis, Spotswood and Williams[80], from n.m.r. spectroscopic data, to be the 2,3-trans isomer (m.p. 138°) which on reduction yielded the 2,3-trans-3,4-trans-bromoflavan-4-ol. The latter compound was converted to the 2,3-cis-3,4-trans-diacetate presumably by trans-opening of the intermediate cis-cis epoxide LIV ($R=OCH_3$) with inversion at C-3. Opening of the epoxide ring by fission of the C-4 oxygen bond rather than the C-3 oxygen bond occurs as expected during the formation of the final diacetate LV. The 2,3-cis-3-bromoflavanone (m.p. 158°) was also formed during cyclization of the chalcone. It was observed that the 2,3-cis isomer was less stable on storage than the 2,3-trans-3-bromoflavanone.

Doifode[81] showed that the 2,3-cis-3-bromoflavanone LVII could be formed by cyclization of 2'-hydroxy-5-methyl-4'-methoxy-chalcone LVI with cupric bromide in dioxane. Initially the cupric bromide in dioxane added a molecule of bromine across the double bond in the chalcone and then cyclized the molecule with an elimination of a molecule of hydrogen bromide to give the 3-bromoflavanone.

The failure of 7,4'-dimethoxy-3-bromoflavanone to yield the required 7,4'-dimethoxy-3,4-trans-2,3-cis-flavan-3,4-diacetate[53] appears to be due to the C-7 substitution of the A-ring which is in the meta-

position with respect to the ether oxygen of the heterocyclic ring. Electron release at C-7 is possible and this confers greater reactivity to the A-ring, causing facile inversion at C-4 of the heterocyclic ring.



(LVI) (LVII)

(d) Flavan-3,4-diols by Epimerization.

In an attempt to assign definite stereochemical configurations to synthetic catechins, Kashikar and Kulkarni[82] attempted the synthesis of the trans isomer of 6-methyl-4'-methoxy-3-hydroxyflavan by hydrogenolysis of the corresponding flavan-3,4-diol with sodium boronhydride and borontrifluoride. Instead of obtaining the required catechin they isolated an epimeric flavan-3,4-diol which they regarded as having the 3,4-trans configuration. From the revised configurations of Bokadia, Brown et al.[48] this compound is now known to have the 3,4-cis configuration.

This method of epimerization of the hydroxyl group at C-4 therefore leads to the facile synthesis of 2,3-trans-3,4-cis-flavan-3,4-diols from corresponding 2,3-trans-3,4-trans-diols. As such, this technique provides an alternative to the reduction of dihydroflavonols

with the "mixed reagent" and "oxime-amine" methods for giving 2,3-trans-3,4-cis-flavan-diols.

Clark-Lewis and Mortimer[44] observed that (-)-melacacidin was converted into (-)-isomelacacidin under mild acidic conditions. Treatment of (-)-melacacidin with 0.5N acetic acid gave a 50% conversion in 1 hr. at 100°, while 0.0 IN HCl gave a 90% conversion in 10 min. at the same temperature. Both of these compounds gave the same anthocyanidin which means that they both have the same hydroxylation pattern of the A and B nuclei. Melacacidin and isomelacacidin were regarded as being epimers differing only in configuration at the C-4 position. The epimerization appeared to be an equilibrium reaction favouring (-)-isomelacacidin. In this case we therefore have conversion of a 2,3-cis-3,4-cis compound into a 2,3-cis-3,4-trans epimer. This reaction was only effective with the free-phenolic compounds.

Selective epimerization at C-4 of (+)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-trans-diol by Saayman and Roux[83] afforded the 2,3-trans-3,4-cis epimer with sodium boronhydride and boron trifluoride under Kashikar and Kulkarni's[82] conditions. Similarly (±)-7,4'-dimethoxy-2,3-trans-flavan-3,4-trans-diol was epimerized to (±)-7,4'-dimethoxy-2,3-trans-flavan-3,4-cis-diol. An interesting observation was that the 2,3-cis-3,4-cis diastereoisomer of the latter compound could not be induced to epimerize to the 2,3-cis-3,4-trans form (see later).

Clark-Lewis and Williams[84] referred to the work of Fujise et al.[68,69,70] who had found that acetylation of flavan-3,4-cis-diols with acetic acid, acetic anhydride and sodium acetate at elevated

temperature for extended periods of time, could lead to the formation of 3,4-trans-diacetates through inversion at the C-4 position. This method was used by Saayman and Roux[53,83] for synthesizing the still outstanding 2,3-cis-3,4-trans isomer of (±)-7,4'-dimethoxyflavan-3,4-diol diacetate from the 2,3-cis-3,4-cis-diol by boiling with acetic anhydride, sodium acetate and acetic acid under controlled conditions (see later).

Epimerization of 2,3-cis-3,4-cis-glycols to 2,3-cis-3,4-trans-diacetates subsequently provided to the most convenient route to 3',4'-dimethoxy- and 4'-methoxy-6-methyl-2,3-cis-flavan-3,4-trans-diacetates[84]. The diacetates of 2,3-cis-3,4-trans-flavan-diols are the least accessible of the four racemates and this novel synthesis by inversion at C-4 during acetylation of cis, cis-diols is a far superior method of preparation of the cis, trans-diacetates than the only previously available route via the 3-bromoflavanones and 3-bromoflavan-4-ols. This method may also be used to convert 2,3-trans-3,4-cis-diols into 2,3-trans-3,4-trans-diacetates in low yield[85].

Drewes and Roux[86,87] epimerized the free-phenolic form of optically pure (+)-mollisacacidin, of 2,3-trans-3,4-trans configuration, by heating in steam under pressure to give three diastereoisomers - identified as the 2,3-trans-3,4-cis, 2,3-cis-3,4-trans and 2,3-cis-3,4-cis isomers, the latter only in low yield. The free-phenolic forms of optically active isomers related to natural flavan-3,4-diols were thus available by epimerization of an optically pure form[88]. Similarly, epimerization of optically pure (+)-leucorobinetinidin yields three

diastereoisomers[89].

The mechanism of epimerization of (+)-mollisacacidin and its diastereoisomers has recently been discussed by Drewes and Roux[88]. The inversion mechanisms at C-2 and C-4 were examined by following the epimerization of each diastereoisomer by means of two-dimensional paper chromatography in 2% acetic acid and water-saturated sec.-butanol. These solvents separated the mixture of four diastereoisomers.

It appeared that the 2,3-cis-3,4-cis isomer was the least favoured configuration under the reaction conditions, while the 2,3-cis-3,4-trans and 2,3-trans-3,4-trans were more stable and therefore more favoured.

The epimerization mechanism may be vizualized as the formation of two diastereoisomers resulting from the inversion of the original 2,3-trans-3,4-trans starting material at C-2 and C-4. The third product might be formed by inversion at the remaining of these two asymmetric centres of one or both of the intermediate products. Thus the 2,3-trans-3,4-trans isomer yields initially the 2,3-trans-3,4-cis and the 2,3-cis-3,4-trans racemates by inversion at C-2 and C-4, respectively. The 2,3-cis-3,4-cis product is formed mainly from the 2,3-trans-3,4-cis isomer as this route appears to be the favoured course of the epimerization.

Methylation of the free-phenolic hydroxyl groups inhibited epimerization as evidenced by a series of epimerizations under similar conditions of the trimethyl ethers of the four diastereoisomeric (+)-leucofisetinidins. Only slight inversions at C-4 occurred of 2,3-trans-

3,4-trans to 2,3-trans-3,4-cis and 2,3-cis-3,4-cis to 2,3-cis-3,4-trans forms.

Summary of the Syntheses of Diastereoisomeric Flavan-3,4-diols.

1. 2,3-trans-3,4-trans-Flavan-diols are readily obtained by catalytic reduction with ~~adams~~ Adams' catalyst of 2,3-trans-dihydroflavonols. These may also be formed from the 2,3-trans-dihydroflavonols by reduction with $LiAlH_4$ or $NaBH_4$.

2. 2,3-trans-3,4-cis-Flavan-diols are prepared by hydrogenation of 2,3-trans-dihydroflavonols with $LiAlH_4 + AlCl_3$. An alternative route is from the 2,3-trans-dihydroflavonol via the "oxime-amine" intermediate. 2,3-trans-3,4-trans-Diols also yield the 2,3-trans-3,4-cis isomers by epimerization at C-4 with metal hydride - metal halide reagents such as $NaBH_4$ and BF_3.

3. 2,3-cis-3,4-cis-Flavan-diols are formed by catalytic hydrogenation of flavonols with Raney nickel or copper chromite at elevated temperatures and pressures.

4. 2,3-cis-3,4-trans-Flavan-diol-diacetates may be synthesized from 3-bromoflavanones via the 3-bromo-flavan-4-ols. Flavan-3,4-diols substituted in the 7-position are exceptions to this rule. The cis, trans-diacetates may also be prepared from 2,3-cis-3,4-cis-diols by acetylation with acetic anhydride, potassium acetate and acetic acid. By means of this reaction the 2,3-trans-3,4-trans-flavan-diol-diacetates may be formed in low yield from the corresponding 2,3-trans-3,4-cis-diols.

5. Epimerization of optically pure, free-phenolic forms of flavan-3,4-diols yield the remaining three diastereoisomers by inversions at C-2 and C-4 of the parent compound, followed by further inversions of these two products to give the third. The reaction is effected at high temperature and pressure by autoclaving aqueous solutions of the free-phenolic diols.

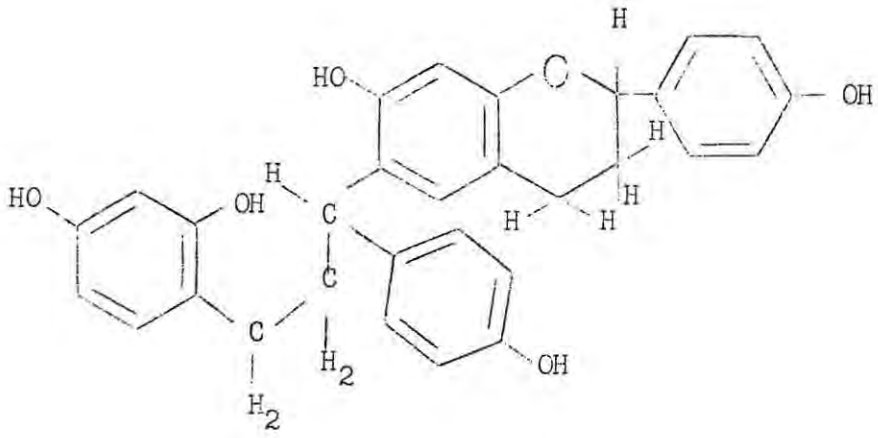
PART II.

THE DIMERIC PROANTHOCYANIDINS.

The name "proanthocyanidin" was proposed by Freudenberg and Weinges[90] to designate a group of colourless compounds which are capable of forming coloured anthocyanidin pigments. Proanthocyanidins would therefore include the flavan-2,3-diols, -3,4-diols, -2,3,4-triols and their possible glycosides. Dimeric proanthocyanidins may be divided into (a) synthetic products formed by condensation of catechins or flavan-3,4-diols, for example, (b) natural products where the two moieties are linked by an ether bond, and (c) natural products having a carbon to carbon linkage between the two nuclei.

Freudenberg *et al.*[91,92] investigated the self-condensation of catechins in aqueous solution, under the influence of heat, with mineral acids. Hydroxyl groups at C-3, C-5 and C-3' were found to be unnecessary for such self-condensations, while all catechins which were prone to condensation possessed hydroxyl groups at C-7 and C-4'. These hydroxyls are in positions *para* to the attachment of the A-and B-rings to the carbon atoms of the heterocyclic ring.

During the polycondensation of hydroxylated flavans the number of hydroxyl groups increase. Freudenberg and Maitland[93] suggested that this was attributable to an opening of the heterocyclic ring. Subsequent condensation reactions with 7,4'-dihydroxyflavan strengthened this notion and on this basis they assigned structure LVIII to the primary condensation product.

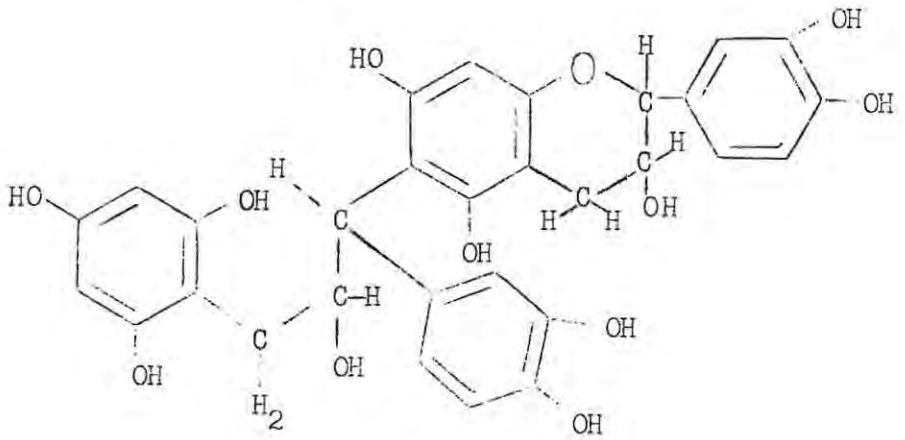


(LVIII)

Catechins with substituents at C-6 and C-8 failed to condense - indicating that these positions were involved in the reaction. The other participating position was considered to be C-2, and the 7,4'-dihydroxy compounds were therefore regarded as bifunctional molecules with reactive centres at C-2 and C-6 or C-8.

The condensation of catechin resulted in the isolation of "dicatechin" which they considered a true tannin because it precipitated gelatin. The condensation product yielded a crystalline acetate.

According to Freudenberg[36], during the acid-catalysed polymerization of catechin, the molecule reacted bifunctionally - electrophilic at C-2 and nucleophilic at either C-6 or C-8. Dicatechin LIX still possessed both these functions and could therefore undergo further condensation to yield branched polymers[94]. The crystalline acetate of dicatechin was the condensation product of two molecules of catechin without the loss of water.

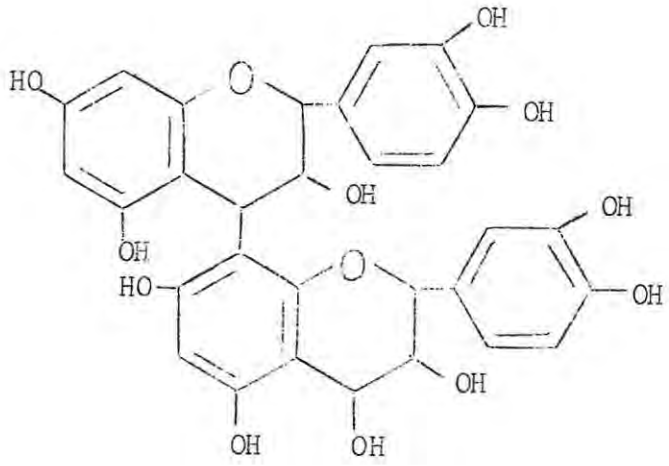


(LIX)

The polyhydroxyflavan-3,4-diols underwent self-condensation more rapidly than catechins, this greater reactivity being due to the direct participation of the 4-hydroxyl group in the condensation. It was thought that this hydroxyl group reacted between the 4-position of one molecule and the 6- or 8-position of the second with the loss of one molecule of water thus giving rise to dimers such as LX.

Mayer and Merger[94] investigated the reactivity of certain sites for the acid condensation of catechin nuclei. Polyphenols such as phloroglucinol were reacted with catechin to give condensation products which served as models for the condensation of catechins and catechin tannins. They considered that during self-condensation (+)-catechin was converted by epimerization to (+)-epicatechin in the initial stages and finally formed two epimeric dimers, one of which was isolated in a crystalline form, thus being the first crystalline dicatechin isolated[95]. No loss of water occurred and from the composition, $C_{30}H_{26}O_{11}$, of the

dicatechin they proposed two alternative structures LXI and LXII for these dimeric catechins.

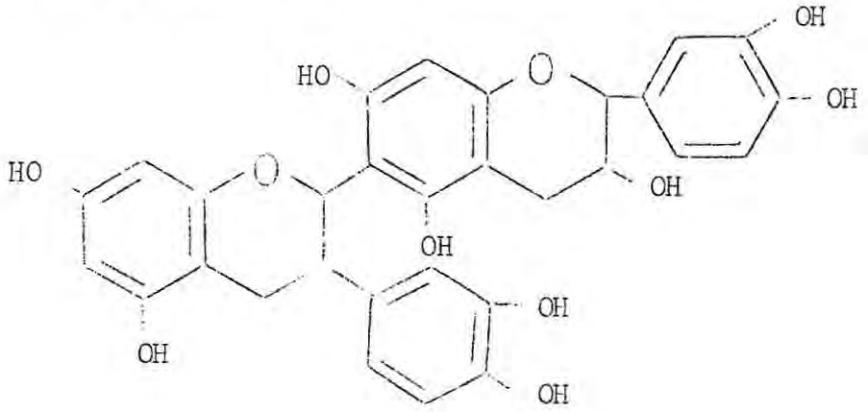


(LX)

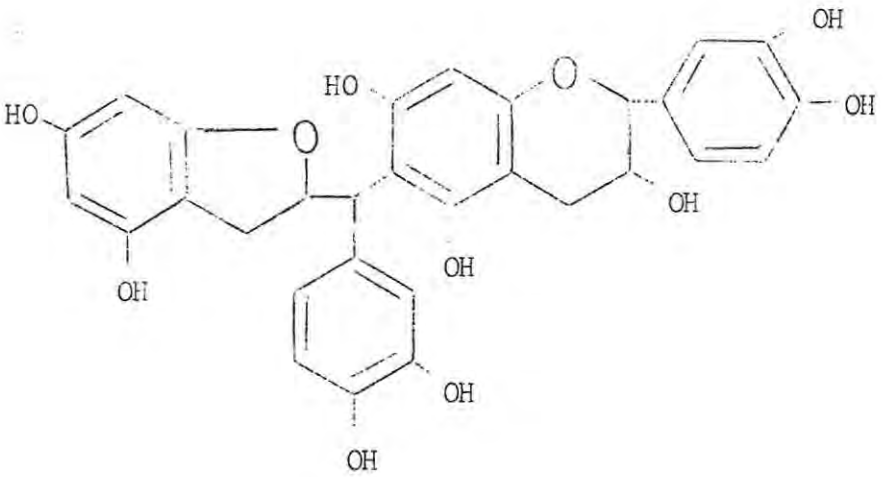
(+)-Catechin was considered to be epimerized to (+)-epi-catechin, which takes part in the reaction. (+)-Catechin reacts as the electrophilic component and reversal of substituents at C-2 and C-3 yields the iso-derivative. Ring contraction during condensation may be responsible for the formation of the benz-pyran structure LXII.

In 1953 Forsyth[96] isolated a "leuco-cyanidin" from cacao beans in chromatographically pure form. Acid hydrolysis of this compound gave cyanidin and a red-brown precipitate as major products. Leuco-cyanidin (5,7,3',4'-tetrahydroxyflavan-3,4-diol) under similar conditions yielded a catechin and a brown precipitate. (-)-Epicatechin under acid hydrolysis produced 64% unchanged starting material, 16% (-)-catechin and a polymeric "phlobaphene". These results led Forsyth to believe that a very close relationship existed between (-)-epicatechin and an integral

part of the "leucocyanidin" molecule. He further suggested that the production of cyanidin from the leuco-compound by strong mineral acid was due to a secondary reaction between two parts of the molecule.



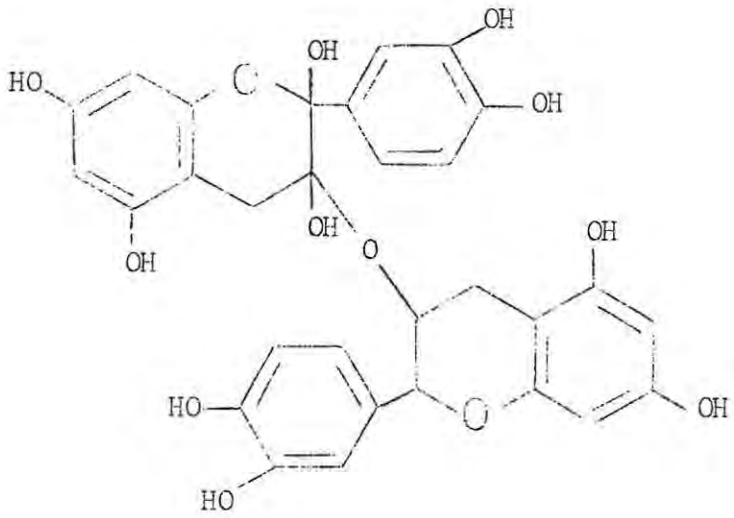
(LXI)



(LXII)

Forsyth and Roberts[97] formed crystalline deca-acetate and octamethyl ether (M = 710 - 725) derivatives of the cacao "leuco-cyanidin". Oxidation with periodate and lead tetra-acetate of the

leuco-compound suggested the presence of a ditertiary α-glycol, indicating that a flavan-3,4-diol structure was unlikely. They tentatively proposed structure LXIII for the "leucocyanidin" and suggested that it was composed of (-)-epicatechin and 5,7,3',4'-tetrahydroxyflavan-2,3-diol joined by an acetal-type of linkage between the alcoholic oxygen atom of (-)-epicatechin and the C-3 atom of the flavan-2,3-diol.

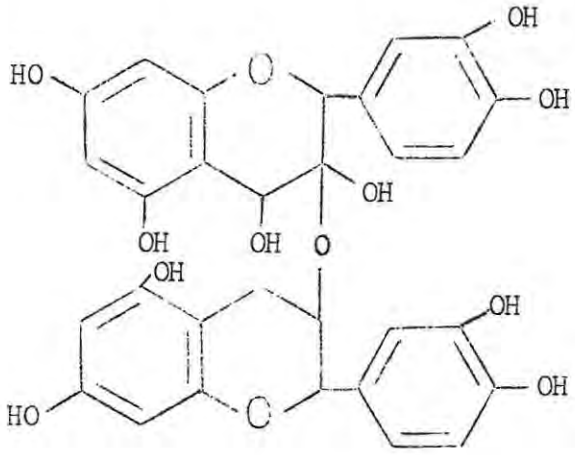


(LXIII)

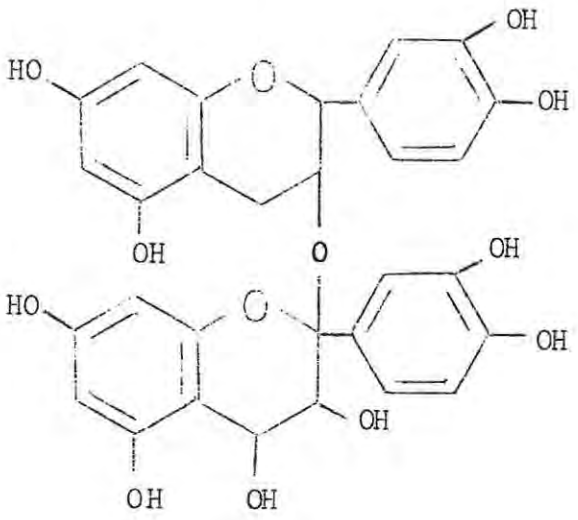
In 1960 Forsyth and Roberts[98] revised their structure for the cacao "leucocyanidin" in the light of work by Gramshaw, Johnson and King[99] on 5,7,3',4'-tetramethoxyflavan-2,3-diol, and proposed two alternative structures LXIV and LXV.

Freudenberg and Weinges[100] isolated two compound proanthocyanidins from hawthorn berries (Crataegus oxyacantha). One of these gave (-)-epicatechin and cyanidin on acid hydrolysis. It contained eight phenolic and two aliphatic hydroxyl groups in addition to three ether oxygen atoms. The octamethylether on hydrolysis yielded tetramethyl-

epicatechin and tetramethyl-cyanidin. On this basis they proposed LXVI for this compound.



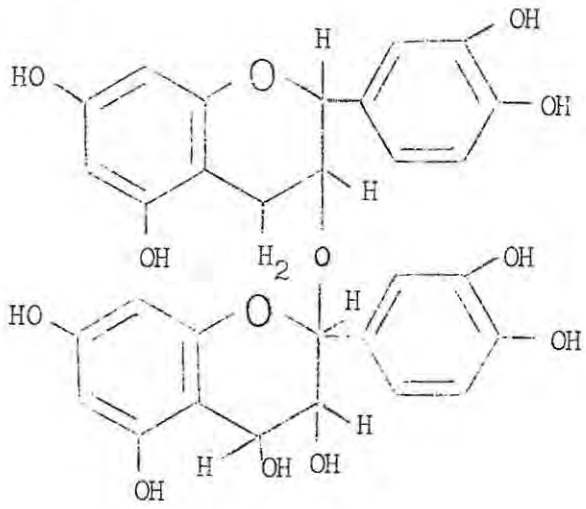
(LXIV)



(LXV)

The other compound proanthocyanidin was regarded as being constituted of (-)-epicatechin and leuco-delphinidin. A related proanthocyanidin was isolated from the fruit of Gleditshia triacanthos[101].

(+)-Catechin and cyanidin resulted from its acid hydrolysis.



(LXVI)

In 1964 Lewak[102] reported the isolation of a new dimeric "leucocyanidin" from the leaves of hawthorn. The reactions and properties of this dimer differed from those of the compound LXVI isolated by Weinges[103] from hawthorn berries.

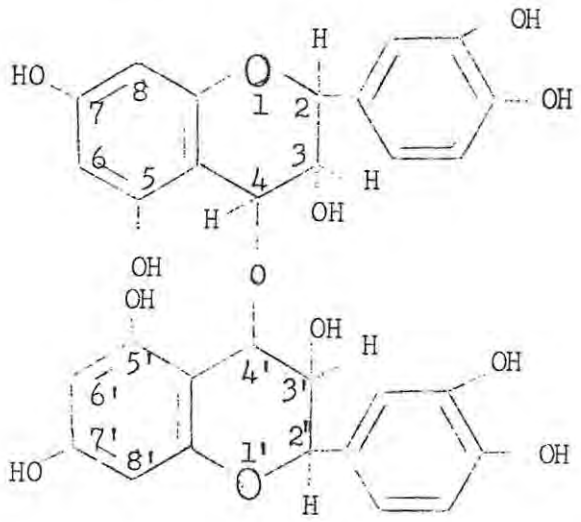
The dimer was found to be very unstable in the free-phenolic form and evaporation of solutions of chromatographically pure compound resulted in the formation of a substantial amount of polymeric or oxidized material. Changes in the ultra-violet spectrum were also detected. For these reasons all structural studies were carried out with derivatives of the dimer.

Results of molecular weight determinations and elemental analyses of derivatives of the "leucocyanidin" showed that the molecule comprised two flavan (C_{15}) units. Methylation showed the presence of

eight phenolic hydroxyl groups. Acetylation yielded a deca-acetate indicating two alcoholic hydroxyl groups, while the remaining three oxygen atoms were located in ether systems.. Lewak assumed that one of these oxygen atoms formed an ether link between the two flavan moieties.

Reaction of the methylated dimer with p-toluenesulphonyl chloride resulted in the formation of an octa-O-methyl-di-O-tosyl derivative which confirmed the dimeric structure of the compound.

These results allowed Lewak to propose a dimeric structure LXVII consisting of two 5,7,3',4'-tetrahydroxyflavan-3,4-diol units linked by an ether bond between the secondary alcoholic hydroxyl groups of the heterocyclic ring systems.



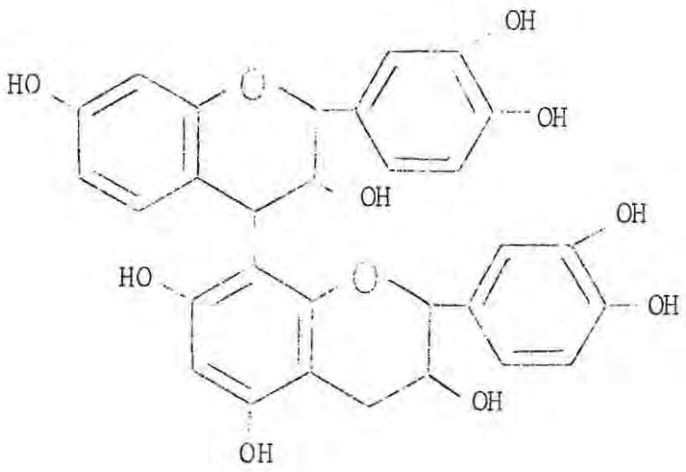
(LXVII)

Alternative structures for the dimer were also proposed with the link between C-3 and C-4' and C-3 and C-3'. This was claimed to be the first isolation of a dimeric leucoanthocyanidin consisting entirely of two

flavan-3,4-diol moieties.

Geissman and Dittmar[104] isolated an amorphous proanthocyanidin from the seeds of the avocado (Persea gratissima Gaertn.). Controlled hydrolysis in acid media yielded catechin, epicatechin and polymeric material. The compound gave amorphous deca-acetate, octamethyl ether and octamethyl ether diacetate derivatives. Analysis of these derivatives indicated a parent compound resulting from the bonding of a tetrahydroxyflavan-3,4-diol with a tetrahydroxyflavan-3-ol, with the accompanying loss of one molecule of water.

The nuclear-magnetic-resonance spectra of the deca-acetate and the octamethyl ether showed appreciable line-broadening. From a consideration of available data they proposed structure LXVIII for the avocado proanthocyanidin.



(LXVIII)

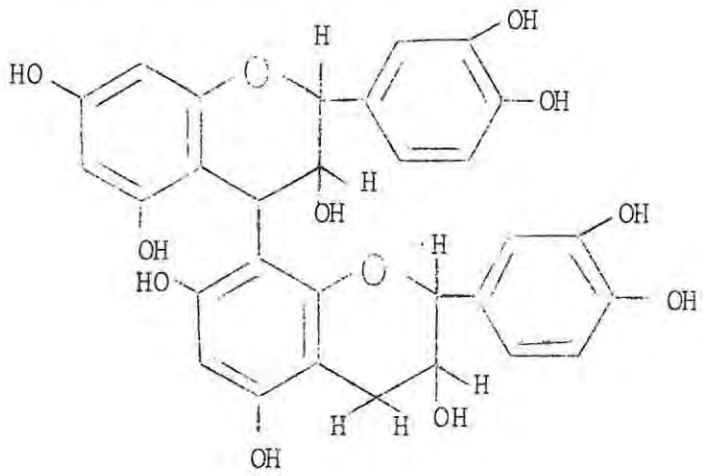
Recently Weinges and Freudenberg[105] obtained crystalline derivatives of two condensed proanthocyanidins and thus laid the foundation for a more accurate study of this class of compounds.

From cranberries (Vaccinium vitis-idaea) a condensed proanthocyanidin was isolated which gave a crystalline heptamethyl ether diacetate, m.p. 189-190°, of molecular weight 758, by mass spectroscopy. The free-phenolic form contained seven phenolic and two aliphatic hydroxyl groups, three ether oxygen atoms, and an olefinic double bond. The corresponding nona-acetate was also crystalline. Assuming that two of the oxygen atoms were allocated to the heterocyclic ring systems, the remaining one was assigned to an ether link between the two flavanoid units. One half of the molecule afforded a product which resembled cyanidin (chromatography). The n.m.r. spectrum of the heptamethyl ether diacetate indicated the presence of a methylene group suggesting that the other half of the molecule consisted of a catechin.

The second condensed proanthocyanidin was isolated from cola nuts (Cola acuminata). On treatment with acid the compound decomposed into catechin and cyanidin. Analyses of derivatives indicated that it contained eight phenolic and two aliphatic hydroxyl groups and two ether oxygen atoms, thus suggesting structure LXIX for the parent compound. The bond between the two flavanoid nuclei need not necessarily be between C-4 and C-8', but could well be between C-2 and C-8', C-4 and C-6' or C-2 and C-6'.

Creasy and Swain[106] studied the physiology of tannin formation in the leaves of wild strawberries (Fragaria vesca) and showed that

besides (+)-catechin and catechin tannins a condensed proanthocyanidin was formed. This compound yielded (+)-catechin and cyanidin on acid treatment and was thus similar to the avocado "dimer"[104] and the

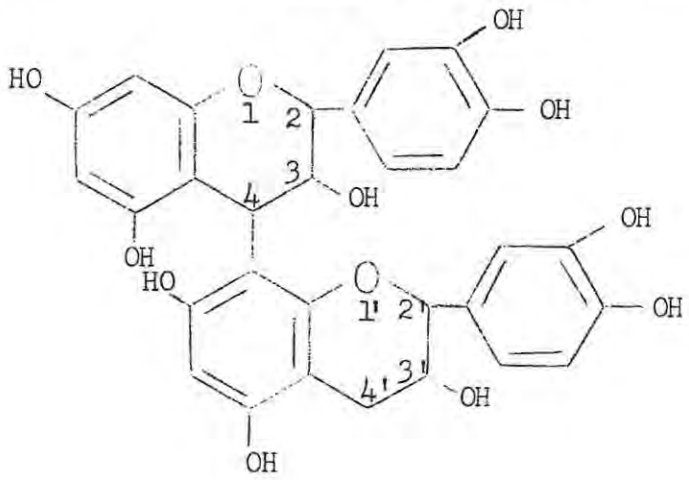


(LXIX)

proanthocyanidin from Gleditschia triacanthos[101]. Although certain physical constants of the avocado "dimer" and the proanthocyanidin from strawberry leaves were very similar, they showed significantly different R_F values in most solvent systems. Hence it was assumed that the two compounds were stereoisomers. This assumption was supported by the observation that on epimerization of the strawberry proanthocyanidin in water at elevated temperature, six components were formed. These were separable on thin-layer cellulose plates and three of them showed R_F values identical with the epimerization products of the avocado "dimer".

Condensation reactions in acid media of (+)-catechin and flavan-3,4-diol racemates and subsequent thin-layer chromatography of

the condensates led Creasy and Swain to believe that the strawberry proanthocyanidin was the 2,3-trans-3,4-trans-2',3'-trans isomer LXX.



(LXX)

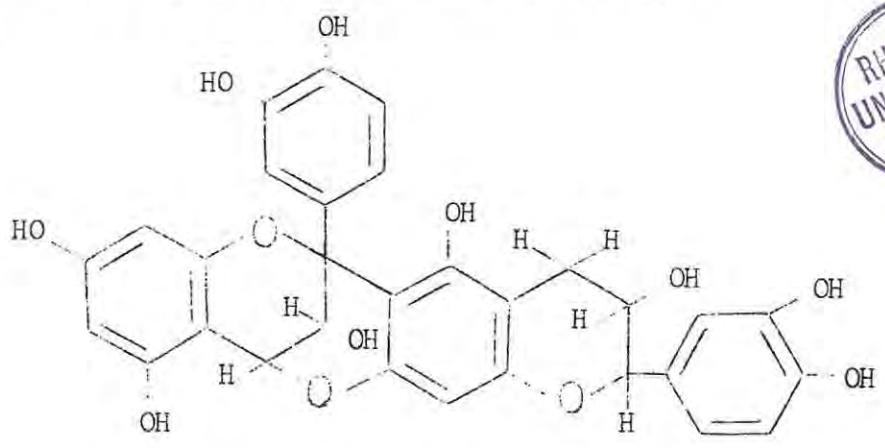
Their assignment of this relative configuration to the strawberry biflavanol appears to be based on insufficient evidence. The resolution of stereoisomers on thin-layer chromatoplates was regarded as sufficiently diagnostic for proving the identities of the epimerization products. Experience has shown that thin-layer chromatography on silica gel could possibly be a misleading criterion, and further proof must be provided to ascertain the identity of components. However, a significant contribution by these authors was the series of condensation reactions of catechins with flavan-3,4-diol isomers to yield biflavanols.

Mayer et al.[107] isolated a novel double-linked condensed proanthocyanidin from unripe seed pods and bark of horse chestnut (Aesculus hippocastanum). In concentrated hydrochloric acid the component

yielded cyanidin, while with dilute acid (-)-epicatechin was detected by chromatographic methods.

The product crystallized from water and had the composition $C_{30}H_{24}O_{12}.\ 2H_2O$. The absence of carbonyl groups was shown by infrared spectrometry. Acetylation gave a nona-acetyl derivative, while methylation with diazomethane yielded both heptamethyl and octamethyl ethers. Further methylation with methyl iodide and silver oxide gave a nona-methyl ether. Mass spectrometry was used to determine the molecular weights of these derivatives.

The above reflected the presence of seven phenolic and two aliphatic hydroxyl groups. The remaining three oxygens were therefore involved in ether linkages, carbonyl groups being excluded. From the above and from considerations of the n.m.r. spectra of derivatives, they proposed structure LXXI for the condensed biflavanol. In the double link between the two nuclei one bond was regarded as a C-C link, while the other was thought to involve an ether linkage.



(LXXI)

Synthetic proanthocyanidins have recently been prepared by Geissman and Yoshimura[108]. Under acidic conditions they condensed phloroglucinol, and (+)-catechin, with 5,7,3',4'-tetramethoxyflavan-3,4-diol to give proanthocyanidins. The n.m.r. spectrum of the condensation product of (+)-catechin and the diol showed appreciable line-broadening and rendered the allocation of benzenoid and heterocyclic protons impossible. Hence the proposed structure for the product was purely hypothetical due to lack of significant structural evidence.

It is evident that, apart from compound LXXI, the chemistry of the condensed proanthocyanidins still remains to be elucidated. Bonds between flavan moieties appear to be variable and open to speculation. The stereochemistry of this class of compounds has thus far received scant attention.

PART III.

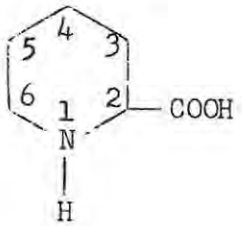
THE NON-TANNINS.

1. The Imino Acids.

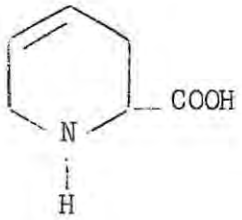
(a) Pipecolic acid and its Derivatives.

Piperidine-2-carboxylic acid, also known as pipecolinic or pipecolic acid LXXII, was originally synthesized by Ladenburg[109] by reduction of picolinic acid with sodium in ethanol. Racemic pipecolic acid was resolved by Mende[110] using optically active tartaric acids, while a partially racemized L-pipecolic acid was prepared by oxidizing conhydrine.

From the water-soluble fraction of Rhodesian teak (Baikiaea plurijuga : family Leguminosae) King et al.[111] isolated baikianin LXXIII. This proved to be a tetrahydropicolinic acid containing one double bond and showing strong negative rotation in aqueous solutions. Baikianin was also synthesized from L-glutamic acid, and its dihydro-derivative was prepared by catalytic reduction. The latter was identical to (-)-pipecolic acid.



(LXXII)

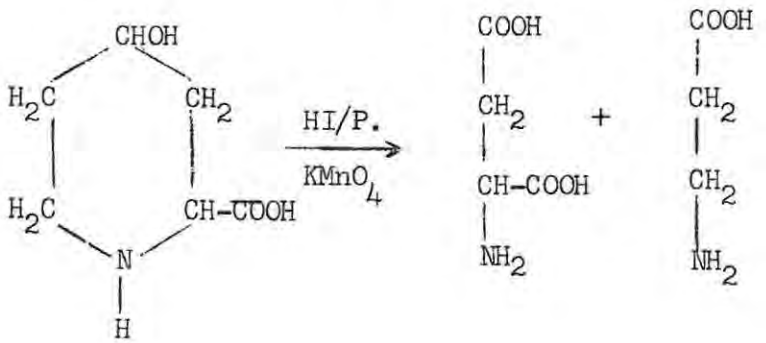


(LXXIII)

Steward and Thompson[112,113] first reported the natural occurrence of (-)-pipecolic acid in green beans (Phaseolus vulgaris). This was isolated as the hydrochloride by means of ion-exchange chromatography. They subsequently showed the presence, by paper chromatography, of (-)-pipecolic acid in fresh fruits and dry seeds of several leguminous plants.

Lysine was shown by Grobbelaar and Steward[114] to be the precursor of pipecolic acid in the developing green bean. Radioactive tracer techniques were used in which labelled lysine was injected into the ovules of young green beans.

4-Hydroxy- and 5-hydroxy-pipecolic acids were originally isolated by Virtanen and Kari[115,116] from Rhapis, Albizzia and Acacia species. Positions of the hydroxyl groups were determined by permanganate oxidation of these compounds. Virtanen and Gmelin[117] reduced 4-hydroxy-pipecolic acid LXXIV with hydrogen iodide and red phosphorus to pipecolic acid, which on subsequent oxidation with potassium permanganate yielded aspartic acid LXXV and β-alanine LXXVI.

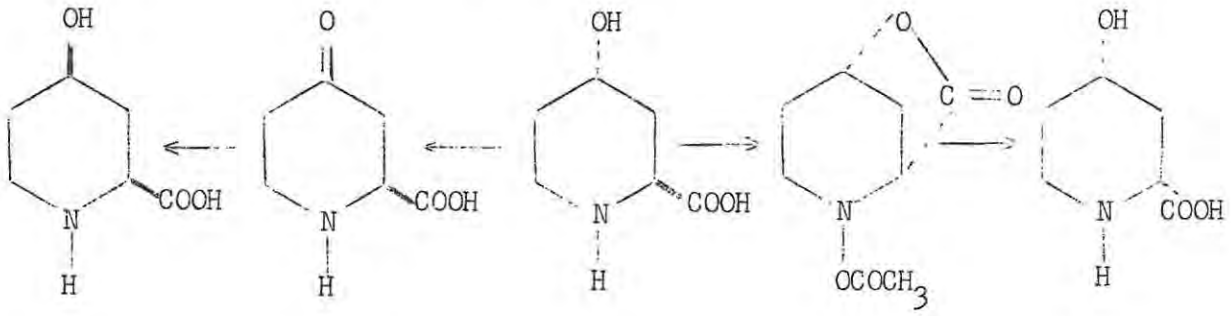


(LXXIV) (LXXV) (LXXVI)

Clark-Lewis and Mortimer[118] isolated trans-4-hydroxy-pipecolic acid from the heartwood of Acacia excelsa, and subsequently showed its presence in several other Acacia species. Acacia oswaldii leaves proved to be a particularly rich source. The acid had a trans configuration as shown by chromatographic comparison with cis-4-hydroxypipecolic acid prepared by reduction of 4-hydroxy-picolinic acid. The imino acid fraction, isolated as N-nitroso derivatives, consisted mainly of proline, (-)-pipecolic acid and trans-4-hydroxy-pipecolic acid. The latter was epimerized with barium hydroxide to a mixture of trans- and cis-4-hydroxy-pipecolic acids. The position of the hydroxyl group was determined by decarboxylation in acetophenone to give dimorphic 4-hydroxy-piperidine LXXVII[119].

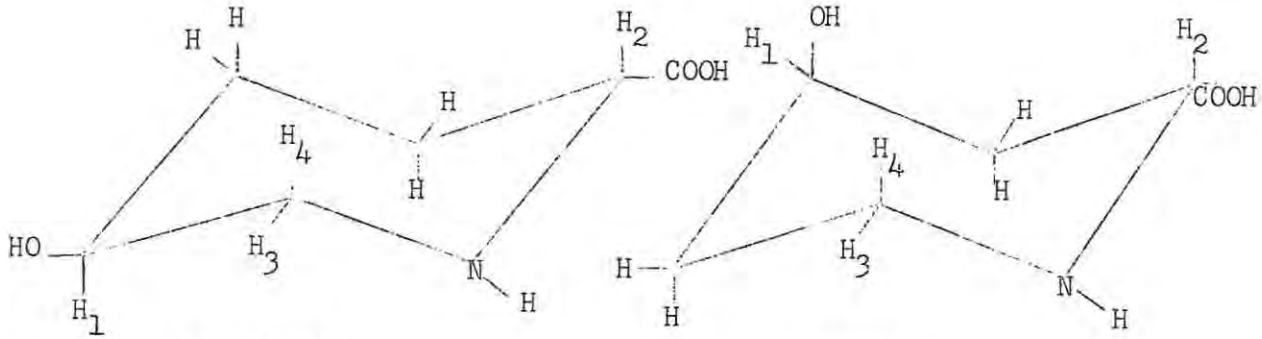
(LXXVII)

The trans-L configuration of 4-hydroxy-pipecolic acid was proved by the transformation of (-)-4-hydroxy-pipecolic acid LXXVIII, via the keto-L-acid LXXIX into (-)-cis-4-hydroxy-L-pipecolic acid LXXX. Compound LXXVIII was also converted to (+)-cis-4-hydroxy-D-pipecolic acid LXXXII by way of the N-acetyl-lactone LXXXI. In this way three of the four possible optically active forms were synthesized.



(LXXX) (LXXIX) (LXXVIII) (LXXXI) (LXXXII)

The conformation of 5-hydroxy-pipecolic acid LXXXIII was established by Shoolery and Virtanen[120] using high-resolution nuclear-magnetic-resonance spectroscopy. Protons H_1 and H_2 were shown to be <u>axial</u> as evidenced by their large coupling constants. H_1 occurred as a septet arising from its coupling with two vicinal <u>axial</u> and two neighbouring <u>equatorial</u> protons.



(LXXXIII) (LXXXIV)

The spectrum of 4-hydroxy-pipecolic acid LXXXIV showed a quintet due to H_1 which has four vicinal protons. Since all four are equally coupled to H_1, it could only be <u>equatorial</u>. H_2 has only 2 neighbouring protons and occurred as a quartet having large coupling which showed that H_2 was <u>axial</u>. Their conclusions regarding the structure of 4-hydroxy-pipecolic acid is therefore in agreement with those of

Clark-Lewis and Mortimer[119].

The natural occurrence of pipecolic acid and its hydroxylated derivatives is widespread, but little is known regarding their functions in plants.

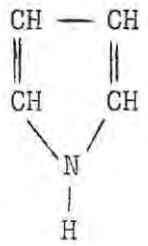
(b) Proline and its derivatives.

Proline is an imino acid constituent of proteins, while hydroxy-proline is unique to collagen. Hydroxyproline has been shown to be derived biosynthetically from proline.

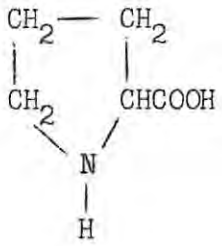
Fischer and Zemplén[121] elegantly resolved synthetic DL-proline into its two optically active forms. Kapfhammer and Eck[122] first isolated L-hydroxy-proline and L-proline from hydrolysed gelatin solution by precipitation with Reinecke's salts.

The synthesis of DL-proline LXXXVI from pyrrole LXXXV was carried out by Signaigo and Adkins[123]. Pyrrole was treated with ethyl-magnesium bromide and ethyl chlorocarbonate at 0° to form 1,2-dicarb-ethoxypyrrole which was first hydrogenated and then hydrolysed to give a 57% yield of crystalline DL-proline. Synthetic proline was also prepared by Gaudry and Berlinguet[124] who cyclized the dihalogenated valeric acids.

Fowden[125] detected both proline and 4-hydroxyproline in aqueous extracts of Armeria leaves. They occurred in association with 4-hydroxy-pipecolic acid and the amino acids aspartic acid, glutamic acid, serine, threonine and homoserine.



(LXXXV)



(LXXXVI)

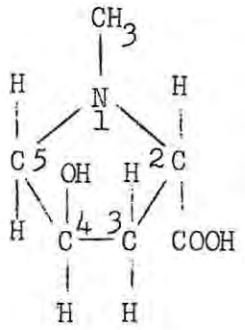
Proline was first detected in the heartwoods of Acacia species by Clark-Lewis and Mortimer[118] who also showed its concurrence in Acacia oswaldii leaves with (-)-pipecolic acid and 4-hydroxy-pipecolic acid.

Myhill and Jackson[126] separated proline and hydroxy-proline, from hydrolysed protein, on cellulose substrates using thin-layer techniques. Nitrous acid was used to destroy amino acids in the protein hydrolysates.

From Afrormosia elata heartwood Morgan[127] isolated 4-hydroxy-N-methyl-L-proline LXXXVII. Although its infrared spectrum was characteristic of an amino acid, it showed no colour with ninhydrin reagent. On heating, the product gave a strong pyrrole reaction. Analyses showed the presence of a methyl group and its physical constants showed close identity with those of 4-hydroxy-N-methyl-proline isolated from the bark of Croton gubourgia by Goodson and Clewer[128].

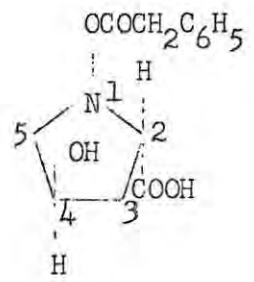
Recently Saayman and Roux[129] isolated amorphous proline from the ethanolic extract of fresh black wattle (Acacia mearnsii) bark. It occurs in association with the imino acids (-)-pipecolic acid and 4-

hydroxy-pipecolic acid, and yielded a crystalline picrate. Although proline was previously detected in Acacia oswaldii leaves[119], this is the first isolation of the compound from Acacia species.

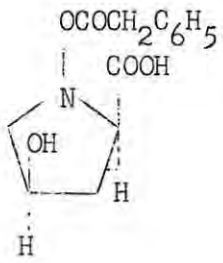


(LXXXVII)

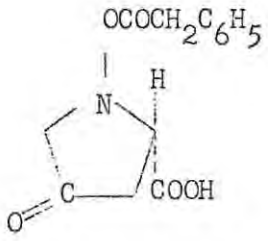
Patchett and Witkop[130] examined L-hydroxy-proline obtained from collagen, which is chemically distinguishable from all other proteins by its high percentage of hydroxy-proline. They prepared the N-carbobenzyloxy derivatives LXXXVIII and LXXXIX of natural L-hydroxy-proline and of D- and L-allohydroxy-proline in crystalline form. These were oxidized to N-carbobenzyloxy-4-keto-L- and D-proline XC and XCI. Stereospecific reduction of 4-keto-L-proline with sodium boronhydride yielded a high percentage of the allohydroxy-L-proline derivative. From these and other results they deduced configuration XCII for natural L-hydroxy-proline.



(LXXXVIII)



(LXXXIX)



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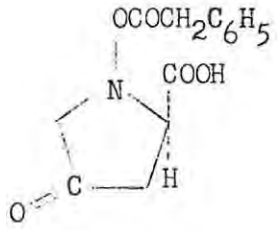

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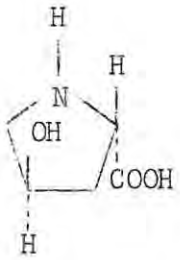


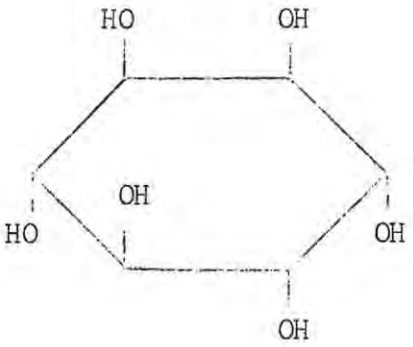
(XCII)

2. (+)-Pinitol.

Pinitol, a monomethylcyclohexanhexol, was first discovered by Berthelot[131] in the exudate of the sugar pine (Pinus lambertiana Dougl.). It was isolated subsequently as sennite from senna leaves[132], as matezite from the juice of Mateza roritina[133] and from the mother liquors resulting from the crystallization of coniferin[134]. Wiley[135] recognized these compounds as being identical to pinitol isolated

from the sugar pine, while Griffin and Nelson[136] prepared its penta-acetyl derivative.

Sherrard and Kurth[137] isolated pinitol from the heartwood of the redwood (Sequoia sempervirens) tree as white, rhombic-hemihedral crystals (m.p. 185°). It showed a large positive rotation and its physical properties were closely related to those of (+)-inositol XCIII. Analysis of the fully acetylated compound showed the presence of one methoxyl and five alcoholic hydroxyl groups. Thus (+)-pinitol was shown to be a monomethyl ether of (+)-inositol.

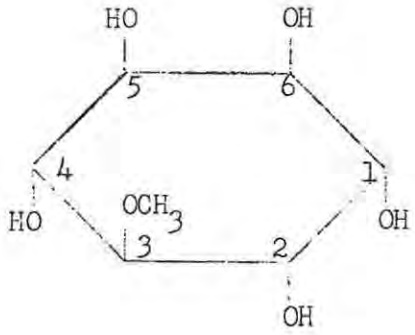


(XCIII)

Since 1950 numerous sources of (+)-pinitol have been cited in the literature. The compound is the most widely distributed methyl ether of the inositols and its presence has been shown in six families of Gymnospermae and thirteen families of Angiospermae[138]. Plouvier[139], in recent reviews, indicated the widespread occurrence of (+)-pinitol amongst the Leguminosae.

(+)-Pinitol XCIV was first reported in Acacia species (A. longissima Wendl., A. stolonifera Burch. and A. lasiopetala Oliv.)

by Rimington[140] in 1935. Stephen[141] isolated it from the heartwood of the black wattle (A. mearnsii) and showed its identity with a sample isolated from Lotononis laxa by de Waal[142]. Its characteristic penta-acetate was prepared and it was also demethylated with hydrogen iodide to (+)-inositol[143]. Sodium meta-periodate oxidation indicated that C-1 and C-4 were the least likely sites for the methoxyl group. Stephen[141] concluded that a study of the oxidation rate of (+)-pinitol was not sufficiently diagnostic for the determination of its structure. Keppler[66] confirmed the presence of (+)-pinitol in black wattle heartwood.

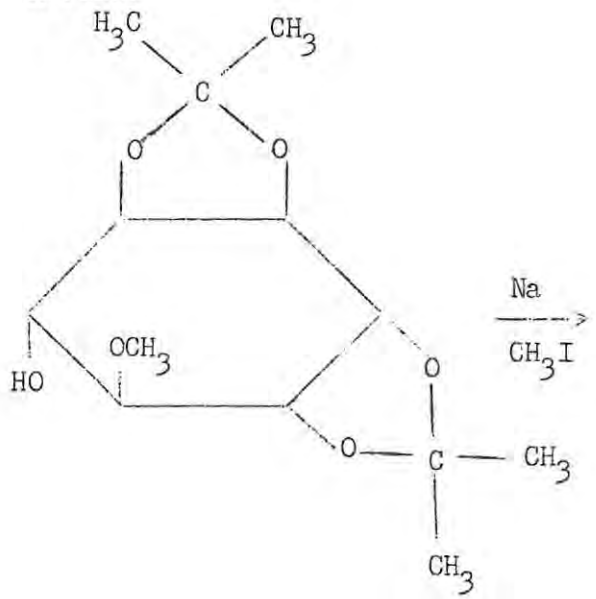


(XCIV)

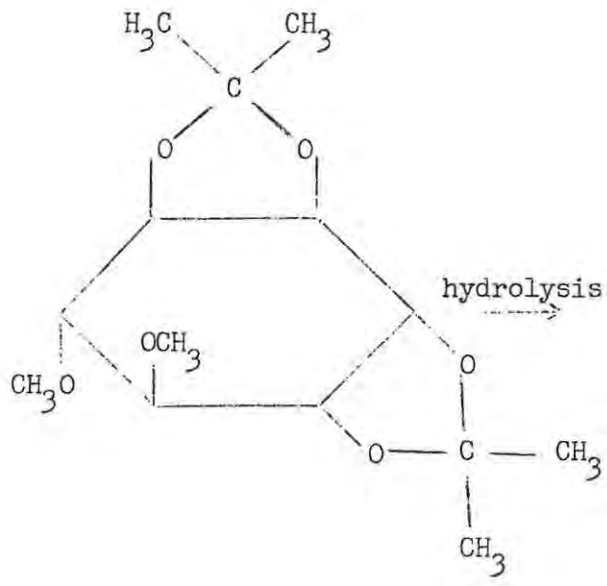
(+)-Pinitol was first isolated by Saayman and Roux[129] from fresh black wattle bark. It occurs as a mixture with sucrose in the acetone extract of the bark, and was isolated from the mother liquor of sucrose. Its presence in the bark extract was not obvious due to poor response to spray reagents. The product was shown to be identical with (+)-pinitol isolated by Appel and Lobos[144] from the naranjillo (Adesmia species : family Leguminosae) of northern Chile.

Posternak[145,146] originally established the configuration of (+)-inositol by physical techniques. Consideration of the two strain-free conformations of the cyclohexane ring - the "boat" and "chair" forms, allowed him to conclude that inositol assumed mainly the latter conformation.

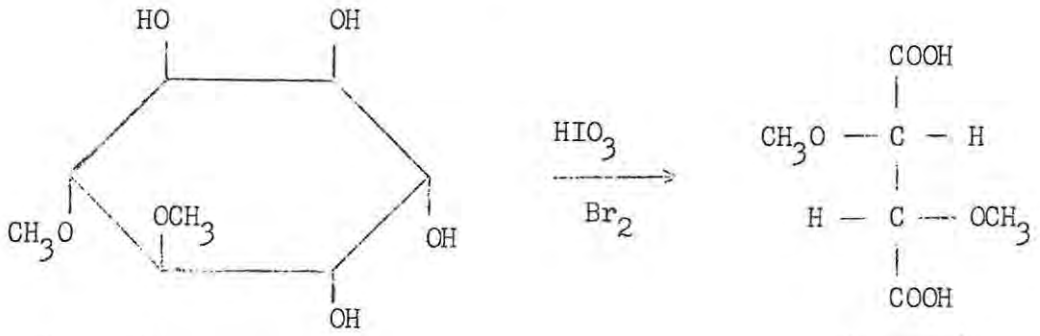
From the known conformation of (+)-inositol Anderson, MacDonald and Fischer[147] and Angyal et al.[148] proceeded to determine the structure of (+)-pinitol. This entailed location of the methoxyl group in the cyclitol. The reaction of (+)-pinitol XCIV with acetone and hydrogen chloride under mild conditions produced a diisopropylidene derivative XCV in high yield. Methylation with sodium and methyl iodide gave the dimethyl diisopropylidene-(+)-inositol XCVI which was hydrolysed to yield a dimethyl-(+)-inositol XCVII. Ring cleavage with periodic acid, followed by oxidation with bromine produced dimethyl-D-(-)-tartaric acid XCVIII.



(XCV)

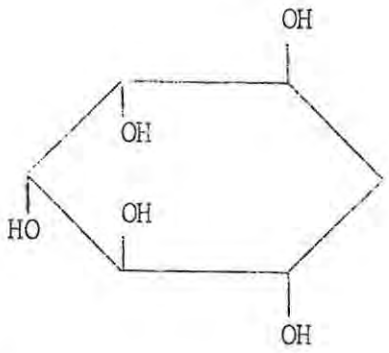


(XCVI)



(XCVII) (XCVIII)

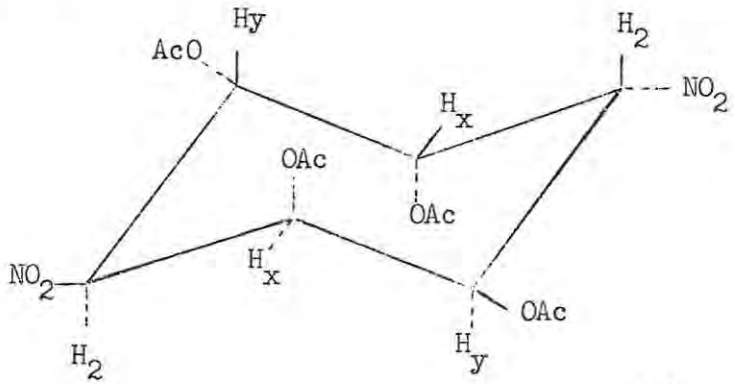
Shoolery et al.[149,150] used nuclear-magnetic-resonance spectroscopy for studying the structures of diastereoisomers of quercitol XCIX in deuterium oxide. They synthesized the ten possible diastereoisomers, six of which were optically active, giving a full series for configurational study. This enabled assignment of stereochemical configurations to the various isomers by n.m.r. spectroscopy.



(XCIX)

1,4-Dinitro-inositol tetra-acetate, which has a possible fourteen isomers, was subjected to n.m.r. spectroscopy by Shoolery[151]. Analysis of the spectrum clearly showed that the molecule has a centre of symmetry because only two acetate and three multiplet resonance

signals, originating from the six ring protons, were observed. From the large spin couplings of two of the multiplets it was deduced that there were four axial protons, each having one axial and one equatorial vicinal proton. The only possible structure for 1,4-dinitro-inositol tetra-acetate was therefore C.



(C)

3. The Long-chain β-Diketones.

The first isolation of natural long-chain β-diketones was by Hamm and Lamberton[152], who found that the β-diketones constituted 50% or more of the leaf and stem waxes of a variety of plants such as Eucalyptus (Myrtaceae), Acacia (Leguminosae), the grass (Festuca glauca) and the carnation (Dianthus caryophyllus).

The β-diketones which conform to the general formula CI were estimated by spectroscopic methods and isolated from petroleum-soluble waxes as insoluble copper complexes[153].

$$n\text{-}C_nH_{2n+1}\text{-}CO\text{-}CH_2\text{-}CO\text{-}C_{n'}H_{2n'+1} \qquad \text{(CI)}$$

The most commonly occurring β-diketone was found to be n-tritriacontan-16,18-dione CII. It gave a copper complex of formula

$C_{66}H_{126}O_4Cu$, and alkaline hydrolysis yielded n-hexadecanoic acid and n-heptadecan-2-one in approximately equal proportions.

$$n\text{-}C_{15}H_{31}\text{-}CO\text{-}CH_2\text{-}CO\text{-}C_{15}H_{31}$$

(CII)

Compositions of β-diketone mixtures were determined by gas chromatography after hydrolysis and esterification of the resulting acids. The chain lengths of β-diketones were determined by reduction with lithium aluminium hydride to give mixtures of unsaturated alcohols. These were further reduced and converted to their iodides which were again hydrogenated to yield fully saturated hydrocarbons suitable for analysis by gas chromatography.

All the Eucalyptus species investigated showed the presence of β-diketones. The waxes of Acacia species, A. prominens, A. brachybotrya and A. cultriformis contained β-diketones, while in A. aneura, A. suaveoleus and A. iteaphylla these were absent.

From the warm, light-petroleum extract of cabbage leaves Horn and co-workers[153] isolated a highly crystalline hydrocarbon fraction which was identified by means of gas chromatography as n-nonacosane. Further elution of the wax fraction on alumina yielded a fraction consisting of a mixture of ketones and esters. From the unsaponifiable fraction a crystalline ketone, m.p. 80-80.5°, was obtained. The breakdown products of this ketone consisted mainly of n-pentadecanoic acid with traces of n-C_{12}, n-C_{14} and n-C_{18} acids.

A chemical examination of Acacia caesia bark by Nigam, Mitra and Kaul[154] showed the presence of stigmasterol, a triterpene alcohol and a waxy product with m.p. 76-78°. No attempt was made to identify the latter compound, but from its properties it is quite likely to be a member of the β-diketone series.

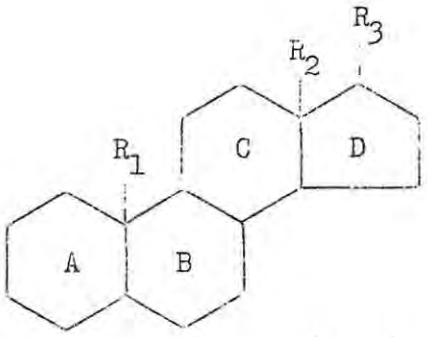
The light-petroleum extract of fresh black wattle (Acacia mearnsii) bark yielded among other components a white crystalline fraction[129] with a waxy appearance and m.p. 80-2°. This compound was initially isolated from the unsaponifiable fraction of the bark wax, but later also obtained as the copper complex.

The plant waxes consist almost entirely of saturated hydrocarbons, long-chain fatty acids and their esters, and long-chain β-diketones. The latter group of compounds constitute a major portion of the waxes of several Acacia species.

Keto-enol tautomerism is very prevalent in these β-diketones and there is resonance stabilization of the enolic form (see Discussion).

4. The Steroid Alcohols.

Steriods are colourless natural products which occur widely and possess the tetracyclic carbon skeleton CIII. The main variations between steriods, apart from the degree of unsaturation of the ring system and the presence of nuclear substituents, are due to differences in the structures of the side-chains R_1, R_2 and R_3. Generally R_1 and R_2 are methyl groups while the side-chain R_3 may be absent, or may consist of

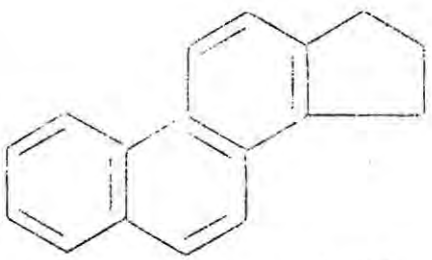


(CIII)

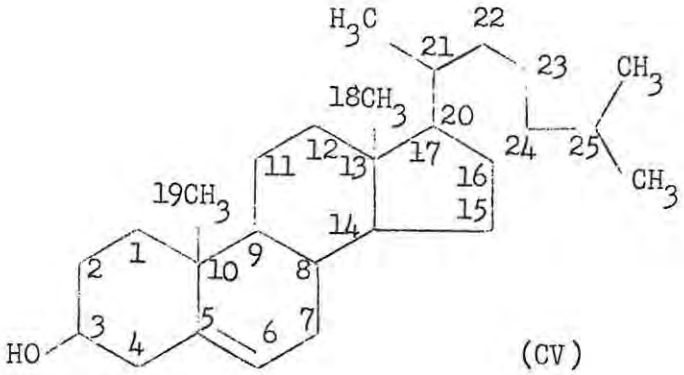
from two to ten carbon atoms.

Early structural investigations of the steriods were mainly concerned with the nature of the nucleus and were carried out with cholesterol and the bile acids. Cholesterol, $C_{27}H_{46}O$, was shown to be a monohydric secondary alcohol with one double bond and a side-chain containing eight carbon atoms. Systematic degradation, by oxidative methods, showed that cholestane, $C_{27}H_{48}$, the parent hydrocarbon of cholesterol, has a tetracyclic structure.

Rosenheim and King[155], and Weiland and Dane[156] proposed that the nuclear structure of cholesterol was derived from 1,2-cyclopentenophenanthrene CIV. This structure was found to accommodate all the available experimental evidence and the proof of its correctness was supplied by the total synthesis of cholesterol CV from 1,6-dihydroxynaphthalene by Robinson, Cornforth et al.[157].



(CIV)



(CV)

β-sitosterol and Stigmasterol.

Stigmasterol was first discovered by Windaus and Hauth[158] in the phytosterol isolated from calabar beans and was also found in soy bean oil by Matthes and Dahle[159] and in the fat extracted from carrots by Beschke[160].

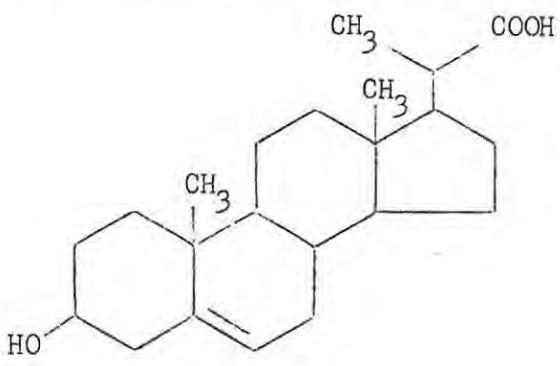
In 1923 Anderson and Moore[161] made a study of the phytosterols isolated from corn, cottonseed and linseed oils. It was found that corn oil contained a relatively high percentage of unsaponifiable matter which consisted of the plant sterol, sitosterol. Cottonseed oil contained a mixture of at least two phytosterols which could not be separated by recrystallization techniques, while linseed oil consisted of a complex mixture of phytosterols.

Anderson and Shriner[162] isolated a saturated sterol, dihydrositosterol, from corn oil. Dihydrositosterol was found to be a constituent of the fat extracted from the endosperm of corn and wheat. After removal of the dihydrositosterol, α - , β- and γ -sitosterols and

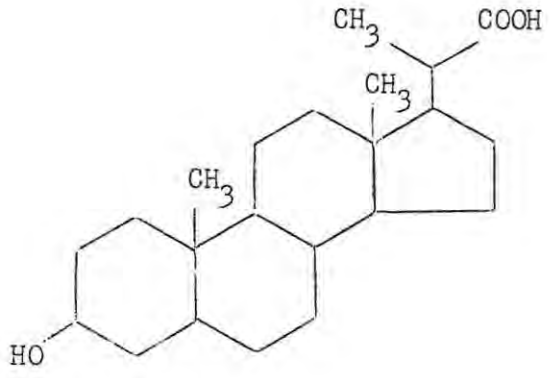
a small quantity of stigmasterol were isolated.

Windaus et al.[163] found that stigmasterol contained two carbon atoms more than cholesterol. Ozonolysis of stigmasterol by Guiteras[164] yielded a volatile aldehyde which formed an optically active semicarbazone, while drastic oxidation of the sterol with chromium trioxide gave acetone. It was therefore concluded that the aldehyde contained an asymmetric carbon atom and a terminal isopropyl group. The aldehyde was identified as α-ethylisopropylacetaldehyde and this proved the presence of a C-24 ethyl group in stigmasterol, and showed the existence of a double bond between carbon atoms C-22 and C-23.

Fernholz[165] brominated stigmasteryl acetate and subjected the dibromide to ozonolysis to give α-ethylisopropylacetaldehyde amongst other products. This showed that the first molecule of bromine entered the nucleus and not the side-chain. Debromination of the main ozonolysis product gave the acetyl derivative of 3-hydroxybisnorcholenic acid CVI. Catalytic reduction of this acid and subsequent saponification gave 3-hydroxybisnorcholanic acid CVII.

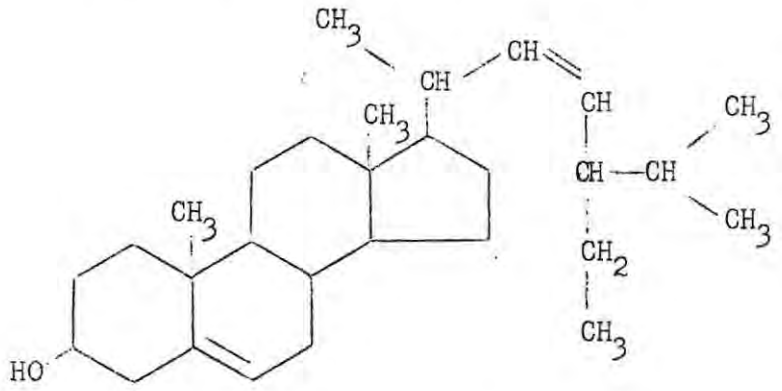


(CVI)



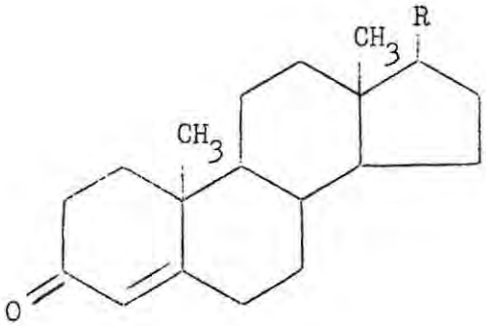
(CVII)

The position of the nuclear double bond in stigmasterol was determined by Fernholz[166] who converted stigmasteryl acetate to the acetate of α-stigmasterol oxide. The latter on heating with hydrochloric acid gave stigmastenetriol which was oxidized with chromium trioxide in acetic acid to dionol. On treatment with hydrochloric acid gas in chloroform a dienedione was formed. The compound was finally reduced with zinc in acetic acid to the dione. This proved that the nuclear double bond was situated between C-5 and C-6 as originally assumed, by analogy with cholesterol. Stigmasterol CVIII therefore differs from cholesterol only in the structure of the side-chain.



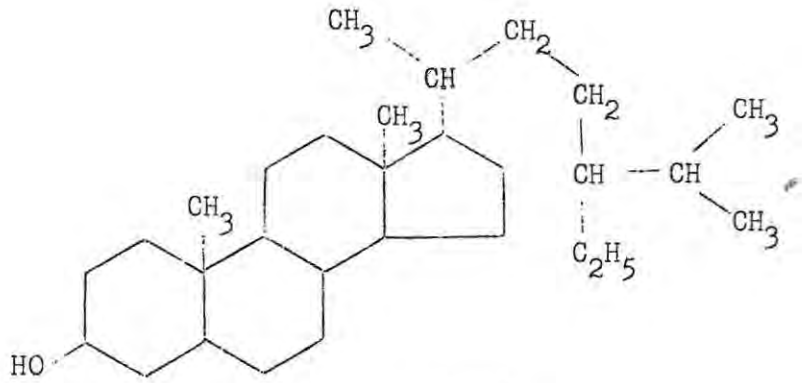
(CVIII)

Marker et al.[167,168] showed that β-sitosterol extracted from pine oil was identical with 22-dihydrostigmasterol. Stigmasterol and β-sitosterol on dehydrogenation with copper powder at elevated temperature under reduced pressure yielded stigmastenone CIX and β-sitostenone CX, respectively. Both of these on catalytic hydrogenation and subsequent treatment with sodium in boiling xylene gave 24-ethyl-epi-coprostanol CXI.



R: -CH-CH=CH-CH-CH (CIX)
(CH3, C2H5, CH3, CH3)

R: -CH-CH2-CH2-CH-CH (CX)
(CH3, C2H5, CH3, CH3)

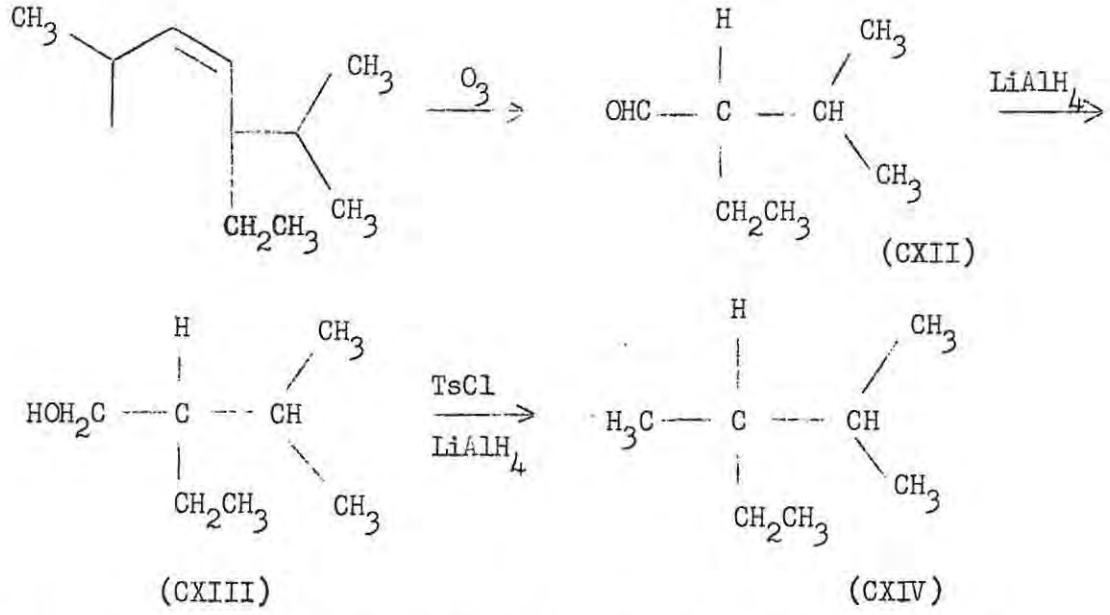


(CXI)

Stigmastenone CIX on treatment with sodium in amyl alcohol gave 5,6-dihydrostigmasterol. Catalytic hydrogenation of the latter yielded stigmastanol which was found to be identical with β-sitostanol prepared from β-sitosterol. Stigmasterol therefore differs from β-sitosterol only in the presence of a double bond between C-22 and C-23 in the side-chain.

The absolute configuration of the C-24 ethyl of stigmasterol was determined by Kishida et al.[169,170]. Ozone oxidation of the 6-methyl ether of stigmasterol yielded 2-ethyl-3-methyl butanal CXII which on reduction with lithium aluminium hydride at room temperature gave the corresponding alcohol, 2-ethyl-3-methyl butanol CXIII. The

tosyl derivative of this alcohol on mild reduction with lithium aluminium hydride in ether finally produced laevorotatory methyl-ethyl-isopropylmethane CXIV. From these degradative studies they concluded



(CXII)

(CXIII) (CXIV)

that the C-24 ethyl group of stigmasterol has the α -configuration.

Nuclear-magnetic-resonance spectroscopy has been applied in structural determinations of steroids. Shoolery and Rogers[171] measured the angular methyl proton shifts of stigmasteryl acetate. The spectrum showed methyl peaks at 216 c./sec. (C-19) and 228 c./sec. (C-18), an acetyl at 175 c./sec. and a signal at 42-48 c./sec. Slomp and MacKellar[172] found that for β-sitosterol the C-18 methyl signal was at 239 c./sec., the C-19 at 220 c./sec. and the C-21, C-26 and C-29 resonance peaks in the range 223-234 c./sec.

Mass spectrometry has been applied in recent years to a wide variety of steroids[173,174]. The mass spectra of hydroxylated steroids

are characterized by the presence of a large peak 18 mass units below the parent peak, while the length of the side-chain at C-17 may be calculated from the mass of the most prominent peak in the range 205-245.

After the loss of one molecule of water in the case of steroids containing an hydroxyl group at C-3, or acetic acid in the case of acetylated steroids, the loss of methyl groups at C-18 and C-19 occurs. This is followed by loss of side-chain substituents at C-17, after which rupturing of the B- and C-rings takes place.

Fitches[175] determined the molecular weights of both stigmasterol and β-sitosterol by means of mass spectrometry, and studied their fragmentation patterns.

EXPERIMENTAL AND RESULTS.

PART I.

STEREOSPECIFIC SYNTHESES OF THE FOUR ISOMERIC RACEMATES OF 7,4'-DIMETHOXYFLAVAN-3,4-DIOL.

Synthesis of (±)-7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol.

Peonol (2-Hydroxy-4-methoxyacetophenone).

Peonol was prepared by a modified version of Adams'[176] original method. Resacetophenone (20g.) was dissolved in the minimum volume of cold aqueous 10% (w./v.) NaOH and treated with dimethyl sulphate (16 ml.). The reaction mixture was heated for 1.5 hr. at 90° with frequent shaking, while the solution was kept alkaline (litmus) by the addition of more 10% NaOH solution when required. The mixture was cooled, acidified with dilute HCl and the organic phase extracted with ether. The ethereal extract was concentrated under vacuum to about 100ml. and the unchanged resacetophenone removed by extraction with aqueous Na_2CO_3 solution. The ether fraction was concentrated at 40° under vacuum in a rotatory evaporator to give a red-brown oil which crystallized with difficulty from ethanol after treatment with charcoal. Recrystallization from ethanol finally gave colourless needles (15g.), m.p. 50°. Tahara[177] recorded an identical melting point for peonol.

2'-Hydroxy-4,4'-dimethoxychalcone.

The synthesis of 2'-hydroxy-4,4'-dimethoxychalcone was based on the original method used by von Kostanecki and Osius[178], as modified by Crabtree and Robinson[179]. To a warm solution (80°) of peonol (15g.)

and anisaldehyde (14g.) in ethanol (200 ml.) under a nitrogen atmosphere, was added aqueous 50% (w./v.) KOH solution (25g.). The mixture was shaken at frequent intervals and maintained at 60-70° for 4 hr. under nitrogen. After cooling the mixture rapidly under running water, it was diluted with water and acidified with dilute HCl when the crude chalcone precipitated. This was crystallized from ethanol as bright-yellow needles (7.5g.), m.p. 113-4°. (Found: C, 72.0 ; H, 5.9 ; OCH_3, 21.9. Calculated for $C_{17}H_{16}O_4$: C, 71.8 ; H, 5.7 ; OCH_3, 21.8%). von Kostanecki and Osius[178] recorded a melting point of 113-114° for the chalcone.

7,4'-Dimethoxyflavonol.

The preparation of 7,4'-dimethoxyflavonol was achieved by methods similar to those used initially by Juppen and von Kostanecki[180] and later by King and Bottomley[38] for analogous compounds. 2'-Hydroxy-4,4'-dimethoxychalcone (7.5g.) was dissolved in aqueous 50% (v./v.) ethanol (300 ml.) and NaOH (15g.) added with warming to 80° to give a clear solution. The solution was allowed to cool to 70° when 28% (v./v.) H_2O_2 (50 ml.) was added slowly with shaking. The reaction was exothermic and the solution, after initial water-cooling, was allowed to come to room temperature over a period of about 3 hr. The solution was acidified with concentrated HCl and allowed to cool to 10° when the flavonol separated as pale-yellow needles. These were recrystallized from aqueous ethanol (2.25g.), m.p. 195-6°. (Found: C, 68.4 ; H, 4.7 ; OCH_3, 21.8. $C_{17}H_{14}O_5$ requires: C, 68.5 ; H, 4.7 ; OCH_3, 20.8%). A melting point of 196-197° was recorded by Juppen and von Kostanecki[180] for the flavonol.

7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol.

7,4'-Dimethoxyflavonol (200 mg.) in a mixture of ethanol: methanol (3: 1, v./v.) (40 ml.) was hydrogenated for 2 hr. at 125° and 120 kg./cm.^{2} in an electric autoclave using copper chromite catalyst[181] (300 mg.), prepared by the method of Adkins[182]. The residual flavonol was filtered off and the flavan-3,4-diol crystallized from aqueous ethanol as white needles (67.5 mg.), m.p. 86-88° (with water of crystallization), and m.p. 154-155° after drying under vacuum over $CaCl_2$ for two days. (Found: C, 68.1 ; H, 6.2 ; OCH_3, 21.3. $C_{17}H_{18}O_5$ requires: C, 67.5 ; H, 6.0 ; OCH_3, 20.5%).

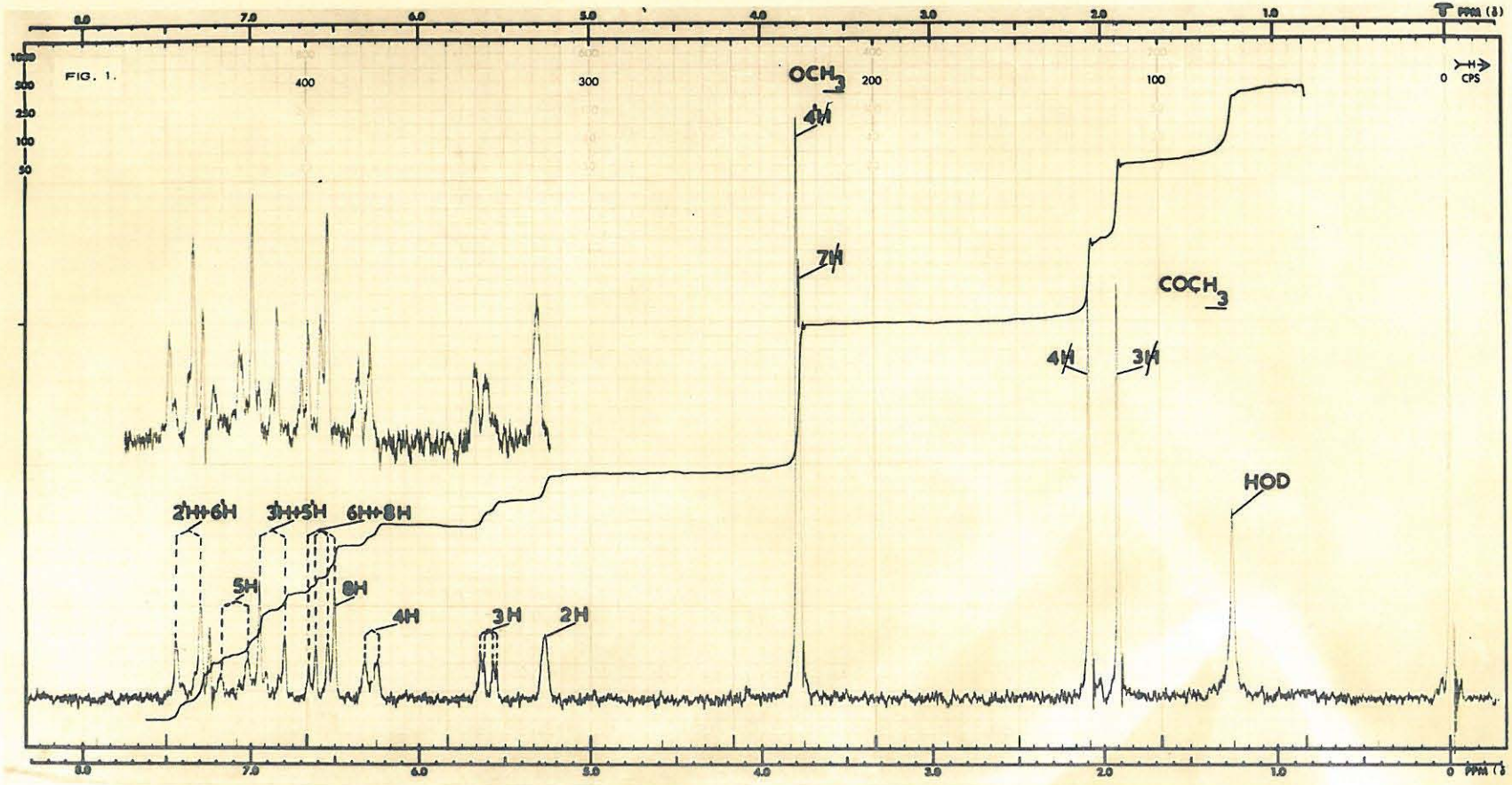
Raney nickel catalyst which had been aged for 2-4 weeks afforded the same flavan-3,4-diol, but in lower yield (about 3%, compared with approximately 35% using copper chromite catalyst), even under optimum conditions.

7,4'-Dimethoxy-3,4-cis-diacetoxy-2,3-cis-flavan (Fig.1).

7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol (108 mg.) was acetylated by dissolving in pyridine (0.4 ml.) and acetic anhydride (0.6 ml.) and leaving the mixture at room temperature overnight. The product was recovered from water (15 ml.), and crystallized from ethanol in white needles (117 mg.), m.p. 143-144°. (Found: C, 65.2 ; H, 5.7 ; OCH_3, 16.1 ; $CO.CH_3$, 22.6. $C_{21}H_{22}O_7$ requires C, 65.3 ; H, 5.7 ; OCH_3, 16.1 ; $CO.CH_3$, 22.3%).

The 2,3-cis-3,4-cis-flavandiol-diacetate was shown, from its nuclear-magnetic-resonance spectrum, to have this configuration

FIG. 1. N.M.R. Spectrum of (±)-7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diacetate.



by comparison of the chemical shifts and analysis of the spin-spin coupling constants of the 2-, 3- and 4-protons of the heterocyclic ring (cf. Table 1).

Synthesis of (±)-7,4'-Dimethoxy-2,3-trans-flavan-3,4-trans-diol.

2'-Acetoxy-4,4'-dimethoxychalcone.

The 2'-acetoxy-4,4'-dimethoxychalcone was synthesized according to the method of von Kostanecki and Osius[178]. 2-Hydroxy-4,4'-dimethoxychalcone (16g.) was dissolved in the minimum volume of pyridine (30 ml.) and acetylated with acetic anhydride (60 ml.) at room temperature. After leaving the solution to stand overnight, it was poured into a large volume of water when a thick, yellow oil resulted. The oil was triturated with CCl_4, when the acetate crystallized as light-yellow needles (20g.), m.p. 107°. (Found: C, 69.9 ; H, 5.8 ; OCH_3, 19.4 ; $CO.CH_3$, 13.5. Calculated for $C_{19}H_{18}O_5$: C, 69.9 ; H, 5.6 ; OCH_3, 19.0 ; $CO.CH_3$, 13.2%). von Kostanecki and Osius[178] recorded a melting point of 103-4° for this chalcone.

2'-Acetoxy-4,4'-dimethoxychalcone dibromide.

2'-Acetoxy-4,4'-dimethoxychalcone (20g.) was dissolved in CCl_4 (350 ml.), and bromine (10g.) in CCl_4 (40 ml.) added cautiously in small portions. The solution was left standing overnight at room temperature and evaporated to a small volume at 50° under reduced pressure. On cooling the crude chalcone dibromide precipitated as orange crystals. These were recrystallized from benzene: light petroleum ether (b.p. 40-60°) (1:1, v./v.) to give clusters of colourless needles (23g.), m.p. 147-8°, with decomposition and reddening. (Found: C, 47.0 ; H, 3.9 ; Br, 33.4 ; OCH_3, 12.9 ; $CO.CH_3$, 8.9. $C_{19}H_{18}Br_2O_5$ requires: C, 46.9 ;

H, 3.7 ; Br, 32.9 ; OCH_3, 12.8 ; $CO.CH_3$, 8.9%).

7,4'-Dimethoxy-2,3-trans-dihydroflavonol.

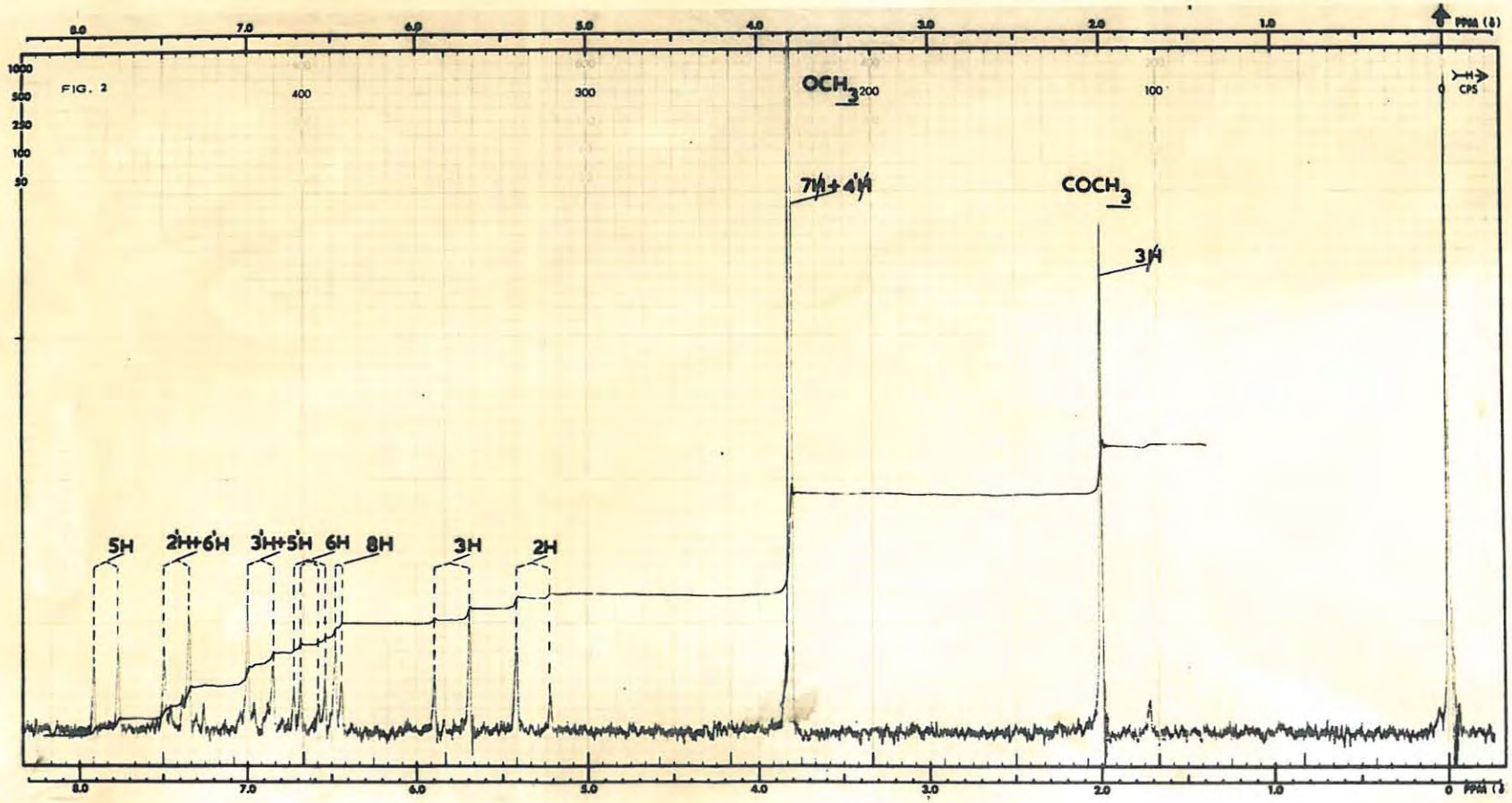
2'-Acetoxy-4,4'-dimethoxychalcone dibromide (5g.) was dissolved in aqueous 80% (v./v.) acetone (100 ml.), boiled for 15 minutes and then for a further 3 minutes with aqueous 10% sodium carbonate (50 ml.). The mixture was cooled and diluted with water when an oil settled out. On standing the oil hardened to form light-yellow needles. These were recrystallized from ethanol giving pale-yellow needles (1.5g.), m.p. 135°. (Found: C, 67.8 ; H, 5.4 ; OCH_3, 20.6. Calculated for $C_{17}H_{16}O_5$: C, 68.0 ; H, 5.4 ; OCH_3, 20.7%). The dihydroflavonol was previously synthesized by Kulkarni and Joshi[184] who recorded a m.p. of 133°.

3-Acetoxy-7,4'-dimethoxy-2,3-trans-dihydroflavonol (Fig. 2).

7,4'-Dimethoxydihydroflavonol (123 mg.) was dissolved in the minimum volume of pyridine and acetic anhydride (0.65 ml.) added. After standing overnight at room temperature the acetate was recovered from water as before and crystallized from ethanol in large shiny plates (60 mg.), m.p. 129-130°. (Found: C, 66.4 ; H, 5.4 ; OCH_3, 18.0 ; $CO.CH_3$, 12.4. $C_{19}H_{18}O_6$ requires: C, 66.7 ; H, 5.3 ; OCH_3, 18.1 ; $CO.CH_3$, 12.6%). Kulkarni and Joshi[184] quoted a m.p. 140-142° for this compound.

The nuclear-magnetic-resonance spectrum of the dihydroflavonol acetate showed, on analysis, that there was a 2,3-trans arrangement of substituents. This was deduced from the large coupling constants ($J_{2,3}$ = 12.0 - 12.1 c./sec.) of the 2- and 3-protons. The chemical

FIG. 2. N.M.R. Spectrum of 7,4'-Dimethoxy-3-acetoxy-2,3-trans-dihydroflavonol.



shifts and spin-spin coupling constants are recorded in Table 2.

7,4'-Dimethoxy-2,3-trans-flavan-3,4-trans-diol.

7,4'-Dimethoxy-2,3-trans-dihydroflavonol (800 mg.) in methanol (40 ml.) was hydrogenated with Adams catalyst (79.86% PtO_2 ; W.C.Hereaus, Hanau, Germany) (550 mg.) for 4 hr. under conditions used originally by Freudenberg and Roux[185] in 1954. The catalyst was filtered off and the solution concentrated to a small volume under vacuum in a rotary evaporator at 60°. The crude 2,3-trans-3,4-trans-flavan-diol crystallized from this solution, and was recrystallized from aqueous ethanol in colourless needles (452 mg.), m.p. 119-120°. (Found: C, 67.1 ; H, 6.3 ; OCH_3, 20.8. Calculated for $C_{17}H_{18}O_5$: C, 67.5 ; H, 6.0 ; OCH_3, 20.5%).

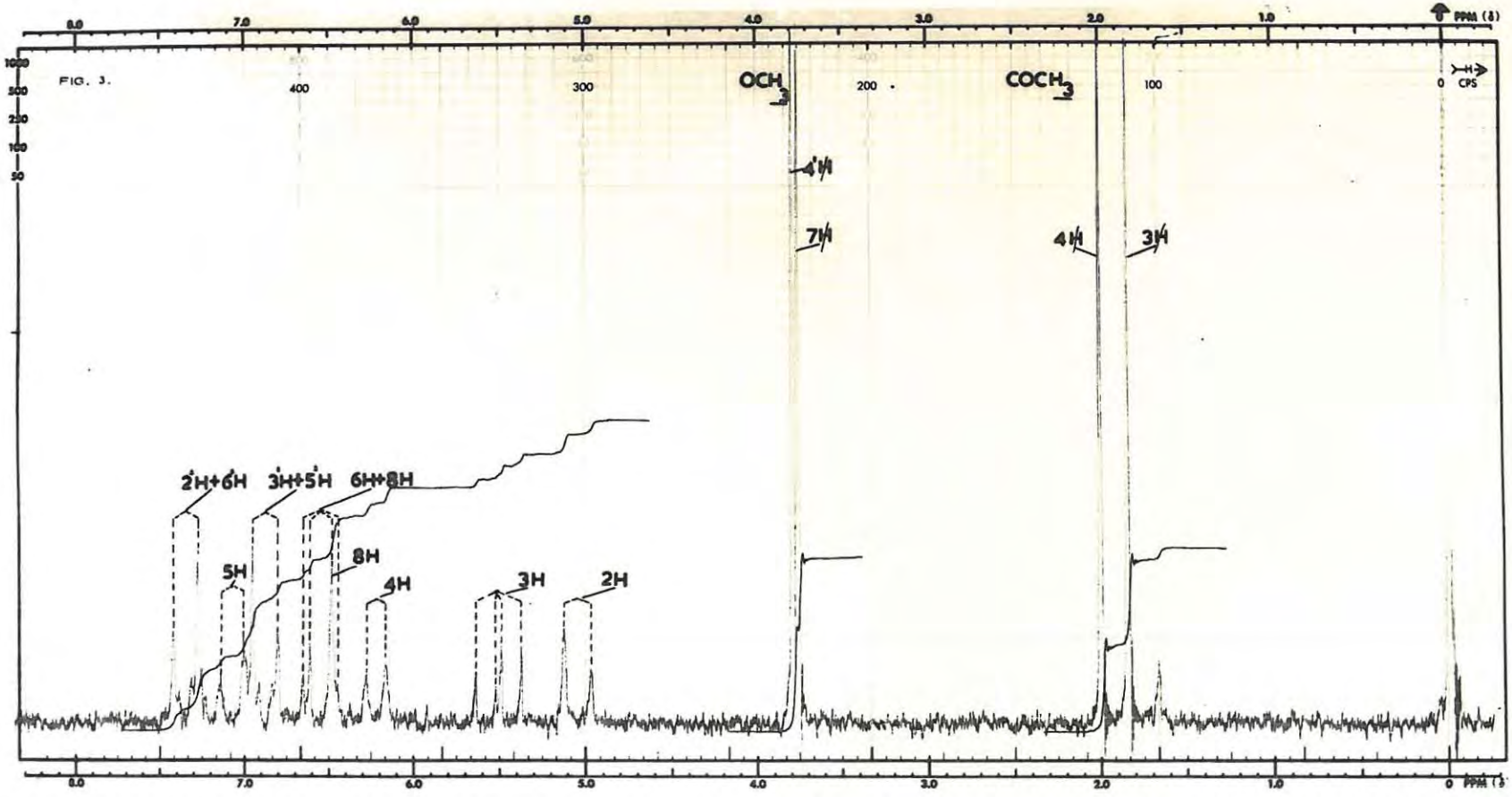
A similar compound was obtained by Phatak and Kulkarni[186] from the reduction of the dihydroflavonol (m.p. 140-142°) with $LiAlH_4$. The 3,4-diol, m.p. 114-115°, was regarded to be the 2,3-trans-3,4-cis-flavan-3,4-diol.

7,4'-Dimethoxy-3,4-trans-diacetoxy-2,3-trans-flavan (Fig. 3)

7,4'Dimethoxy-2,3-trans-flavan-3,4-trans-diol (105 mg.) was acetylated with pyridine (0.4 ml.) and acetic anhydride (0.6 ml.) as before. The product was recovered from water and crystallized from ethanol as colourless needles (100 mg.), m.p. 135-136° with sintering 107-108°. (Found: C, 65.2 ; H, 5.6 ; OCH_3, 16.0 ; $CO.CH_3$, 22.0. Calculated for $C_{21}H_{22}O_7$: C, 65.3 ; H, 5.7 ; OCH_3, 16.1 ; $CO.CH_3$, 22.3%).

Confirmation of the 2,3-trans-3,4-trans arrangement of substituents and purity of the compound was provided by the analysis of its nuclear-magnetic-resonance spectrum (cf. Table 1).

FIG. 3. N.M.R. Spectrum of (±)-7,4'-Dimethoxy-2,3-trans-flavan-3,4-trans-diacetate.



Synthesis of (±)-7,4'-Dimethoxy-2,3-trans-flavan-3,4-cis-diol.

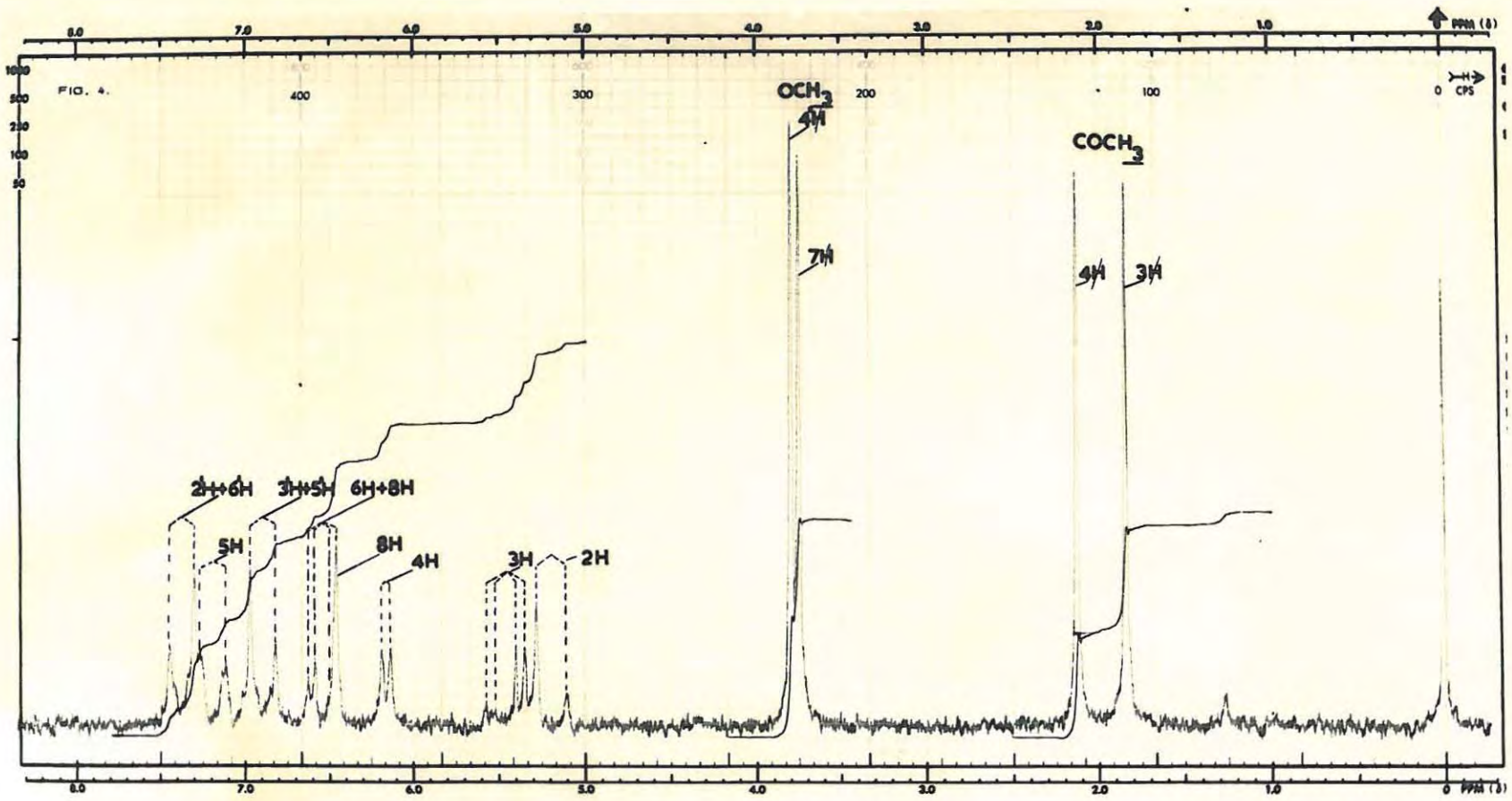
7,4'-Dimethoxy-2,3-trans-flavan-3,4-trans-diol was epimerized selectively at C-4 using Kashikar and Kulkarni's[82] method, $NaBH_4$ + BF_3 in bis-(2-methoxyethyl)-ether (diglyme).

To a suspension of $NaBH_4$ (280 mg.) in dry diglyme (20 ml.) was added an ethereal solution of BF_3 (45%) (1.5 ml.). A solution of 7,4'-dimethoxy-2,3-trans-flavan-3,4-trans-diol (300 mg.) in dry diglyme (15 ml.) was added to the suspension with stirring. The mixture was kept at room temperature for 2 hr., heated at 70° for a further 2 hr. and finally left to stand overnight at room temperature. The excess reagent was decomposed with glacial acetic acid (5 ml.), the mixture diluted with water and the product obtained by repeated ether extractions. The ethereal extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure at 40°. The product crystallized from ethanol as colourless needles (130 mg.), m.p. 138-140°. (Found: C, 67.5 ; H, 6.2 ; OCH_3, 20.7. Calculated for $C_{17}H_{18}O_5$: C, 67.5 ; H, 6.0 ; OCH_3, 20.5%).

7,4'-Dimethoxy-3,4-cis-diacetoxy-2,3-trans-flavan (Fig. 4).

7,4'-Dimethoxy-2,3-trans-flavan-3,4-cis-diol (100 mg.) was acetylated with acetic anhydride (0.4 ml.) and pyridine (0.5 ml.) as before, and the product recovered from water (10 ml.) as an amorphous white powder (114 mg.), m.p. 80-3°. (Found: C, 65.4 ; H, 5.9 ; OCH_3, 16.4 ; $COCH_3$, 22.0. $C_{21}H_{22}O_7$ requires: C, 65.3 ; H, 5.7 ; OCH_3, 16.1 ; $COCH_3$, 22.3%).

FIG. 4. N.M.R. Spectrum of (±)-7,4'-Dimethoxy-2,3-trans-flavan-3,4-cis-diacetate.



Phatak and Kulkarni[186] synthesized a diol, m.p. 136°, which they regarded as the 2,3-trans-3,4-trans isomer. N.m.r. spectrometric analysis of their diacetate showed, however, that it was a mixture of 2,3-trans-3,4-trans and 2,3-trans-3,4-cis isomers (cf. Fig. 5).

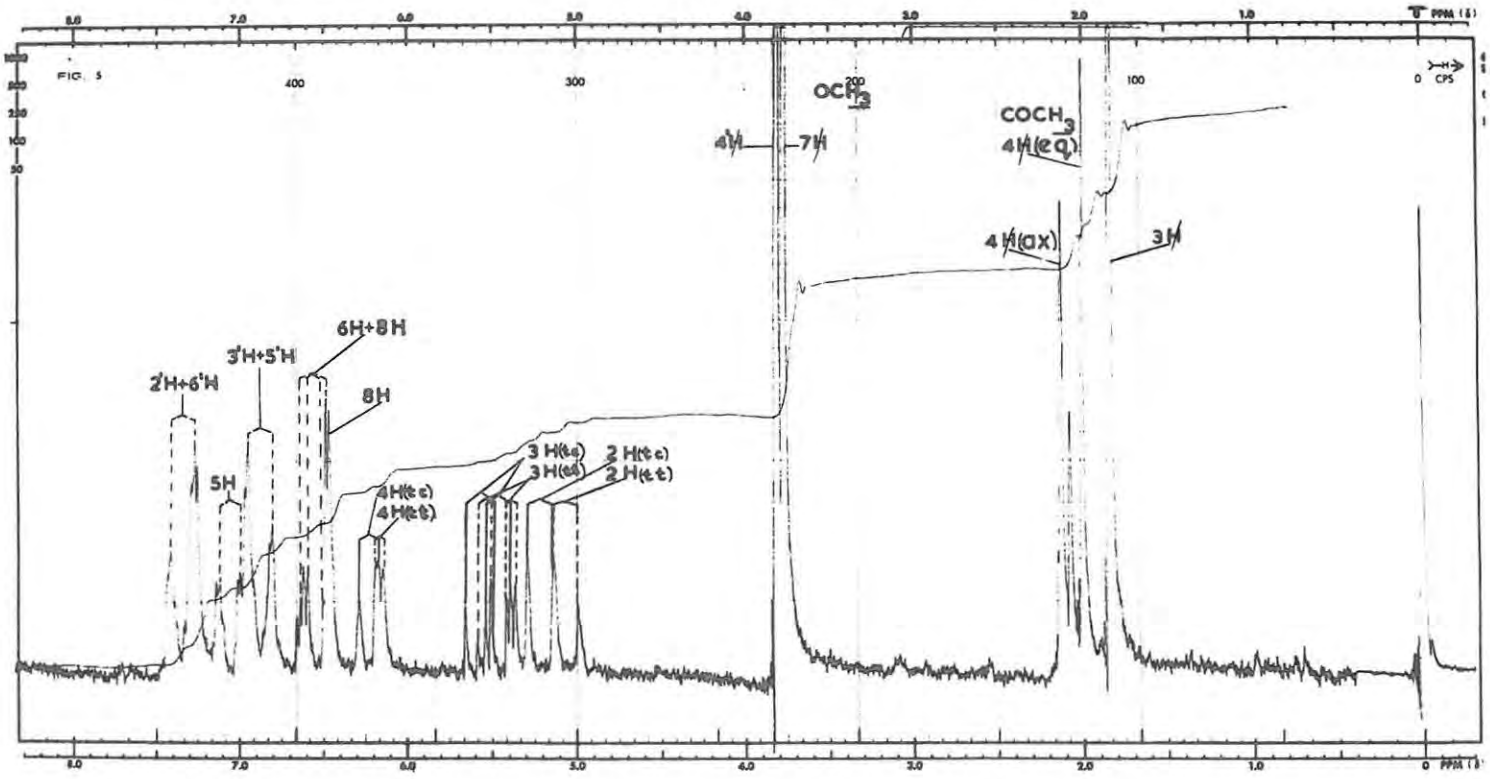
The nuclear-magnetic-resonance spectrum of the amorphous 7,4'-dimethoxy-3,4-cis-diacetoxy-2,3-trans-flavan confirmed its 2,3-trans-3,4-cis arrangement of substituent groups (cf. Table 1; Discussion).

Synthesis of 7,4'-Dimethoxy-2,3-cis-flavan-3,4-trans-diol.

Attempted Epimerization of 7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol.

An ethereal solution of boron trifluoride (1.5 ml.) (45%) was added to a suspension of sodium boronhydride (290 mg.) in dry diglyme (20 ml.). A solution of 7,4'-dimethoxy-2,3-cis-flavan-3,4-cis-diol (300 mg.) in dry diglyme (15 ml.) was added to the above suspension with stirring. The reaction was carried out as before for the conversion of the 2,3-trans-3,4-trans into the 2,3-trans-3,4-cis isomer. The product crystallized from an ethanolic solution to which water (2 drops) had been added, and formed colourless needles on recrystallization (106 mg.), m.p. 155-6°. A mixed m.p. with authentic 2,3-cis-3,4-cis-diol was undepressed at 155-156°. The mother liquor was examined by paper electrophoresis (cf. Table 5) and by thin-layer chromatography on Kieselgel G (E. Merck A.-G., Darmstadt, Germany) (cf. Table 3). This indicated that the desired 2,3-cis-3,4-trans isomer had not formed. A trace of a high R_F (0.94) component was evident, possibly the 4-ethyl ether of the 2,3-cis-3,4-trans compound. Darkening of the reaction

FIG. 5. N.M.R. Spectrum of Phatak and Kulkarni's Mixture of Two Racemic (±)-7,4'-Dimethoxy-flavan-3,4-diol-diacetates.



mixture was observed during heating and therefore milder conditions were attempted. A repetition of the reaction at 50° for 1 hr. again gave the 2,3-cis-3,4-cis racemate with higher recovery (84%, compared with 32% for the first attempt).

Epimerization at C-4 of the 2,3-cis-3,4-cis diol evidently does not occur under the same conditions as for the conversion of the 2,3-trans-3,4-trans diol to the 2,3-trans-3,4-cis isomer.

Attempted Total Synthesis of (±)-7,4'-Dimethoxy-2,3-cis-flavan-3,4-trans-diol.

Using 2'-acetoxy-4,4'-dimethoxychalcone dibromide as starting material, an attempt was made to prepare the 2,3-cis-3,4-trans diol by way of the 3-bromoflavanone and 3-bromoflavan-4β-ol. The synthetic route developed by Kulkarni et al.[184,187] as modified by Clark-Lewis, Jackman and Williams[75] was used.

3-Bromo-7,4'-dimethoxyflavanone.

2'-Acetoxy-4,4'-dimethoxychalcone dibromide (18g.) was dissolved in aqueous acetic acid (85%, 200 ml.) at 60-70° and left overnight at room temperature. The solution was diluted with water when a yellow oil formed. After repeated changes of water the oil hardened and the product crystallized from ethanol as light-yellow plates (5g.), m.p. 156-158°. (Found: C, 55.1 ; H, 3.6 ; Br, 26.7 ; OCH_3, 17.5. $C_{17}H_{15}BrO_4$ requires: C, 56.2 ; H, 4.2 ; Br, 22.0 ; OCH_3, 17.1%).

The product on being sprayed with a 1% solution of $NaBH_4$ in isopropanol gave a light-magenta colour on paper, indicating the

conversion of the chalcone dibromide to the corresponding flavanone.

3-Bromo-7,4'-dimethoxyflavan-4β-ol.

3-Bromo-7,4'-dimethoxyflavanone (5g.) in dry tetrahydrofuran (100 ml.) was added slowly to a stirred mixture of $LiAlH_4$ (1.5g.) and $AlCl_3$ (10.5g.) in dry tetrahydrofuran (60 ml.) at 0°. The excess reagent was decomposed with water after 1 hr. and the greenish-grey mixture acidified with 1.5N HCl (30 ml.). The mixture was extracted with ether (3 times), dried over anhydrous Na_2SO_4 and the ethereal solution taken to dryness under reduced pressure at 40°. A brown residue of crude 3-bromo-7,4'-dimethoxyflavan-4β-ol resulted. This crystallized from ethanol in buff-coloured needles (900 mg.), m.p. 185-7°. The product was spotted on filter paper and sprayed with a solution of toluene-p-sulphonic acid in ethanol. After heating at 100° for 2 minutes a blue colour appeared, slowly fading to a pink on standing, indicating a positive reaction for flavan-4-ols (cf. Roux and Paulus[188] and Row et al.[189]).

7,4'-Dimethoxy-2,3-cis-flavan-3,4-trans-diol.

3-Bromo-7,4'-dimethoxyflavan-4β-ol (500 mg.) was boiled for 70 hr. in a mixture of acetic anhydride (5 ml.), acetic acid (25 ml.) and anhydrous potassium acetate (2g.). After reaction, the mixture was diluted with ice-water (200 ml.) and the brown oil left to harden. A dark-brown precipitate, which failed to crystallize from aqueous ethanol in the usual way, was subjected to chromatography on an activated alumina column using gradient elution. A mixture of isohexane: benzene (5:3, v./v.) was used initially, the ratio changing to (1:1) and finally to

(1:2). Four main fractions were collected of which only the last showed slight reaction characteristic of leucoanthocyanidins. Concentration of this fraction afforded a brown precipitate which failed to crystallize.

3-Bromo-7,4'-dimethoxyflavanone.

In order to investigate the failure of flavan-4β-ol to yield the desired 2,3-cis-3,4-trans-diol, the synthesis of the corresponding flavan-4α-ol by the elegant method of Doifode[81] was attempted.

2'-Hydroxy-4,4'-dimethoxychalcone (1g.) and cupric bromide (2.4g.) in dioxane (60 ml.) were refluxed for 2 hr. The mixture was cooled, diluted with water and the precipitate crystallized from ethanol giving bright-yellow needles, m.p. 110-113°. Paper chromatography confirmed that the product consisted of unchanged chalcone.

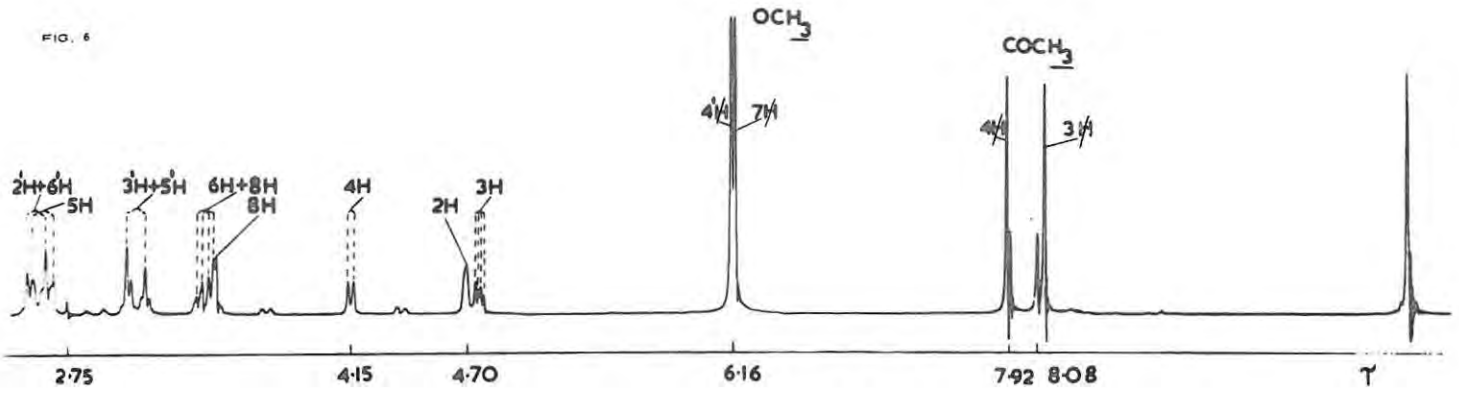
The reaction of cupric bromide with 2'-hydroxy-4,4'-dimethoxychalcone apparently does not proceed along a similar route to that of a chalcone methylated in the 5- and 4'-positions. In the case of 2'-hydroxy-5-methyl-4'-methoxychalcone, under these conditions, 3-bromo-6-methyl-4'-methoxyflavanone was formed.

Failure of the synthesis of (±)-7,4'-dimethoxy-2,3-cis-flavan-3,4-trans-diol, via the 3-bromoflavanone and 3-bromoflavan-4β-ol intermediates, appears to be due to methoxyl substitution at C-7 (see Discussion).

Synthesis of (±)-7,4'-Dimethoxy-3,4-trans-diacetoxy-2,3-cis-flavan (Fig. 6).

The epimerization of the 2,3-cis-3,4-cis-diol to yield the 2,3-cis-3,4-trans-diacetate by the acetylation technique was performed

FIG. 6. N.M.R. Spectrum of (±)-7,4'-Dimethoxy-2,3-*cis*-flavan-3,4-*trans*-diacetate.



according to the method of Fujise et al.[68,69,70].

(±)-7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol (400 mg.) and anhydrous sodium acetate (110 mg.) were added to acetic anhydride (4 ml.) and acetic acid (1 ml.). The mixture was heated under reflux for 4 hr., poured into ice-water and allowed to harden overnight. The product was examined by thin-layer chromatography on Kieselgel G (0.25 mm. thickness) using 10 cm. migration and benzene: acetone (19:1, v./v.) as solvent. The compounds were detected by spraying with concentrated H_2SO_4 : 40% formaldehyde (20:1, v./v.) reagent. The two main components had R_F values of 0.75 and 0.51, while at lower R_F (0.25-0.42) four minor impurities were detected. The product (R_F 0.51) was isolated by means of preparative thin-layer chromatography on six plates (20 x 20 cm.) on Kieselgel G (1 mm.) in the same solvent system. Development was extended to 1.5 - 2.0 hr. to give 18 cm. migration to the top of the plates. The band at R_F 0.51 was located by spraying a strip along the side of the plate with a mixture of concentrated H_2SO_4 and 40% formaldehyde (20:1, v./v.) and heating for 5 minutes at 110°. The product was stripped off the Kieselgel and extracted with benzene: ethanol (9:1, v./v.). It crystallized from ethanol as colourless prisms (213 mg.) m.p. 109-110°. (Found: C, 65.3 ; H, 5.9 ; OCH_3, 17.0 ; $CO.CH_3$, 22.6. $C_{21}H_{22}O_7$ requires: C, 65.3 ; H, 5.7 ; OCH_3, 16.1 ; $CO.CH_3$, 22.3%).

Analysis of the n.m.r. spectrum of the diacetate confirmed its 2,3-cis-3,4-trans configuration of substituents. A small portion of the 2,3-cis-3,4-cis starting material was detectable. This was not removed by repeated recrystallization. The chemical shifts and spin-spin coupling constants for the four racemates are given in Table 1.

TABLE 1.

Nuclear-magnetic-resonance Spectra of 3,4-Diacetoxy-7,4'-dimethoxy-flavan Racemates.

(a) Chemical Shifts : τ values (p.p.m.).

Racemate	CH_3 (Acetyl)		CH_3 (Methoxyl)		H						
	3-	4-	7-	4'-	2-	3-	4-	5-	6+8-	3'+5'-	2'+6'
	(s)	(s)	(s)	(s)	(d)	(q)	(d)	(d)	(m)	(d)	(d)
2,3-trans-3,4-trans	8.15	8.00	6.24	6.21	4.95	4.49	3.78	2.91	3.43	3.13	2.65
2,3-trans-3,4-cis	8.16	7.87	6.25	6.20	4.82	4.53	3.84	2.80	3.47	3.09	2.63
2,3-cis-3,4-trans	8.11	7.90	6.23	6.19	4.70	4.77	4.15	2.65	3.44	3.07	2.56
2,3-cis-3,4-cis	8.08	7.92	6.22	6.20	4.73	4.40	3.73	2.89	3.43	3.13	2.64
Guibourtacacidin*	8.12	7.89	6.23	6.19	4.70	4.70	4.13	2.65	3.45	3.11	2.62

(b) Spin-spin Coupling Constants for 2-, 3- and 4-Protons (c./sec.).

Racemates	$J_{2,3}$	$J_{3,4}$
2,3-trans-3,4-trans	9.0	6.9
2,3-trans-3,4 cis	10.3	3.1
2,3-cis-3,4-trans	0.9x	2.8
2,3-cis-3,4-cis	1.2x	4.2
Guibourtacacidin*	$\angle$ 1.0	2.7

s = singlet, d = doublet, q = quartet, and m = multiplet.

*Natural product obtained from Guibourtia Coleosperma heartwood[190].

xValues obtained by analysis of the 3-proton quartet.

TABLE 2.

Nuclear-magnetic-resonance Spectrum of 3-Acetoxy-7,4'-dimethoxy-2,3-trans-dihydroflavonol.

(a) Chemical Shifts : τ values (p.p.m.).

CH_3(Acetyl)	CH_3(Methoxyl)		H						
3-	7-	4'-	2-	3-	5-	6-	8-	3'+5'-	2'+6'-
s	s	s	d	d	d	q	d	d	d
8.00	6.19	6.19	4.68	4.21	2.16	3.38	3.55	3.07	2.58

s = singlet, d = doublet, and q = quartet.

(b) Spin-spin Coupling Constant for the 2- and 3-Protons (c./sec.)

$$J_{2,3} = 12.0, 12.1$$

Thin-layer Chromatography of (±)-7,4'-Dimethoxyflavan-3,4-diol Racemates.

The trans-trans, trans-cis and cis-cis racemates of (±)-7,4'-dimethoxyflavan-3,4-diol were chromatographed on Kieselgel G (E. Merck A.-G., Darmstadt, Germany) chromatoplates (0.25 mm. thick). A mixture of chloroform : ethyl acetate (2:1, v./v.) was used with 10 cm. migration. The chromatoplates were dried in a current of warm air and sprayed with toluene-p-sulphonic acid[191] or concentrated H_2SO_4 : 40% aqueous formaldehyde (20:1, v./v.). The colours were developed by heating the plates for 5-10 minutes at 120°. The former reagent gave pinks, while the latter spray gave deep-reds. Under ultraviolet light all showed golden-yellow fluorescent spots. R_F values are listed in Table 3.

TABLE 3.

Thin-layer Chromatography of 7,4'-Dimethoxyflavan-3,4-diol Racemates.

Racemate	R_F
2,3-trans-3,4-trans	0.28
2,3-trans-3,4-cis	0.31
2,3-cis-3,4-cis	0.43
Product of attempted epimerization of 2,3-cis-3,4-cis racemate:	
(i) crystals	0.43
(ii) mother liquor	0.46 0.96*

*Possibly the 4-ethyl ether of the desired 2,3-cis-3,4-trans racemate.

Thin-layer Chromatography of the 3,4-Diacetoxy-7,4'-dimethoxyflavans.

The four racemates of (±)-7,4'-dimethoxy-3,4-diacetoxyflavan were developed (10 cm. migration) on Kieselgel G chromatoplates (0.25 mm. thick) with benzene : acetone (19:1, v./v.). The plates were sprayed with concentrated sulphuric acid : 40% formaldehyde (20:1, v./v.) and heated at 120° for 5-10 minutes. The 2,3-cis-3,4-cis racemate was the only derivative giving a pink colour with this spray reagent - all three other racemates showing brick-red spots. The R_F value of the 2,3-cis-3,4-trans racemate was appreciably lower than the others. R_F values of the four racemates and the colours produced with the above spray reagent are summarized in Table 4.

TABLE 4.

Thin-layer Chromatography of 3,4-Diacetoxy-7,4'-dimethoxyflavans.

Racemate	R_F	Colour
2,3-*trans*-3,4-*trans*	0.65	Brick-red
2,3-*trans*-3,4-*cis*	0.60	Brick-red
2,3-*cis*-3,4-*cis*	0.64	Pink
2,3-*cis*-3,4-*trans*	0.51	Brick-red
Acetylation products of 2,3-*cis*-3,4-*cis* isomer	0.51 0.75	Brick-red Pink

Paper Ionophoresis of (±)-7,4'-Dimethoxyflavan-3,4-diols.

The apparatus used was of the horizontal open-strip type (*cf.* Grassman and Hannig[192]) in which the paper is allowed to dip at each end into cells containing 0.1M sodium borate solution. The atmosphere was maintained near saturation by enclosing the apparatus with a tight-fitting "Perspex" lid. The paper [Sleicher and Schull brand no.2043 (4 x 41 cm.)] was wetted with borate solution and equilibrated in position for 1 hr. before use. The compound was applied as a streak on the origin, located 17 cm. from the one end of the paper. A "Shandon Vokam" power supply, type 2541, was used to apply a potential of 70-75 volts across the electrodes, giving a current of 5 m.A., for 18 hr. The strips were dried at 50° in an oven and heavily sprayed with toluene-*p*-sulphonic acid to liberate the diols from the borate complex. Heating for 5-10 minutes at 80-100° produced pink bands on a grey back-ground. The ionophoretic mobilities of the 7,4'-dimethoxyflavan-3,4-diols are listed in Table 5.

TABLE 5.

Paper Ionophoresis of (±)-7,4'-Dimethoxyflavan-3,4-diols.

Racemate	Ionophoretic mobility (cm.)
2,3-trans-3,4-trans	-2.1
2,3-trans-3,4-cis	+3.0
2,3-cis-3,4-cis	+1.7
Products of attempted epimerization of 2,3-cis-3,4-cis racemate:	
(i) crystals, m.p. 155-156°	+1.7
(ii) mother liquor	+1.9 -1.7*

*Possibly the 4-ethyl ether of the desired 2,3-cis-3,4-trans racemate.

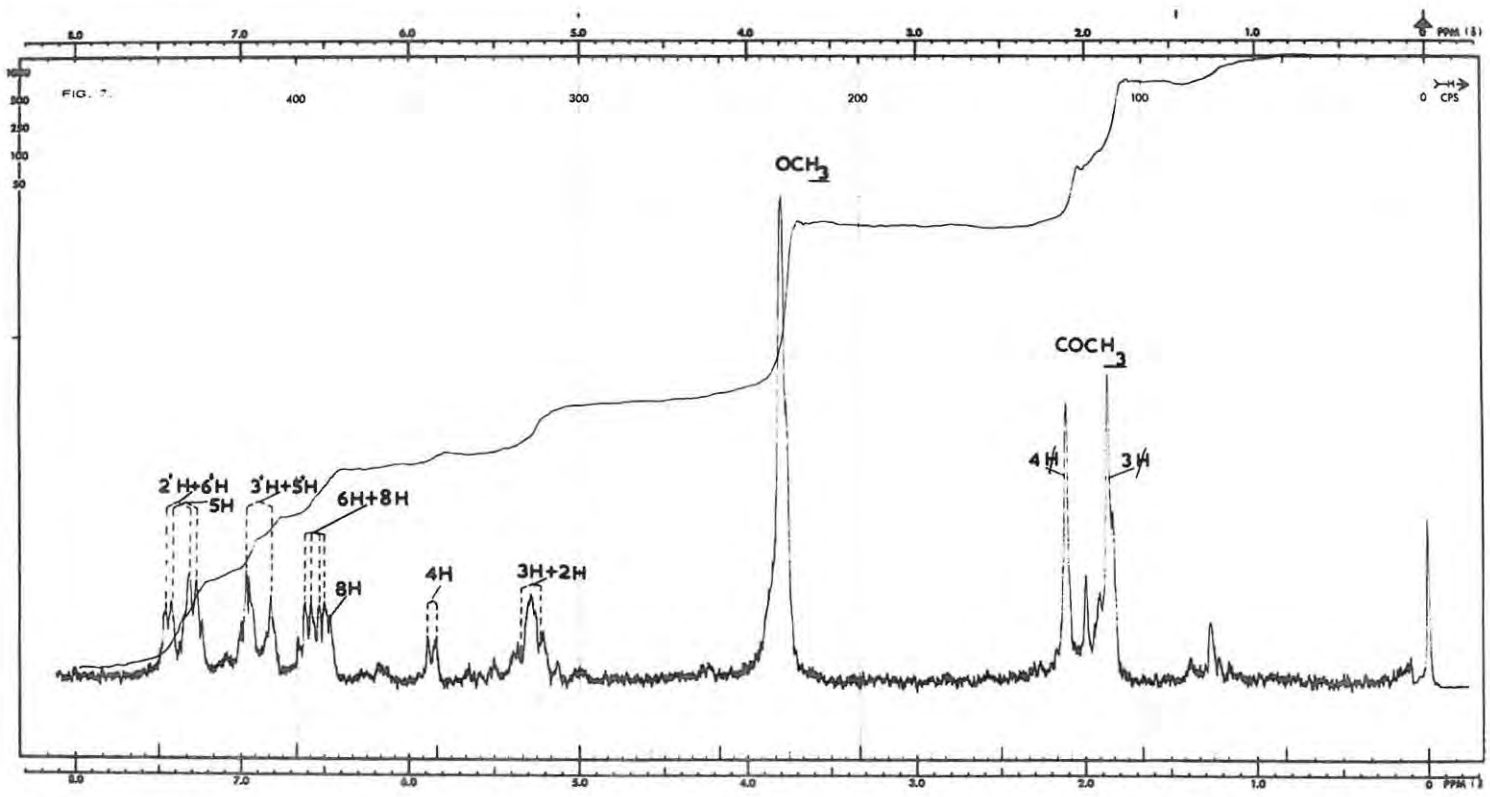
Paper Ionophoresis of the Dimethyl Ether of Guibourtacacidin.

Paper ionophoresis of methylated guibourtacacidin, the natural product extracted from the heartwood of Guibourtia coleosperma (Rhodesian copalwood), in sodium borate under the conditions developed by Drewes and Roux[56] showed a main band at -2.10 cm. and a weak band at +3.0 cm. These correspond to 2,3-cis-3,4-trans and/or 2,3-trans-3,4-trans (-2.10 cm.), and 2,3-trans-3,4-cis (+3.20 cm.) isomers (cf. Drewes and Roux[56]). A band corresponding to the 2,3-cis-3,4-cis isomer (+1.70 cm.) was absent.

Nuclear-magnetic-resonance Spectrum of the Diacetyl-dimethoxy derivative of Guibourtacacidin (Fig. 7).

The n.m.r. spectrum of the amorphous diacetyl dimethyl ether of guibourtacacidin from G. coleosperma (cf. Table 1) showed that this

FIG. 7. N.M.R. Spectrum of Dimethyl Ether Diacetate of (+)-Guibourtacacidin.



derivative of the flavan-3,4-diol has the predominantly 2,3-cis-3,4-trans arrangement of substituents.

When the spectrum was run at high amplitude, however, weak signals of the 2- and 4-protons of the 2,3-trans-3,4-trans and 2,3-trans-3,4-cis isomers were also observed. These were recognized from the signals of the corresponding protons of the pure racemates (cf. Table 1) and from their mixture. Signals from the 2- and 4-protons of the 2,3-cis-3,4-cis isomer were absent.

An examination of the integral curve of the signals from the 4-protons, which were well separated, indicated that the isomers are present in the proportions 2,3-cis-3,4-trans (5) : 2,3-trans-3,4-trans (1) : 2,3-trans-3,4-cis (1).

Nuclear-magnetic-resonance Spectrum of a $LiAlH_4$ Reduction Product of (±)-7,4'-Dimethoxydihydroflavonol.

A mixed diol, m.p. 113-121°, obtained by Phatak and Kulkarni[186] through reduction of the above dihydroflavonol with $LiAlH_4$, was resolved by them by acetylation and fractional crystallization of the diacetates. These diacetates were hydrolysed to give two diols, m.p. 114-115° (also obtainable by reduction with $NaBH_4$) and 136°.

The latter diol, kindly made available by Dr. A.B. Kulkarni, was acetylated as before, and its n.m.r. spectrum analysed (Fig. 5). A comparison of this spectrum with those of the 2,3-trans-3,4-trans and* 2,3-trans-3,4-cis diacetates (Table 1) showed that the product consists

of these two racemates in the ratio 11:9. This was shown by the signals of the well-separated 4-acetyl protons.

The Epimerization of (+)-7,3',4'-Trimethoxy-2,3-trans-flavan-3,4-trans-diol at C-4 by $NaBH_4$ + BF_3.

(+)-7,3',4'-Trimethoxy-2,3-trans-flavan-3,4-trans-diol.

(+)-Leucofisetinidin [(+)-7,3',4'-trihydroxy-2,3-trans-flavan-3,4-trans-diol] (2.085g.), m.p. 126°, from black wattle heartwood[193] was methylated with ethereal diazomethane for 36 hr. at -10°. The solution was evaporated under reduced pressure at 40° to a small volume (10 ml.) and a few drops of water were added when the (+)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-trans-diol crystallized as colourless needles. The compound was recrystallized from aqueous ethanol (1.5g.), m.p. 129-130°. (lit. m.p. 130° [193]).

Epimerization of (+)-7,3',4'-Trimethoxy-2,3-trans-flavan-3,4-trans-diol.

Ethereal boron trifluoride (1.5 ml., 45%) was added to a suspension of sodium boronhydride (273.6 mg.) in dry diglyme (20 ml.). A solution of (+)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-trans-diol (315 mg.) in dry diglyme (15 ml.) was then added with stirring to the suspension and the reaction carried out as for the epimerization of (+)-7,4'-dimethoxy-2,3-trans-flavan-3,4-trans-diol to the 2,3-trans-3,4-cis isomer. The residue, after reaction, was dissolved in the minimum volume of ethanol and the 2,3-trans-3,4-cis isomer crystallized after the addition of 2 drops of water. The material was recrystallized from aqueous ethanol to give colourless needles (156 mg.), m.p. 186-187°. A mixed m.p. with authentic (+)-7,3',4'-trimethoxy-2,3-trans-flavan-3,4-

cis-diol[86], kindly supplied by Dr. S.E. Drewes, was undepressed, m.p. 186-188°, $[\alpha]_D^{20}$ + 28.7° (tetrachloroethane ; $\overset{c}{\phi}$, 8.3) ; $[\alpha]_D^{20}$ + 47.2° [acetone: water (2.25 : 1.25) ; $\overset{c}{\phi}$, 4.9]. (Found: C, 64.6 ; H, 6.2 ; OCH_3, 27.9. Calculated for $C_{18}H_{20}O_6$: C, 65.0 ; H, 6.0 ; OCH_3 28.0%).

PART II.

ISOLATION, STRUCTURE AND STEREOCHEMISTRY OF BIFLAVANOLS B AND D FROM BLACK WATTLE (ACACIA LEARNSII) BARK.

Two-dimensional paper chromatography of enriched low molecular weight fractions of black wattle bark extract (cf. Fig. 8) on spraying with toluene-p-sulphonic acid reagent, show a number of components giving orange (B and D) or orange-pink (C and E) spots. These constituents are therefore considered to be leucoanthocyanidins[194]. From the positions occupied by B and D on these chromatograms, i,e, higher R_F values than the highly condensed bark tannins and lower R_F's than the monomeric components, these two products were originally assumed to be dimeric leucoanthocyanidins[195].

Isolation of B and D from Fresh Black Wattle Bark Extract.

Fresh black wattle bark (3 Kg.) from young trees (6-10 years old) was cut into small slivers with a stainless steel knife and extracted with ethyl acetate (10 l.) for 4 days. Ethyl acetate extraction selectively excludes a considerable percentage of the highly condensed bark tannins and therefore has a certain fractionating effect. Gums are absent in ethyl acetate extracts and the percentage of extracted sugars is less than in the case where alcohols are used. On the other hand, ethyl acetate is a good solvent for waxes and hence the extract has to be de-waxed prior to fractionation.

After decantation of the solution, the bark cuttings were re-extracted with fresh ethyl acetate (10 l.) for a further period of

5 days. The total extract (20 l.) was concentrated to dryness under reduced pressure in a rotary evaporator at 60°, yielding a buff-coloured solid (962g.), which was de-waxed by warming and shaking with isohexane in small portions (2 l. total).

The extract (100g.) was dissolved in the aqueous phase (1 l.) of an ethyl acetate : water (1:1, v./v.) mixture, and placed in the first of a series of 10 separating funnels each containing aqueous phase (1 l.) saturated with ethyl acetate. The extract was partitioned in the ethyl acetate-water system by countercurrent separation in which each funnel was shaken for 2 minutes and the phases allowed to separate for 10 minutes. After a 10-stage separation the contents of funnels 1 to 4 were concentrated to dryness, while for the remaining funnels (5 to 10) the aqueous phase was extracted with ethyl acetate (3 times), and this extract added to the organic phase for concentration to dryness under reduced pressure at 60-70°.

Typical yields of the various fractions from 100g. extract and the distribution of components are shown in Table 6 (cf. Fig. 8).

The manual countercurrent partition procedure was repeated six times to give approximately 200g. of enriched low molecular weight fraction from tubes 1 to 4, for further partitioning in the automatic Craig countercurrent machine using a different solvent system.

A mixture of sec-butanol : isohexane : water (4.5 : 0.5 : 5.0, by vol.) was shaken thoroughly to saturate the aqueous (lower) and organic (upper) phases. The 160-tube Craig machine was charged with 50 ml. lower phase per tube. The buff-coloured solids of the fractions 1 to 4

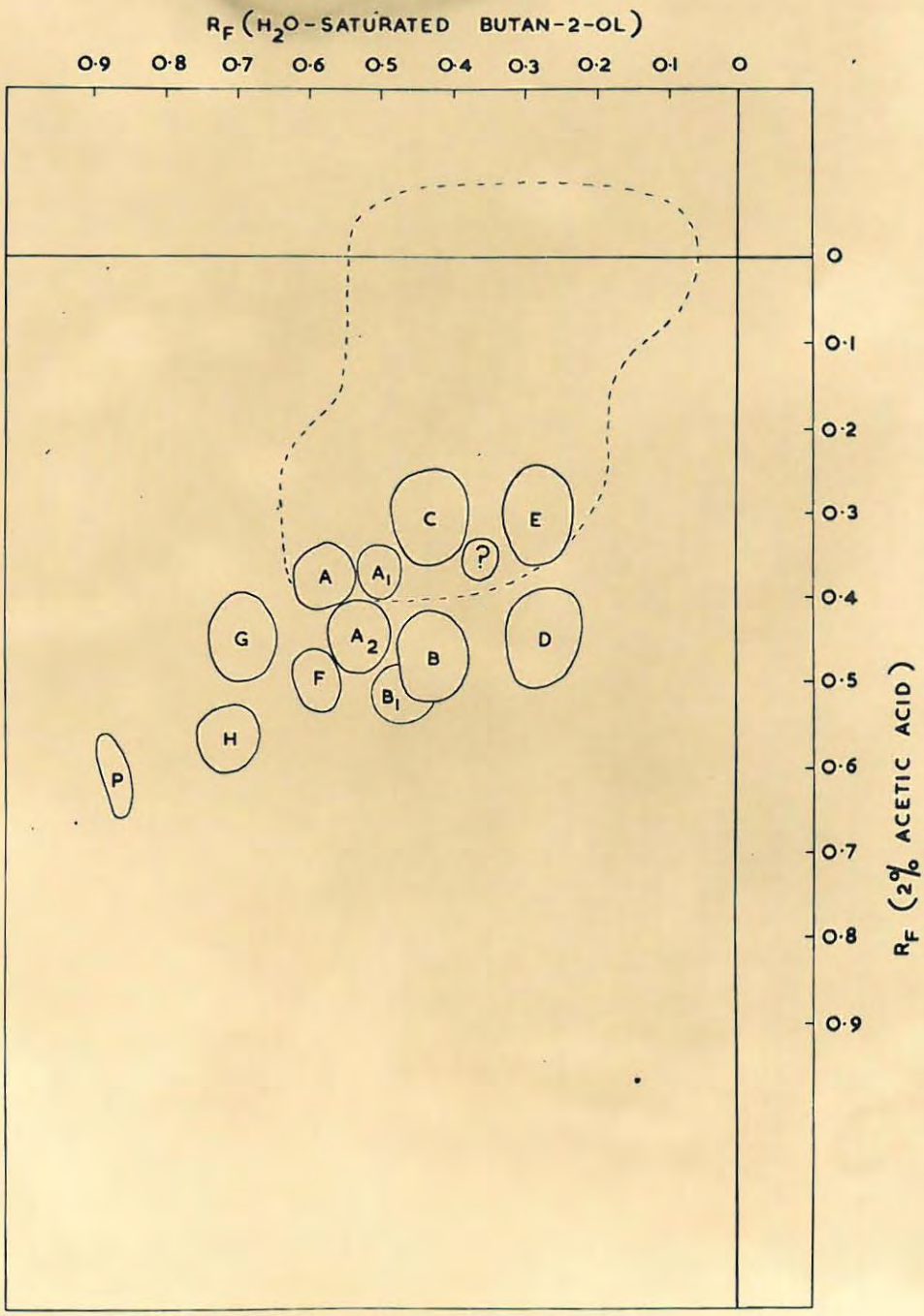


FIG. 8. Two-way Chromatogram of Fresh Black Wattle Bark Components.

(100g.) were dissolved in lower phase (400 ml.) and introduced into the first 8 tubes of the machine. After 150 transfers the upper and lower phases of every fifth tube were examined by two-dimensional chromatography on Whatman no. 1 paper in water saturated sec.-butanol, and then in 2% aqueous acetic acid. Chromatograms were run in duplicate, one being sprayed with ammoniacal $AgNO_3$, while bis-diazotized benzidine spray was used for the other.

TABLE 6.

The Distribution of Components and Yields of Various Fractions after a Manual Countercurrent Separation of Black Wattle Bark Extract.

Fraction	1	2	3	4	5	6	7	8	9	10
Yield (g.)	5.6	7.6	8.1	7.9	5.4	5.5	7.1	8.0	10.1	6.1
Low R_F tannins	+	+	+	+	+	+	x	x	x	-
A	-	-	-	-	-	+	+	+	+	+
A_1	-	-	-	-	-	+	+	+	+	x
A_2	-	-	x	x	+	+	+	+	x	x
B	x	x	+	+	+	+	x	x	-	-
C	-	-	x	+	+	+	+	x	x	-
D	+	+	+	+	x	x	-	-	-	-
E	+	+	+	+	+	x	x	x	-	-
F	-	-	-	-	-	+	+	+	x	-
G	-	-	-	-	x	x	+	+	+	x
H	-	-	-	-	-	x	+	+	+	x

(-) = absent, (+) = present in fair conc., (x) = present in low conc.
A_2 = (+)-Gallocatechin, G = (+)-Catechin, H = Robinetinidol.

The chromatograms, showing the distribution of components, were used to group the contents of the Craig tubes into several fractions (cf. Table 7).

TABLE 7.

Distribution of Components and Grouping of Craig Tube Contents.

Craig Tubes	Distribution of Components.
1-29	Low R_F tannins
30-40	D and E
41-70	D, B, E and C
71-80	E, B, C and A
81-90	B, C and A

The contents of the tubes were further grouped together to give 3 main fractions : I - tubes 30-45 (D predominant), II - tubes 46-64 (B and D in approximately equal concentrations) and III - tubes 65-75 (B predominant).

The fractions I to III were applied to Whatman no. 3 paper (250 mg. per sheet in 5 ml. ethanol), and developed with 2% aqueous acetic acid for 15 hr. Strips along the side were cut and sprayed with toluene-p-sulphonic acid. An orange band at R_F 0.44 (4.5 cm. wide), corresponding to components B and D, and an orange-pink band at R_F 0.34 (5 cm. wide), corresponding to constituents C and E, were located. The higher R_F band was cut, eluted with 70% aqueous ethanol and the eluates concentrated to dryness under reduced pressure at 60°. The fraction B + D (7g.) was light,

buff-coloured.

In order to separate components B and D, the fraction was applied to pre-washed sheets of Whatman no. 3 paper at concentrations of 60-70 mg. per sheet. The fraction was partitioned in water-saturated sec.-butanol for 24 hr., and after drying, side-strips were cut and sprayed with toluene-p-sulphonic acid. Bands at R_F 0.47 (4.7 cm. wide), due to B, and at R_F 0.32 (4.6 cm. wide), corresponding to D, were located. These were cut, eluted with 70% aqueous ethanol and the eluates concentrated to dryness under reduced pressure at 60°. Light-brown residues of relatively pure (by paper chromatography) B (2g.) and D (3g.) were obtained.

Neither B nor D crystallized and both tended to condense on handling in the free-phenolic forms. The products were accordingly stabilized by methylation prior to their purification by thin-layer chromatography.

Octamethyl ether of B.

Compound B (900 mg.) in methanol (200 ml.) was methylated with ethereal diazomethane, generated from nitrosomethylurea (25g.), at -10°. After 48 hr. the solution was concentrated to dryness under reduced pressure in a rotary evaporator at 50°. The yellow residue was examined by thin-layer chromatography on Kieselgel G (0.25 mm. thick) using chloroform : ethyl acetate (1:1, v./v.). The chromatoplate was sprayed with concentrated sulphuric acid : 40% formaldehyde (20:1, v./v.) and heated at 120° for 5-10 minutes. Three components at R_F values of 0.70, 0.36 and 0.21 were shown. Approximately 30% of residue remained at the origin and an extensive trail at low R_F was observed. The main component

was located at R_F 0.36, and showed a brick-red spot having slight red-brown fluorescence under ultraviolet light.

The crude methylated B in ethyl acetate : ethanol (1:2, v./v.) (30 ml.) was applied to 30 chromatoplates (30 mg./plate) using Kieselgel G (1 mm. thick) and chloroform : ethyl acetate (1:1, v./v.). The main band was located by spraying a side-strip on the plate with the sulphuric acid-formaldehyde reagent. The band was stripped mechanically and the product eluted with ethyl acetate : ethanol (1:2, v./v.). The solution was concentrated under reduced pressure at 50° to give a light buff-coloured precipitate. This was taken up in ethanol and precipitated with water (0.05 ml.). Further precipitation gave a light-cream powder (212 mg.). The compound settled over the range 130-135°, after sintering at 126°. $[\alpha]_D^{19}$ - 98.8° (c, 1.84 in acetone). (Found: C, 65.3 ; H, 6.3 ; OCH_3, 35.2. $C_{38}H_{42}O_{12}$ requires : C, 66.1 ; H, 6.1 ; OCH_3, 36.0%).

Diacetyl-octamethyl ether of B.

The octamethyl ether of B (52.7 mg.) was dissolved in the minimum of pyridine (0.3 ml.) and acetic anhydride (0.3 ml.) added. The mixture was left overnight at room temperature, and then poured into water. The product was recovered to give an off-white precipitate. Several re-precipitations with water from ethanolic solutions gave a white amorphous powder (43 mg.), settling at 120-125°. $[\alpha]_D^{19}$ - 71.9° (c, 0.76 in acetone). (Found: C, 64.8 ; H, 6.1 ; OCH_3, 31.6 ; $CO.CH_3$, 10.0. $C_{42}H_{\cancel{64}\,46}O_{14}$ requires : C, 65.1 ; H, 6.0 ; OCH_3, 32.1 ; $CO.CH_3$, 10.9%). The molecular weight by mass spectrometry was 774.

Deca-acetate of B.

Component B (110.5 mg.) was acetylated with acetic anhydride (0.6 ml.) and a minimum (0.4 ml.) of pyridine. The product was recovered from water as above, and purified by thin-layer chromatography on Kieselgel G (1 mm. thick) using benzene : acetone (7:1, v./v.). The product was eluted with acetone and precipitated with water from ethanol : acetone (9:1, v./v.). A white amorphous powder (83 mg.) which sintered at 168° and settled over the range 178-185° was obtained. $[\alpha]_D^{19}$ - 51.3° (c, 1.53 in acetone). (Found: C, 60.0 ; H, 4.7 ; $CO.CH_3$, 41.8. $C_{50}H_{46}O_{22}$ requires : C, 60.1 ; H, 4.6 ; $CO.CH_3$, 42.1%).

Nonamethyl ether of D.

Compound D (1g.) was methylated with ethereal diazomethane as above to give a light-yellow precipitate. This was examined by thin-layer chromatography on Kieselgel G (0.25 mm. thick) using chloroform : ethyl acetate (1:1, v./v.). Concentrated sulphuric acid : 40% formaldehyde (20:1, v./v.), followed by heating at 120° for 10 minutes, was used for locating the product. The spray reagent showed a major product (brick-red) at R_F 0.49 with extensive trailing at lower R_F and an appreciable residue at the origin.

The crude methylated D was dissolved in ethyl acetate : ethanol (1:2, v./v.) (32 ml.) and run on 32 Kieselgel G (1 mm. thick) plates using the same solvent as above. The main band was located with the sulphuric acid spray as before, and stripped off. Ethyl acetate and ethanol were used to elute the product. The solution was concentrated to dryness under reduced pressure at 50°, giving a light-yellow precipitate.

This was dissolved in the minimum ethanol, cooled to 0° and 2 drops of water added when a white flaky precipitate appeared. Several such precipitations yielded an amorphous cream-coloured product (340 mg.) which sintered at 140° and settled at 161°. $[\alpha]_D^{19}$ - 86.0° (c, 2.08 in acetone). (Found: C, 62.5 ; H, 6.1 ; OCH_3, 37.0. $C_{39}H_{44}O_{13}$ requires: C, 62.9 ; H, 6.0 ; OCH_3, 37.5%).

Diacetyl-nonamethyl ether of D.

The nonamethyl ether of D (51.4 mg.) was dissolved in a minimum of pyridine (0.3 ml.) and acetic anhydride (0.3 ml.) added. The mixture was left overnight at room temperature and the product recovered from water as an off-white precipitate. Several re-precipitations from aqueous ethanol resulted in a light cream-coloured powder (39 mg.) which settled at 140°. $[\alpha]_D^{19}$ - 65.7° (c, 2.4 in acetone). (Found: C, 63.9 ; H, 6.1 ; OCH_3, 35.6 ; $CO.CH_3$, 9.8. $C_{43}H_{48}O_{15}$ requires: C, 64.2 ; H, 6.0 ; OCH_3, 34.7 ; $CO.CH_3$, 10.5%). The molecular weight, determined by mass spectrometry, was 804.

Undeca-acetate of D.

Compound D (106.5 mg.) was acetylated with acetic anhydride (0.5 ml.) in the minimum of pyridine (0.4 ml.). The product was recovered from water as before, and purified by precipitation with water from ethanol : acetone (9:1, v./v.). A white, amorphous powder (91 mg.), sintering at 130° and settling at 148° was obtained. $[\alpha]_D^{19}$ - 42.7° (c, 1.96 in acetone). (Found: C, 58.7 ; H, 4.8 ; $CO.CH_3$, 42.2. $C_{52}H_{48}O_{24}$ requires: C, 59.2 ; H, 4.6 ; $CO.CH_3$, 43.8%).

Qualitative Paper Ionophoresis of B and D.

The apparatus was of the open-strip, horizontal type[192] in which the paper was allowed to dip at both ends into cells containing borate buffer [sodium borate : boric acid : water (63:15:5, w./w./v.)][196]. The atmosphere was maintained near saturation by enclosure of the apparatus with a "Perspex" lid. The paper [Sleicher and Schull brand no. 2043 (4x41 cm.)] was wetted with buffer and equilibrated in position for 1 hr. before use. The free-phenolic compound was applied as a streak on the origin, 17 cm. from the one end of the paper. A "Vokam" power supply (Shandon Co.) was used to apply a potential of 200 volts across the electrodes, giving a current of 5 m A. The ionophoretic development lasted 4 hr. The strips were dried at 50° in an oven and heavily sprayed with toluene-p-sulphonic acid to liberate the product from the borate complex. Ammoniacal silver nitrate proved a more satisfactory spray reagent, and was only reduced during the washing of the papers after the excess buffer had been removed. The ionophoretic mobilities of the free-phenolic forms of B and D are shown in Table 8.

TABLE 8.

Ionophoretic Mobilities of Free-phenolic Forms of B and D.

Product	Ionophoretic Mobility (cm.)	Width of band (cm.)
B	+6.0	2.9
	+8.4*	1.9
D	+6.0	1.7
	+8.4*	3.0

*Minor impurities.

Preparative Paper Ionophoretic Purification of B and D.

For the preparative paper ionophoresis of the free-phenolic forms of B and D pre-washed sheets of Whatman no. 3 paper in the same buffer as above was used. A larger "Perspex" cabinet (45 x 52 cm.), with proportionately larger cells at each end, was used to accommodate the paper (21 x 18 cm.). A potential of 100 volts was applied across the paper to give a current of 10 m.A. for 15 hr. The compound (30 mg.) was streaked on to each sheet, which had previously been wetted with borate buffer and equilibrated in position for 1 hr. before the start. After completion of the ionophoretic run the sheets were dried in a warm current of air and side-strips cut and sprayed with silver nitrate. Bands, having mobilities 9.6 cm. (3.5 cm. wide) for B and 7.5 cm. (3.0 cm. wide) for D, were cut and eluted with water for 24 hr. The solutions were concentrated to dryness under vacuum in a desiccator over calcium chloride, giving light, buff-coloured precipitates. The products, after liberation from the borate complexes, were shown to be pure by two-dimensional paper chromatography in water-saturated sec.-butanol and 2% aqueous acetic acid, on spraying with toluene-p-sulphonic acid or ammoniacal silver nitrate.

Alkali Fusions of B and D.

Components B and D, purified by paper ionophoresis, were fused with alkali[197]. The compound (10 mg.) was placed in a hard glass test tube (15 x 0.7 cm.) and tapped to the base of the tube. Holding the tube horizontally, 3 pellets of solid KOH were introduced and pushed to a position 2 cm. from the base of the tube. The pellets were melted in a small flame and the tube tilted upright so that the molten KOH fused with

the compound. The molten mixture was gently heated, without boiling, for 1.5 minutes, and then rapidly cooled in a strong current of cool air. The contents of the tube were acidified with sulphuric acid (6N) and the solution treated with solid sodium bicarbonate until just alkaline to litmus. The phenolic fraction was extracted with ether (6 times), and after acidification with 6N sulphuric acid the acidic fraction was also extracted with ether (6 times). Both ethereal extracts were dried over anhydrous sodium sulphate for 2 hr. and evaporated to dryness. The residues were examined by one-dimensional paper chromatography (Whatman no. 1) with reference phenols and acids. The chomatograms were developed with n-butanol : acetic acid : water (6:1:2, by vol.) for 16 hr. Chromatograms of the phenolic fractions were sprayed with bis-diazotized benzidine reagent, while those of the acidic fractions were sprayed with silver nitrate and ferric alum reagents (cf. Table 9).

Generation of Anthocyanidin Pigments from B and D.

Anthocyanidin pigments[198] were generated from products B and D after their purification by paper ionophoresis. The product (4 mg.) was dissolved in a mixture of 3N hydrochloric acid : isopropanol (1:4, v./v.) (2.5 ml.) and heated in a pressure vessel in a water-bath for 1 hr. The resultant colour of the solutions were brownish-red instead of the usual bright red or pink-red of anthocyanidins. The solutions were spotted on Whatman no. 1 paper and developed in 90% formic acid : 3N HCl (1:1, v./v.)[194] mixture for 2-3 hr., with solutions of anthocyanidins generated from reference compounds. Product B yielded an orange pigment (R_F 0.66), an impurity (R_F 0.46) and robinetinidin chloride (R_F 0.30), while component D only gave the orange pigment (R_F 0.65) and robinetinidin chloride (R_F 0.33).

TABLE 9.

Fission Products Formed by Microfusion of the Free-phenolic Forms of B and D with Alkali.

Product	Fraction	Spray Reagent and Colours produced		
		$AgNO_3$	Ferric Alum	Bis-diazotized benzidine
B	Acidic	Protocatechuic acid (grey) $R_F = 0.74$ Gallic acid (brown) $R_F = 0.62$	β-resorcylic acid (brick-red) $R_F = 0.83$ Protocatechuic acid (olive-green) Gallic acid (blue-grey)*	
	Phenolic			Resorcinol (amber) $R_F = 0.84$ Phloroglucinol (violet) $R_F = 0.72$
D	Acidic	Gallic acid (brown)	β-resorcylic acid (brick-red) Gallic acid (blue-grey)	
	Phenolic			Resorcinol (amber) Phloroglucinol (violet)

*Low concentration. R_F values refer to reference compounds.

Generation of Anthocyanidin Pigments from the Methyl ethers of B and D.

The octamethyl ether of B and nonamethyl ether of D, previously purified by thin-layer chromatography, were used for generating anthocyanidin pigments as above. The resultant pigments were barely discernable, but clearly visible as fluorescent spots under ultraviolet light. The tetramethyl ether of (+)-leucorobinetinidin (from Robinia pseudacacia[62]) was used as a reference compound for the generation of pigments. The octamethyl ether of B gave two pigments; R_F 0.43 (golden-

yellow fluorescence) and R_F 0.86 (brick-red fluorescence), while the nonamethyl ether of D showed similar pigments at R_F 0.40 and R_F 0.84.

Acid-induced Fission of Products B and D to give Catechin Residues.

Samples of B and D, purified by paper electrophoresis, were used for acid hydrolysis. The compound (5 mg.) was dissolved in ethanol (1 ml.) and 3N hydrochloric acid (1 ml.) added. The solutions were kept in a test tube in a water-bath at 90° and the volume was kept constant by addition of aqueous ethanol. Aliquots were extracted at intervals of 5, 15 and 30 minutes and spotted on two-dimensional Whatman no. 1 papers which were developed in water-saturated sec.-butanol and then in 2% aqueous acetic acid. (+)-Catechin and (+)-gallocatechin were run as reference compounds. Results are summarized in Table 10.

TABLE 10.

Acid-induced Fission Products of B and D.

Product	Time (min.)	Fission Products.
B	5	Unchanged B, (+)-catechin*, orange pigment*, phloroglucinol, resorcinol and 3 unknown components.
	15	Unchanged B, condensed product and 4 unknown components.
	30	Condensed product* and 2 unknown components.
D	5	Unchanged D, (+)-gallocatechin*, orange pigment*, phloroglucinol, resorcinol and 2 unknown components.
	15	Unchanged D and condensed product.
	30	Condensed product*.

*Present in high concentration.

Acid-induced Condensation of (+)-Leucorobinetinidin with (+)-Catechin and (+)-Gallocatechin.

In order to elucidate the structures of the biflavonols B and D, the acid-induced condensation products of (+)-leucorobinetinidin with (+)-catechin and (+)-gallocatechin were examined by paper chromatography, using reference compounds.

(+)-Leucorobinetinidin was obtained by reduction of (+)-dihydrorobinetin isolated from Robinia pseudacacia heartwood[62], (+)-catechin was extracted from Gambier catechu leaves[199] and (+)-gallocatechin obtained from golden wattle (Acacia pycnantha) bark[200].

(+)-Leucorobinetinidin (1 mg.) and (+)-catechin (1 mg.) were dissolved in 0.4N HCl (1 ml.), warmed to 60-70° for 5 minutes and left at room temperature for a further 15 minutes. The solution was made alkaline (litmus) with triethylamine in chloroform (1:10, v./v.). The aqueous phase was spotted on two-dimensional chromatograms and developed in water-saturated sec.-butanol and then 2% aqueous acetic acid. Silver nitrate spray showed five spots, two of which, at R_F's (0.57, 0.53) and (0.67, 0.38), corresponded to the starting materials (+)-leucorobinetinidin and (+)-catechin, respectively. A component (R_F 0.32, 0.36) appeared identical with B, while the remaining two spots were in low concentration at R_F's (0.47, 0.54) and (0.51, 0.36) (cf. Fig. 9).

Similarly, (+)-leucorobinetinidin and (+)-gallocatechin were condensed to give a compound identical by chromatography with D (R_F 0.15, 0.33). The (+)-gallocatechin residue was detected (R_F 0.40, 0.30), but no residual (+)-leucorobinetinidin showed. An additional spot (R_F 0.23,

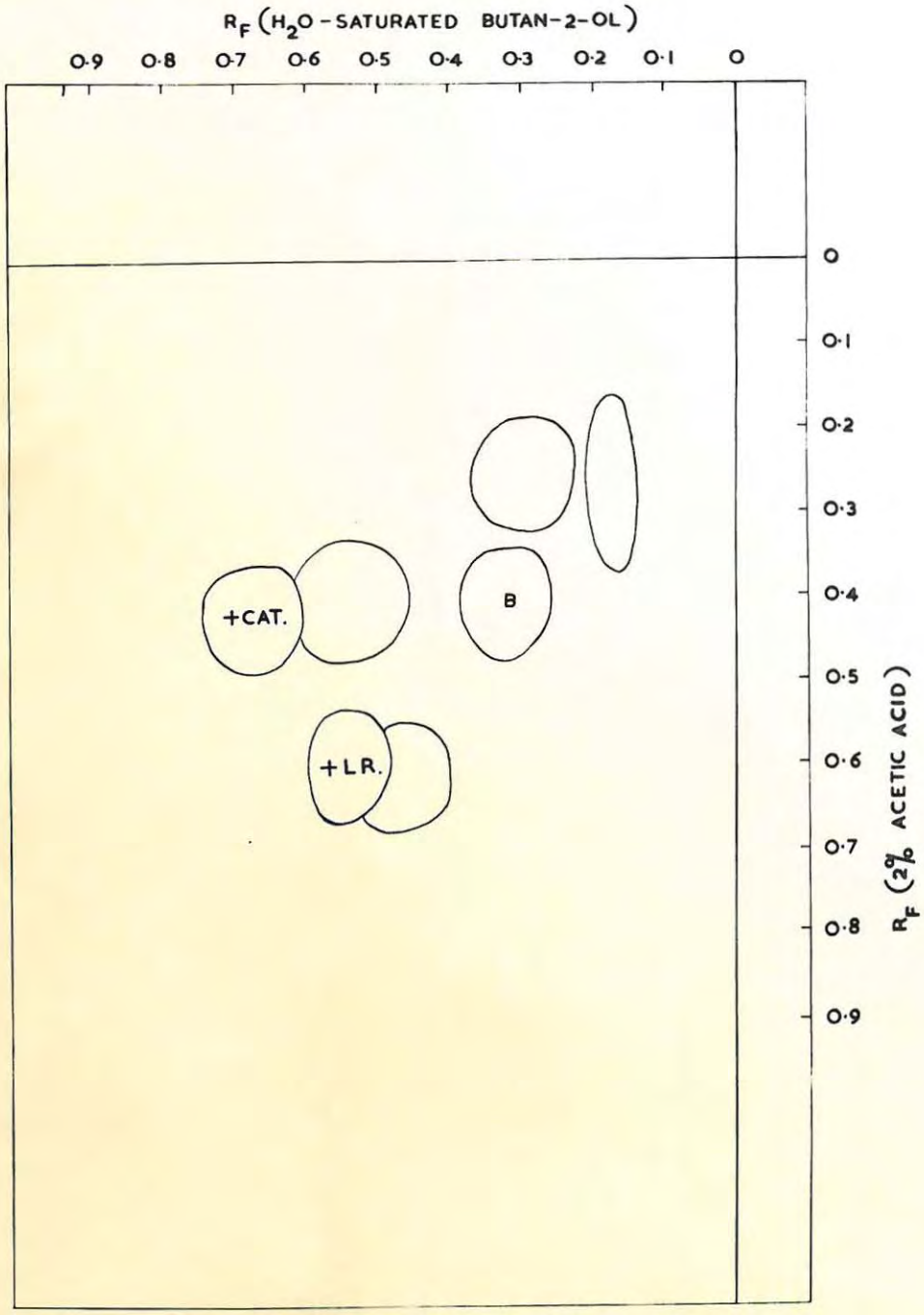


FIG. 9. Two-way Chromatogram of Acid-induced Condensation Products of (+)-Leucorobinetinidin and (+)-Catechin.

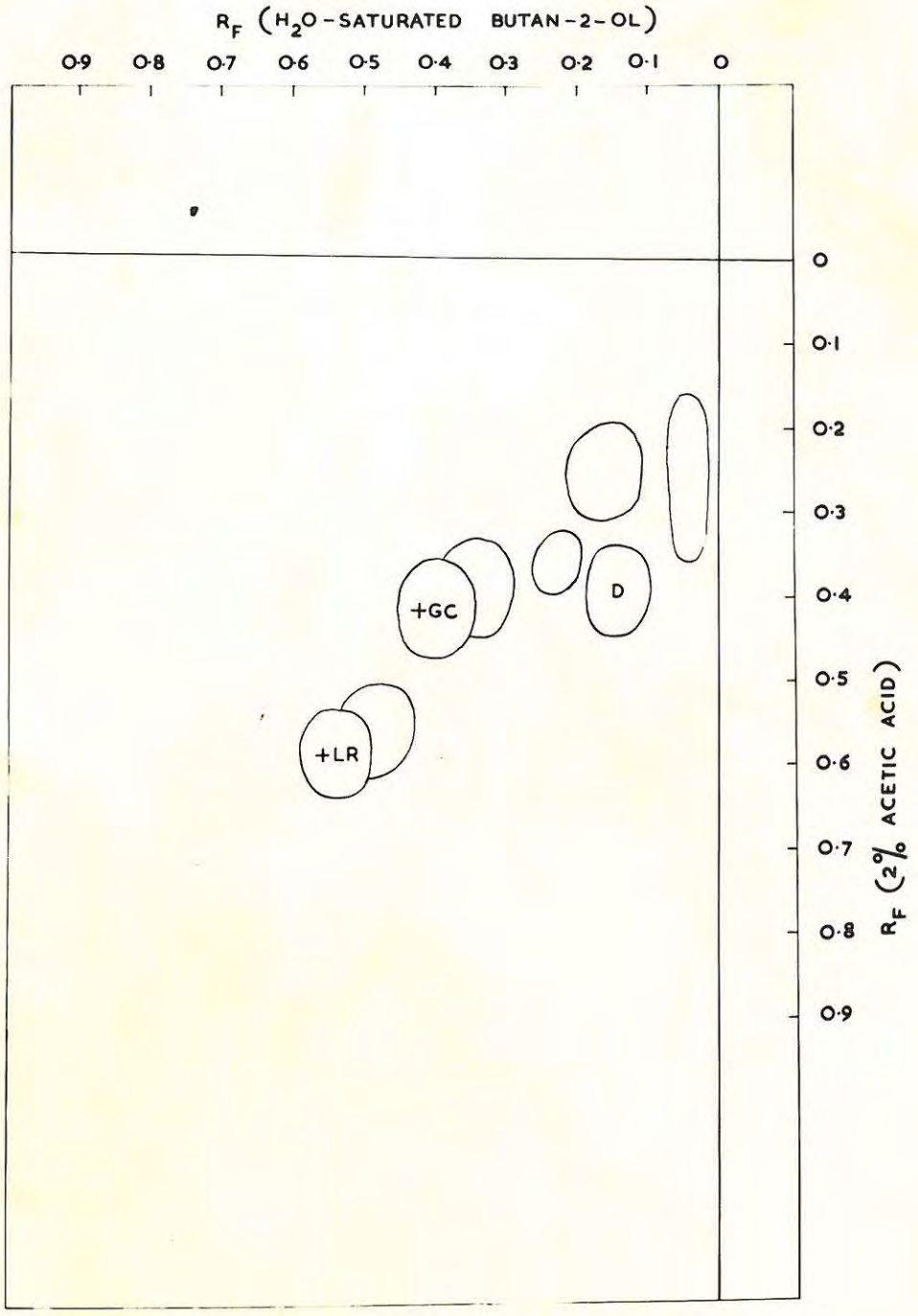


FIG. 10. Two-way Chromatogram of Acid-induced Condensation Products of (+)-Leucorobinetinidin and (+)-Gallocatechin.

0.32) was found in very low concentration (cf. Fig. 1C).

These acid-induced condensations showed that the biflavanols B and D could be formed by the condensation of (+)-leucorobinetinidin with (+)-catechin and (+)-gallocatechin, respectively.

Infrared Spectra of the Octamethyl ether of B and Nonamethyl ether of D.

The infrared spectra of the methyl ethers of B and D were determined in chloroform (c, 0.05) using a "Beckman I R-8" spectrophotometer. For purposes of comparison the spectra of (+)-leucorobinetinidin tetramethyl ether (c, 0.025) and (-)-robinetinidol tetramethyl ether (c, 0.05) were also determined in the same solvent.

The I.R. spectra of the compounds showed the following characteristics:

Octamethyl ether of B (cf. Fig. 11).

3700 $cm.^{-1}$ (weak), 3600 $cm.^{-1}$ (medium), 3000 $cm.^{-1}$ (medium), 2940 $cm.^{-1}$ (medium), 2650 $cm.^{-1}$ (strong), 1700 $cm.^{-1}$ (inflection), 1590 $cm.^{-1}$ (strong), 1500 $cm.^{-1}$ (strong), 1460 $cm.^{-1}$ (strong), 1425 $cm.^{-1}$ (medium), 1350 $cm.^{-1}$ (inflection), 1320 $cm.^{-1}$ (medium), 1260 $cm.^{-1}$ (inflection), 1220 $cm.^{-1}$ (weak), 1160 $cm.^{-1}$ (inflection), 1130 $cm.^{-1}$ (medium), 1070 $cm.^{-1}$ (inflection), 1035 $cm.^{-1}$ (weak), 1000 $cm.^{-1}$ (inflection) and 830 $cm.^{-1}$ (weak).

Nonamethyl ether of D. (cf. Fig. 12).

3700 $cm.^{-1}$ (weak), 3600 $cm.^{-1}$ (medium), 3000 $cm.^{-1}$ (medium), 2940 $cm.^{-1}$ (strong), 2650 $cm.^{-1}$ (strong), 1700 $cm.^{-1}$ (inflection), 1590 $cm.^{-1}$ (strong), 1500 $cm.^{-1}$ (strong), 1460 $cm.^{-1}$ (strong), 1425 $cm.^{-1}$

FIG. 11. Infrared Spectrum of Octamethyl Ether of B.

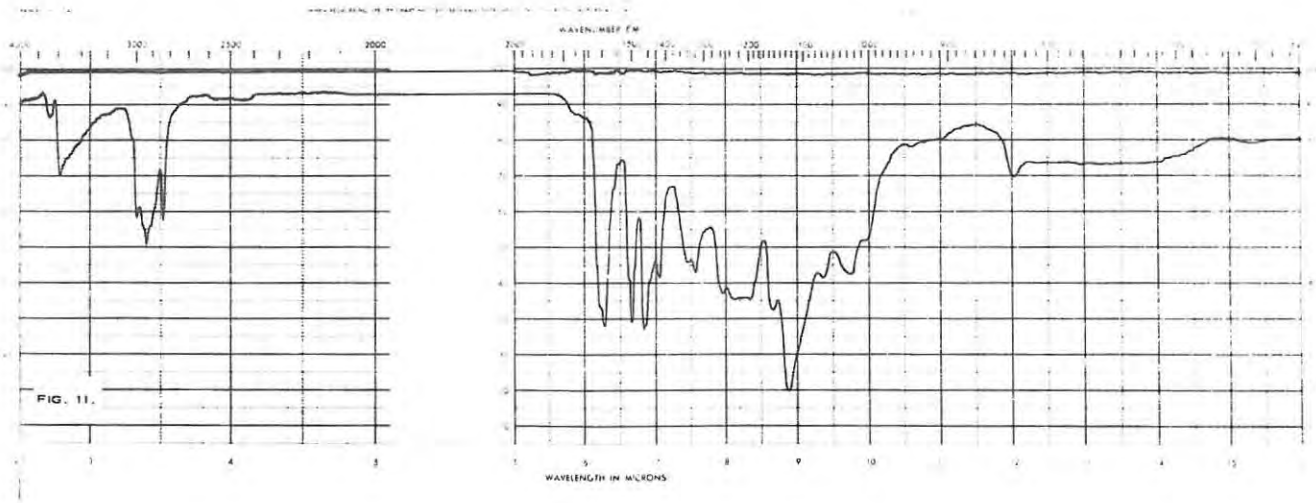
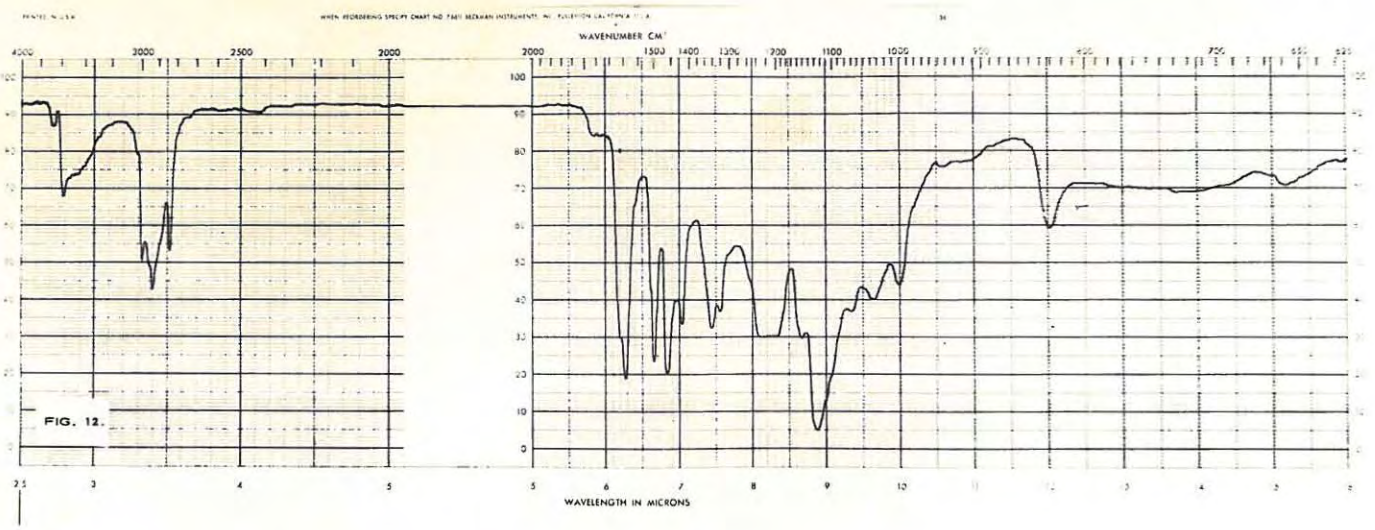


FIG. 12. Infrared Spectrum of Nonamethyl Ether of D.



(medium), 1350 cm.$^{-1}$ (medium), 1320 cm.$^{-1}$ (medium), 1220 cm.$^{-1}$ (weak), 1160 cm.$^{-1}$ (inflection), 1130 cm.$^{-1}$ (medium), 1070 cm.$^{-1}$ (inflection), 1035 cm.$^{-1}$ (weak), 1000 cm.$^{-1}$ (medium) and 830 cm.$^{-1}$ (weak).

(-)-Robinetinidol tetramethyl ether (cf. Fig. 13).

3700 cm.$^{-1}$ (weak), 3600 cm.$^{-1}$ (medium), 3000 cm.$^{-1}$ (medium), 2940 cm.$^{-1}$ (strong), 2650 cm.$^{-1}$ (strong), 1650 cm.$^{-1}$ (strong), 1590 cm.$^{-1}$ (strong), 1500 cm.$^{-1}$ (strong), 1460 cm.$^{-1}$ (strong), 1450 cm.$^{-1}$ (inflection), 1425 cm.$^{-1}$ (strong), 1350 cm.$^{-1}$ (medium), 1290 cm.$^{-1}$ (inflection), 1265 cm.$^{-1}$ (strong), 1220 cm.$^{-1}$ (weak), 1160 cm.$^{-1}$ (strong), 1120 cm.$^{-1}$ (strong), 1110 cm.$^{-1}$ (inflection), 1035 cm.$^{-1}$ (medium), 1000 cm.$^{-1}$ (medium), 945 cm.$^{-1}$ (weak) and 830 cm.$^{-1}$ (medium).

(+)-Leucorobinetinidin tetramethyl ether (cf. Fig. 14).

3700 cm.$^{-1}$ (weak), 3600 cm.$^{-1}$ (medium), 3000 cm.$^{-1}$ (weak), 2900 cm.$^{-1}$ (weak), 2650 cm.$^{-1}$ (weak), 1625 cm.$^{-1}$ (strong), 1590 cm.$^{-1}$ (strong), 1500 cm.$^{-1}$ (strong), 1460 cm.$^{-1}$ (strong), 1450 cm.$^{-1}$ (inflection), 1425 cm.$^{-1}$ (weak), 1325 cm.$^{-1}$ (medium), 1200 cm.$^{-1}$ (weak), 1160 cm.$^{-1}$ (strong), 1130 cm.$^{-1}$ (strong), 1040 cm.$^{-1}$ (weak), 1000 cm.$^{-1}$ (inflection) and 830 cm.$^{-1}$ (medium).

The more prominent peaks in the above spectra may be ascribed to: 3600 cm.$^{-1}$ (free O-H), 3000 cm.$^{-1}$ (CH=CH stretching), 2940 cm.$^{-1}$ (C-H stretching), 1700 cm.$^{-1}$ (C-O stretching), 1590 cm.$^{-1}$ (C=C, conjugated stretching), 1425 cm.$^{-1}$ (aromatic stretching), 1350 cm.$^{-1}$ (OH deformation), 1260-1160 cm.$^{-1}$ (aromatic substitution), 1070 and 1035 cm.$^{-1}$ (aromatic substitution) and 830 cm.$^{-1}$ (R-CHO stretching).

Stretching frequencies due to C=O were absent.

FIG. 13. Infrared Spectrum of (-)-Robinetinidol Tetramethyl Ether.

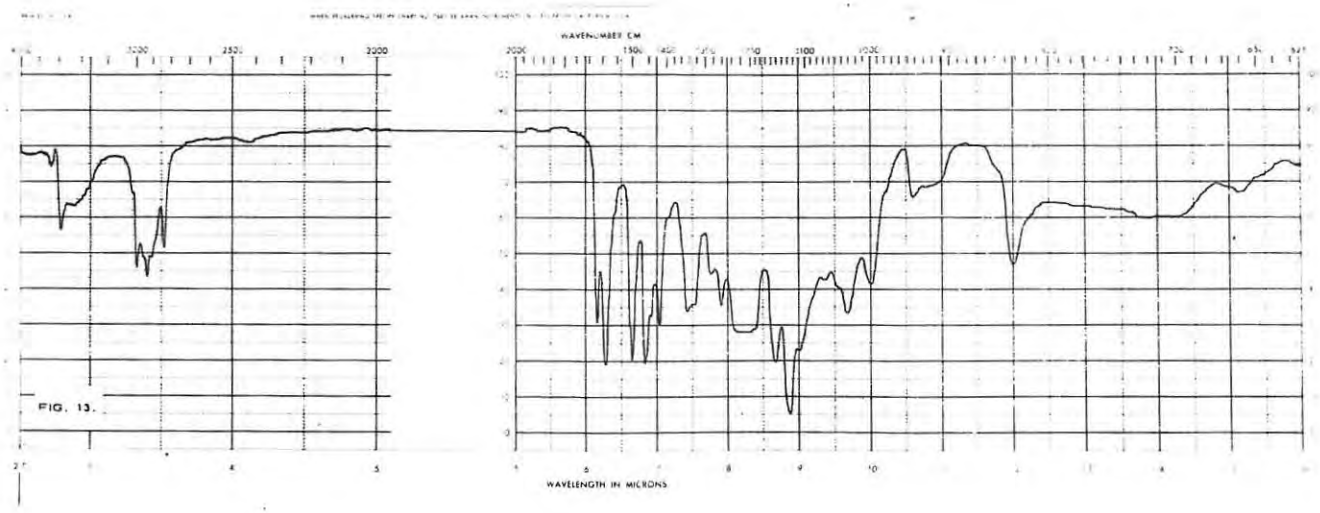
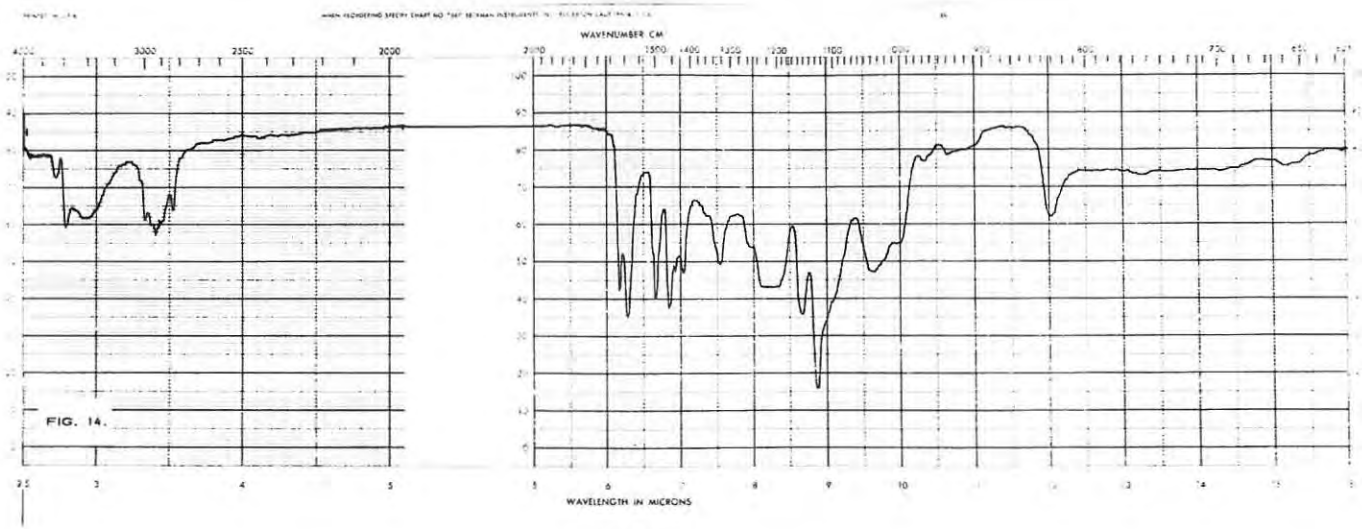


FIG. 14. Infrared Spectrum of (+)-Leucorobinetinidin Tetramethyl Ether.

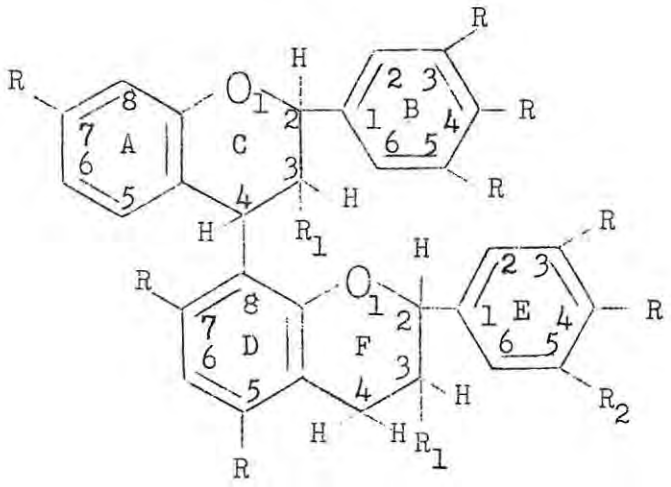


Nuclear-magnetic-resonance Spectra of Derivatives of B and D.

N.m.r. spectra of derivatives of B and D (cf. Figs. 15-20) were determined at 100 Mc./sec. in deuterochloroform on a Varians high-resolution HA-100 spectrometer using trimethylsilane as internal standard. Spin-decoupling experiments were performed by Dr. J.A. Feeney, Varians Associates, Walton-on-Thames, Surrey, England.

Chemical shifts and coupling constants of the derivatives of B and D are listed in Table 11.

Derivatives of B and D are given by structure I.



(I)

BMe_8 : $R=OCH_3$, $R_1=OH$, $R_2=H$.

BMe_8Ac_2 : $R=OCH_3$, $R_1=OCOCH_3$, $R_2=H$.

BAc_{10} : $R=R_1=OCOCH_3$, $R_2=H$.

DMe_9 : $R=R_2=OCH_3$, $R_1=OH$

DMe_9Ac_2 : $R=R_2=OCH_3$, $R_1=OCOCH_3$

DAc_{11} : $R=R_1=R_2=OCOCH_3$

FIG. 15. N.M.R. Spectrum of Octamethyl Ether of B.

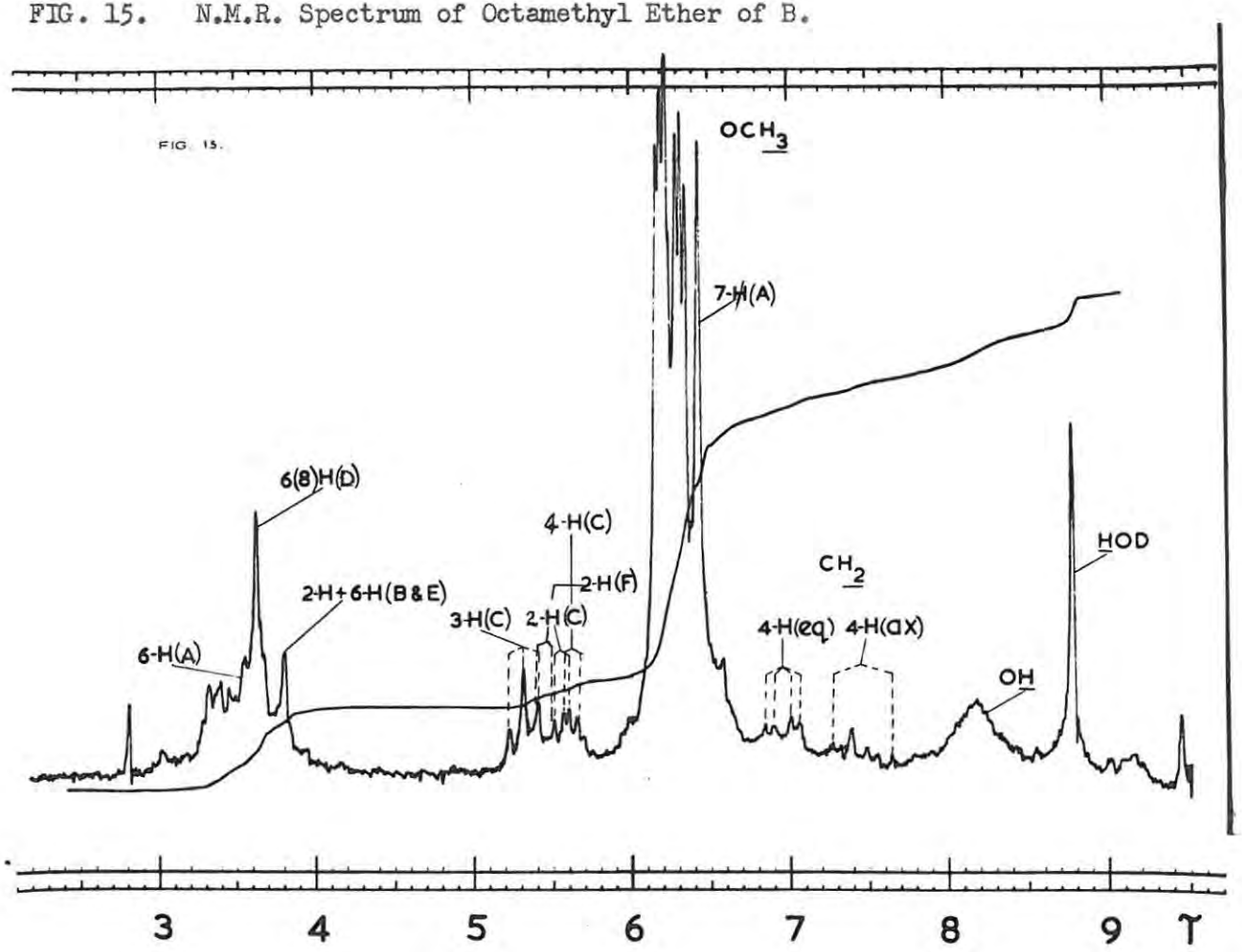


FIG. 16. N.M.R. Spectrum of Nonamethyl Ether of D.

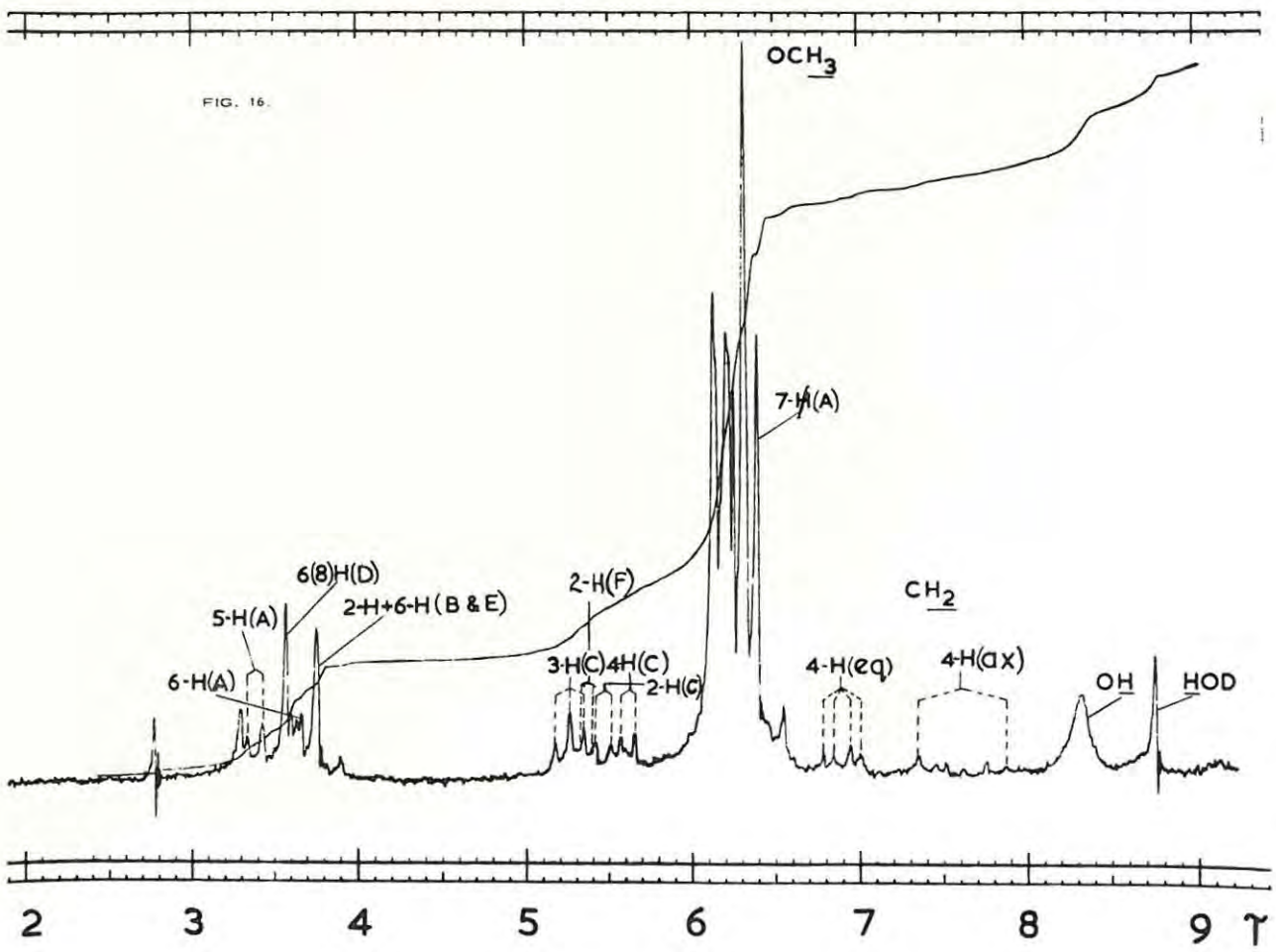


FIG. 17. N.M.R. Spectrum of Octamethyl Ether Diacetate of B.

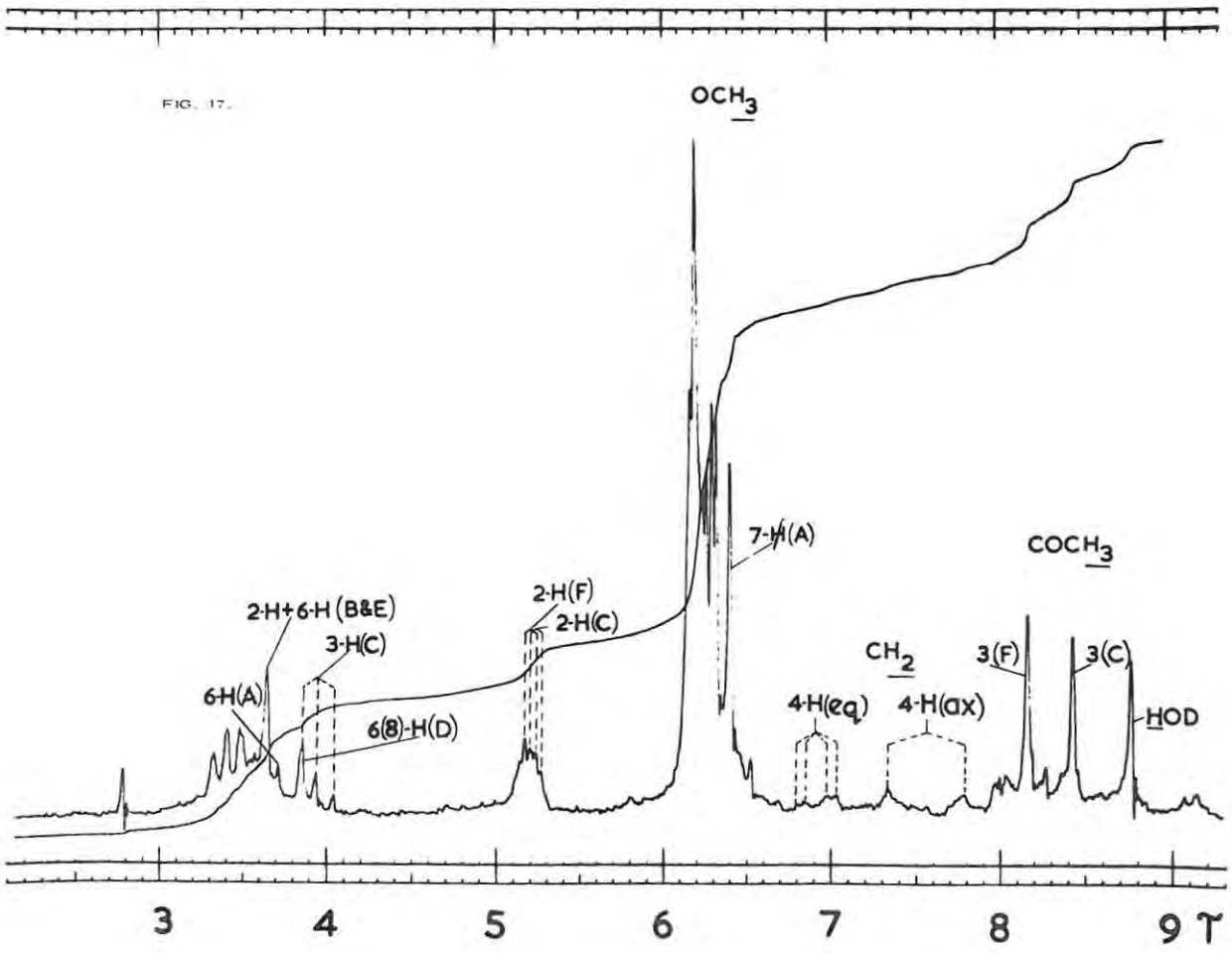


FIG. 18. N.M.R. Spectrum of Nonamethyl Ether Diacetate of D.

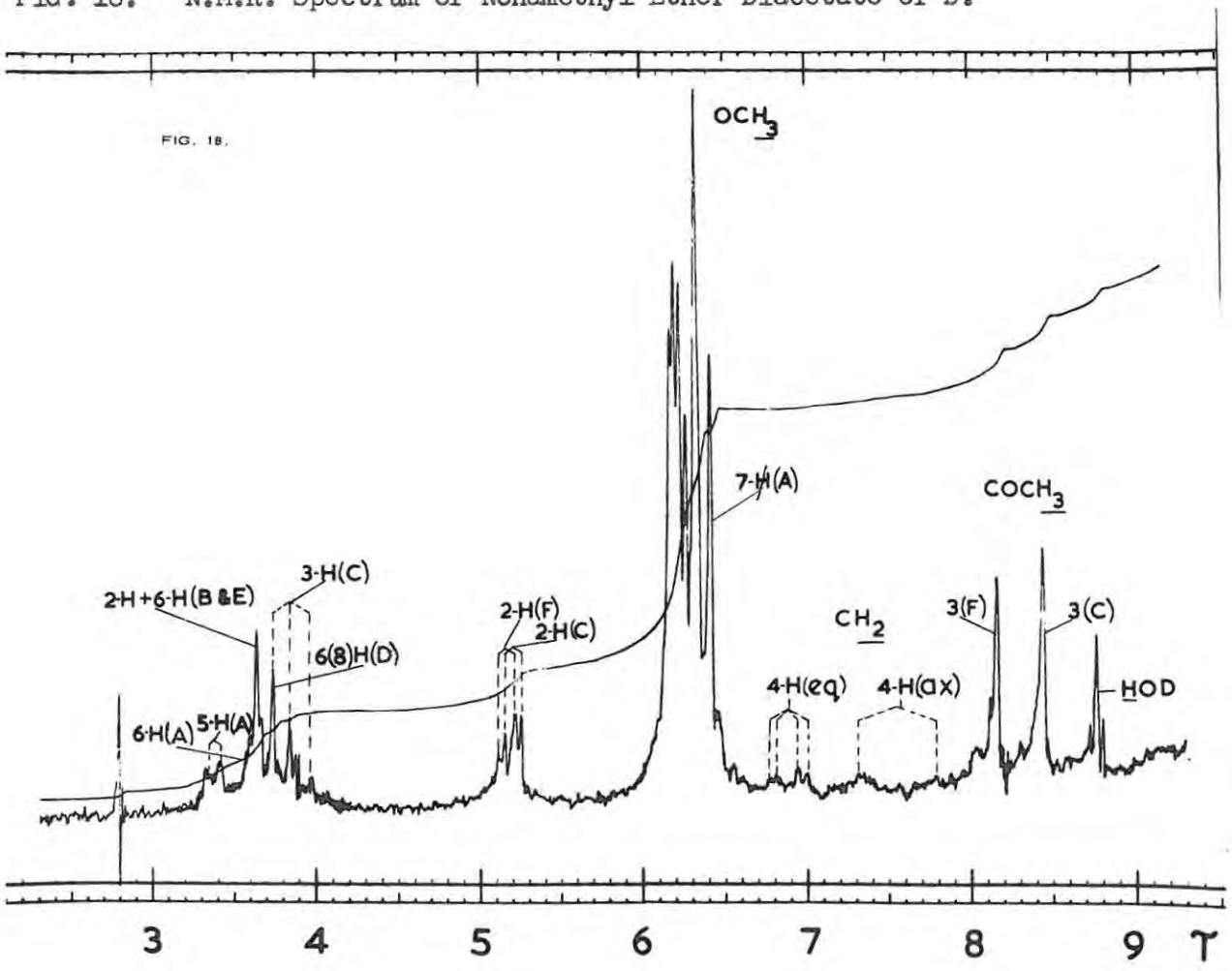


FIG. 19. N.M.R. Spectrum of Deca-acetate of B.

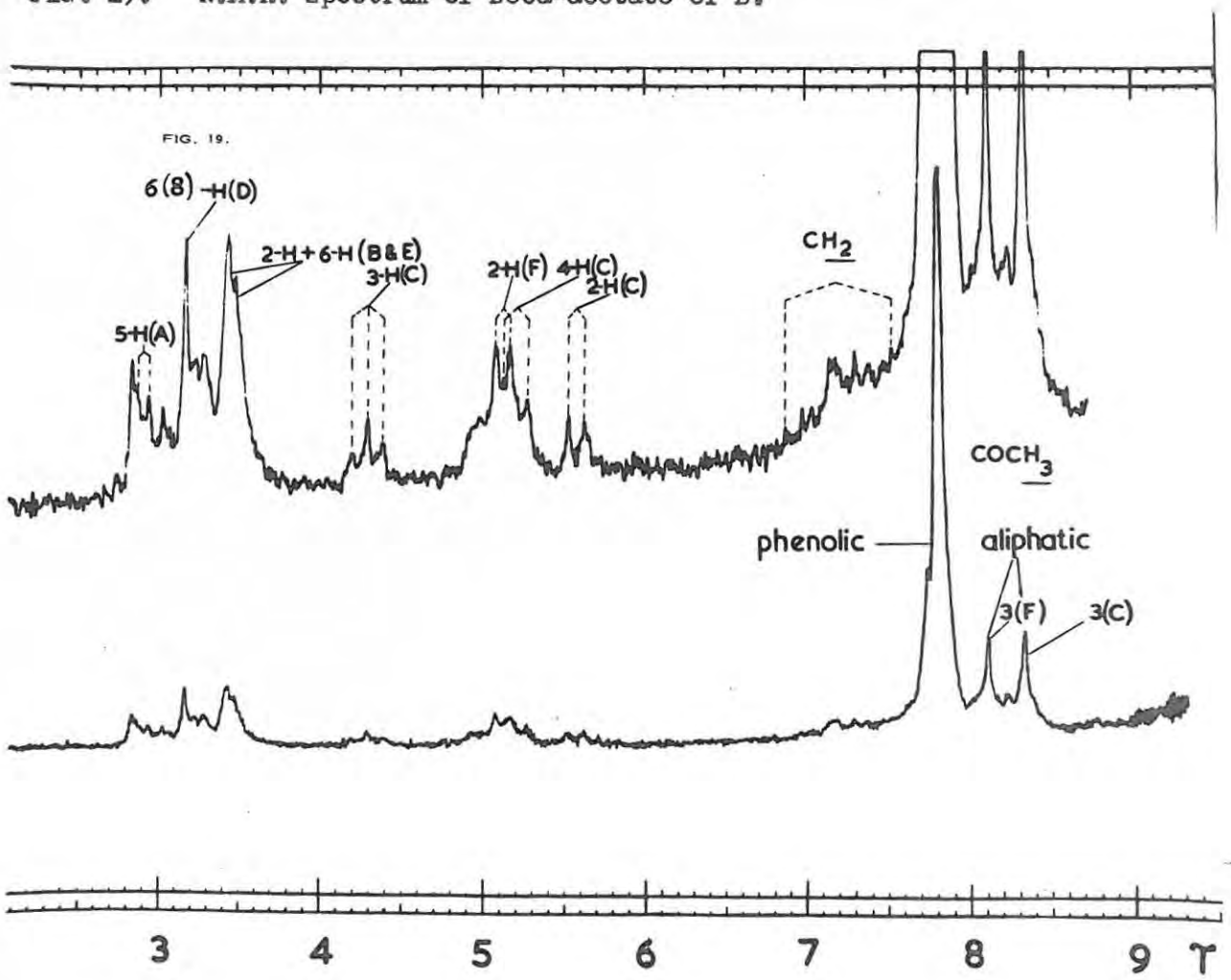


FIG. 20. N.M.R. Spectrum of Undeca-acetate of D.

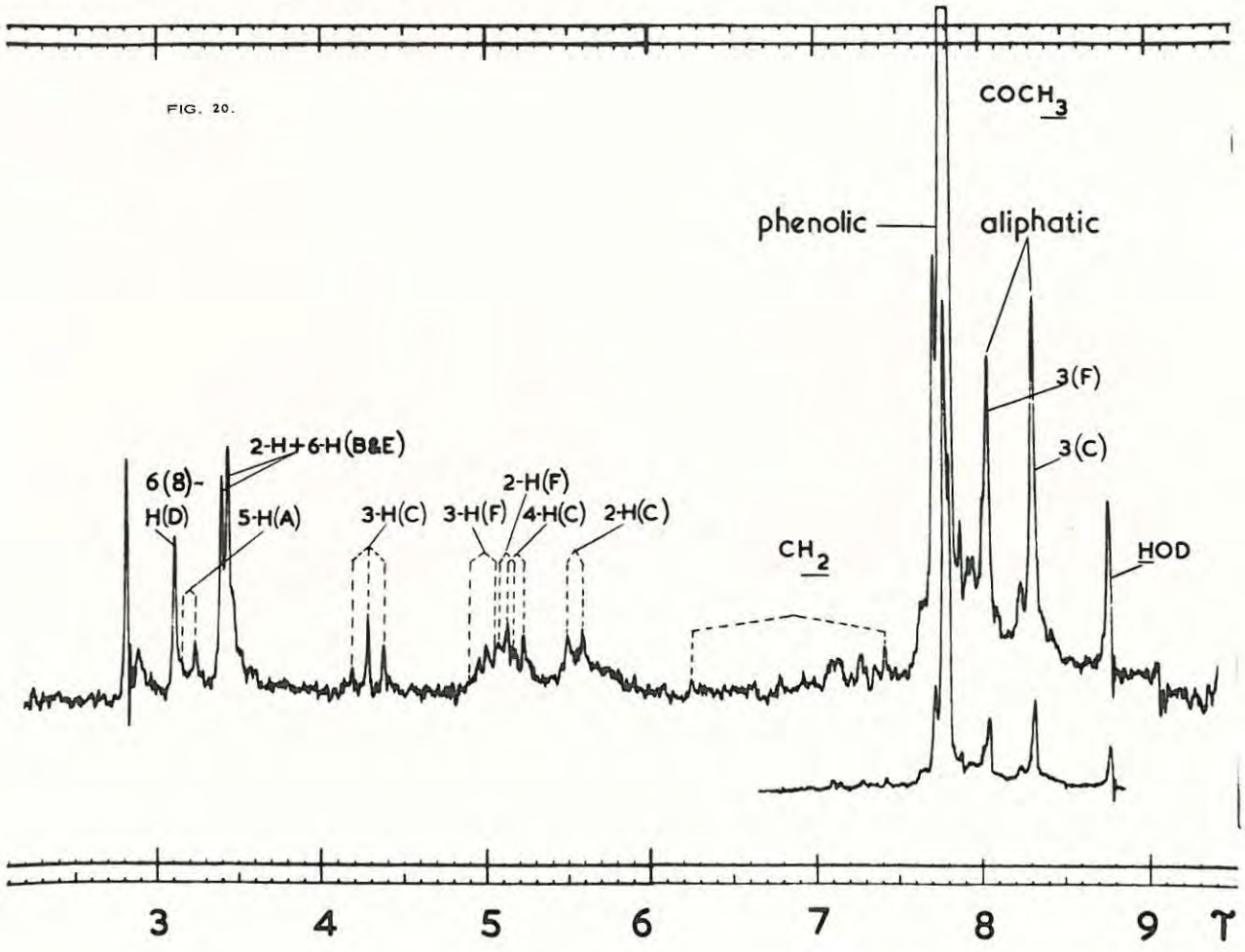


TABLE 11.

N.m.r. Spectra of Derivatives of B and D.

(a) Chemical Shifts ; τ values (p.p.m.)

	Heterocyclic Protons.						Benzenoid Protons.							-OH
Ring	C			F			A			B and E		D	E	C and F
Protons.	2	3	4	2	3	4	5	6	8	2+6		6(8)	5	3
	d	t	d	d	m	m	d	q	d	s	s	s	d	m
BMe_8	~~5.55~~ ~~5.61~~ (1)	5.31 (1)	~~5.61~~ ~~5.55~~ (1)	~~5.37~~ ~~5.67~~ (1)	5.28 (1)	7.23 (2)	~3.35 (1)	3.60 (1)	3.66 (1)	3.81 (2)	3.63 (2)	3.31 (1)	3.47 (1)	7.92-8.45 (2)
BMe_8Ac_2	5.25 (1)	3.97 (1)	~5.28 (1)	5.20 (1)	~5.13 (1)	7.17 (2)	3.41 (1)	3.60 (1)	3.66 (1)	3.89 (2)	3.58 (2)	~~3.88~~ ~~3.44~~ (1)	3.51 (1)	
BAC_{10}	5.24 (1)	4.29 (1)	~5.57 (1)	5.13 (1)	~4.94 (1)	7.09 (2)	2.95 (1)	~3.38 (1)	~3.50 (1)	3.51 (2)	3.47 (2)	3.17 (1)	3.25 (1)	
DMe_9	~~5.46~~ ~~5.61~~ (1)	5.26 (1)	~~5.62~~ ~~5.45~~ (1)	~~5.30~~ ~~5.57~~ (1)	~5.22 (1)	7.18 (2)	3.38 (1)	3.69 (1)	3.73 (1)	3.75 (2)	3.58 (2)	3.29 (1)		8.08-8.45 (2)
DMe_9Ac_2	5.18 (1)	3.87 (1)	~5.15 (1)	5.17 (1)	~5.10 (1)	7.15 (2)	3.41 (1)	3.76 (1)	3.85 (1)	3.74 (2)	3.65 (2)	~~3.75~~ ~~3.42~~ (1)		
DAc_{11}	5.17 (1)	4.28 (1)	5.54 (1)	5.12 (1)	~4.97 (1)	7.23 (2)	3.18 (1)	~3.42 (1)	~3.46 (1)	3.43 (2)	3.39 (2)	3.11 (1)		

s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Numbers in brackets denote the number of protons per signal.

TABLE 11 (Continued).

N.m.r. Spectra of Derivatives of B and D.

(a) Chemical Shifts ; τ values (p.p.m.).

	Methoxyl Protons								Acetyl Protons					
Ring	A													
Protons									Aliphatic		Phenolic			
	s	s	s	s	s	s	s	s	s		s			
BMe_8	6.43 (3)	6.35 (3)	6.31 (3)	6.29 (3)	6.25 (3)	6.23 (3)	6.19 (3)	6.16 (3)						
BMe_8Ac_2	6.43 (3)	6.34 (3)	6.32 (3)	6.29 (3)	6.27 (3)	6.22 (6)	6.19 (3)		8.43 (3)	8.18 (3)				
BAc_{10}									8.35 (3)	8.11 (3)	7.81 7.74 (24)			
DMe_9	6.40 (3)	6.31 (6)	6.23 (3)	6.22 (3)	6.21 (3)	6.19 (3)	6.17 (3)	6.11 (3)						
DMe_9Ac_2	6.40 (3)	6.33 (3)	6.31 (6)	6.26 (3)	6.22 (6)	6.18 (3)	6.16 (3)		8.42 (3)	8.14 (3)				
DAc_{11}									8.32 (3)	8.04 (3)	7.89 (3)	7.80 (6)	7.78 (15)	7.72 (3)

s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Numbers in brackets denote the number of protons per signal.

TABLE 11.

(b) Spin-spin Coupling Constants of 2-,3- and 4-Protons of C-Ring and 2- and 3-Protons of F-Ring; J values (c./sec.).

Ring	C			F
Coupling Constants	$J_{2,3}$	$J_{3,4}$	$J_{2,3}+J_{3,4}$	$J_{2,3}$
BMe_8	9.0	9.0	18.0	8.0
BMe_8Ac_2	10.0	~ 9.8	19.5	~ 7.0
BAc_{10}	10.0	~ 9.5	19.5	~8.0
DMe_9	9.0	9.0	18.0	~7.0
DMe_9Ac_2	10.0	9.8	19.5	~7.0
DAc_{11}	10.0	9.5	19.5	~8.0

Mass Spectra of Derivatives of B and D.

Mass spectra of the octamethyl ether diacetate of B and the nonamethyl ether diacetate of D were recorded to obtain accurate molecular weights and fragmentation patterns of these derivatives. The mass spectrum of the tetramethyl ether diacetate of (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol [(+)-leucorobinetinidin] was recorded for purposes of comparison with the corresponding derivatives of B and D. (+)-Leucorobinetinidin (m.p. 172-175°), obtained from the methanolic extract of Robinia pseudacacia heartwood[62], was methylated with diazomethane and then acetylated to give (+)-7,3',4',5'-tetramethoxy-3,4-diacetoxyflavan (m.p. 121-122°).

The mass spectra were recorded on an MS-9 double-focussing mass spectrometer by Dr. S.H. Eggers, National Chemical Laboratories, Pretoria, South Africa. Dr. Eggers kindly gave aid in the interpretation of these spectra.

The spectrum of (+)-7,3',4',5'-tetramethoxy-3,4-diacetoxy-flavan (cf. Fig. 21) showed a parent peak (molecular ion) at mass 446 and prominent peaks at 386, 344, 328, 327, 210, 195, 181, 44 and 43.

The octamethyl ether diacetate of B gave a mass spectrum (cf. Fig. 22) showing a molecular ion peak at mass 774 (relative intensity 5%) and prominent peaks at 714 (100%), 683 (15%), 654 (34%), 623 (26%), 521 (8%), 503 (38%), 492 (34%), 491 (22%), 477 (18%), 473 (16%), 462 (12%), 461 (18%), 447 (8%), 431 (8%), 327 (24%), 269 (60%), 210 (22%), 195 (20%), 181 (50%), 180 (50%), 165 (13%) and 151 (32%).

The mass spectrum of the nonamethyl ether diacetate of D (cf. Fig. 23) gave a molecular ion peak at mass 804 (relative intensity 9%) with prominent peaks at masses 744 (100%), 713 (14%), 684 (21%), 653 (22%), 521 (12%), 503 (37%), 492 (27%), 477 (18%), 461 (14%), 327 (14%), 269 (60%), 210 (39%), 195 (37%) and 181 (80%).

FIG. 21. Mass Spectrum of (+)-Leucorobinetinidin Tetramethyl Ether Diacetate.

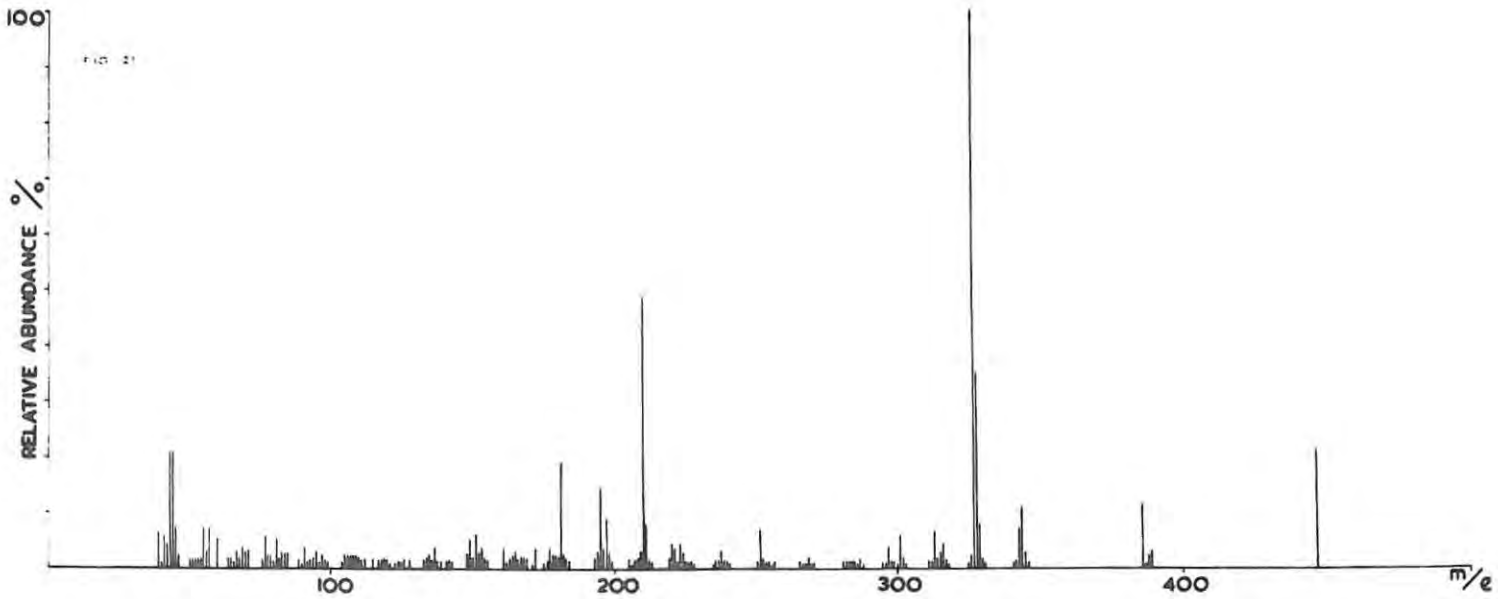


FIG. 22. Mass Spectrum of Octamethyl Ether Diacetate of B.

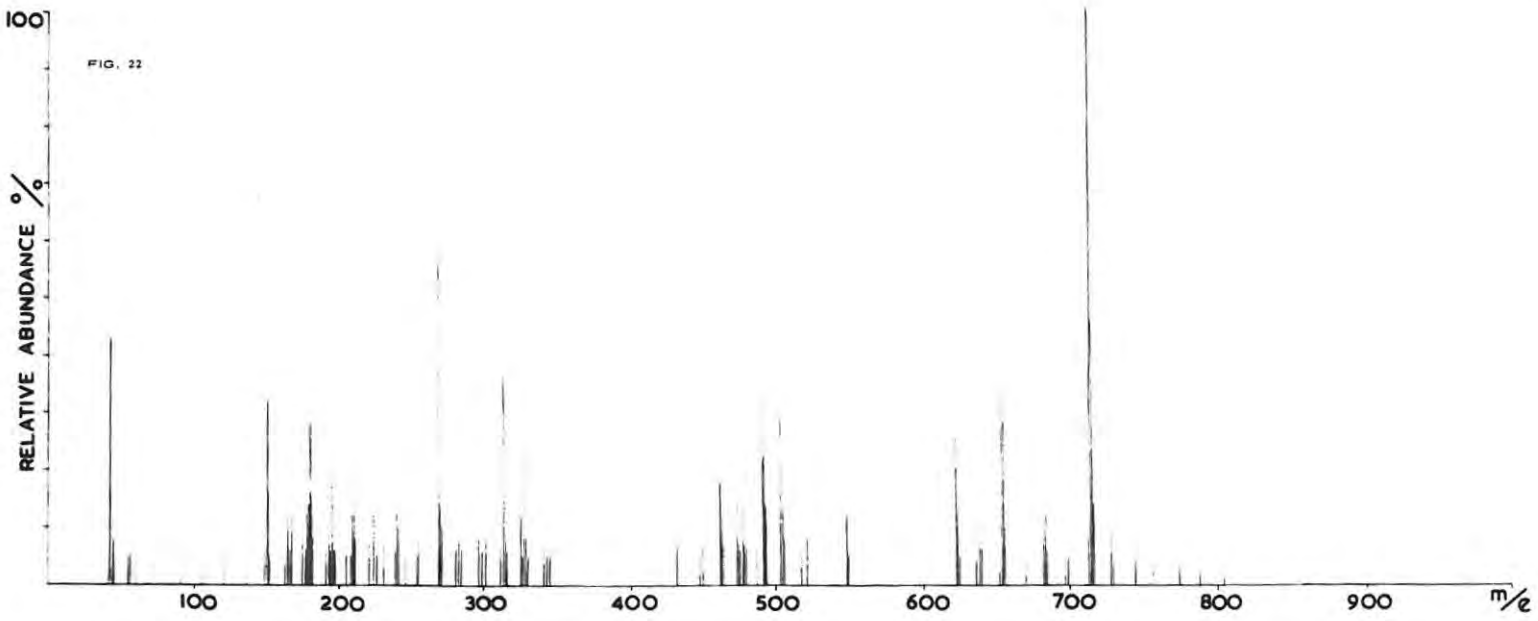
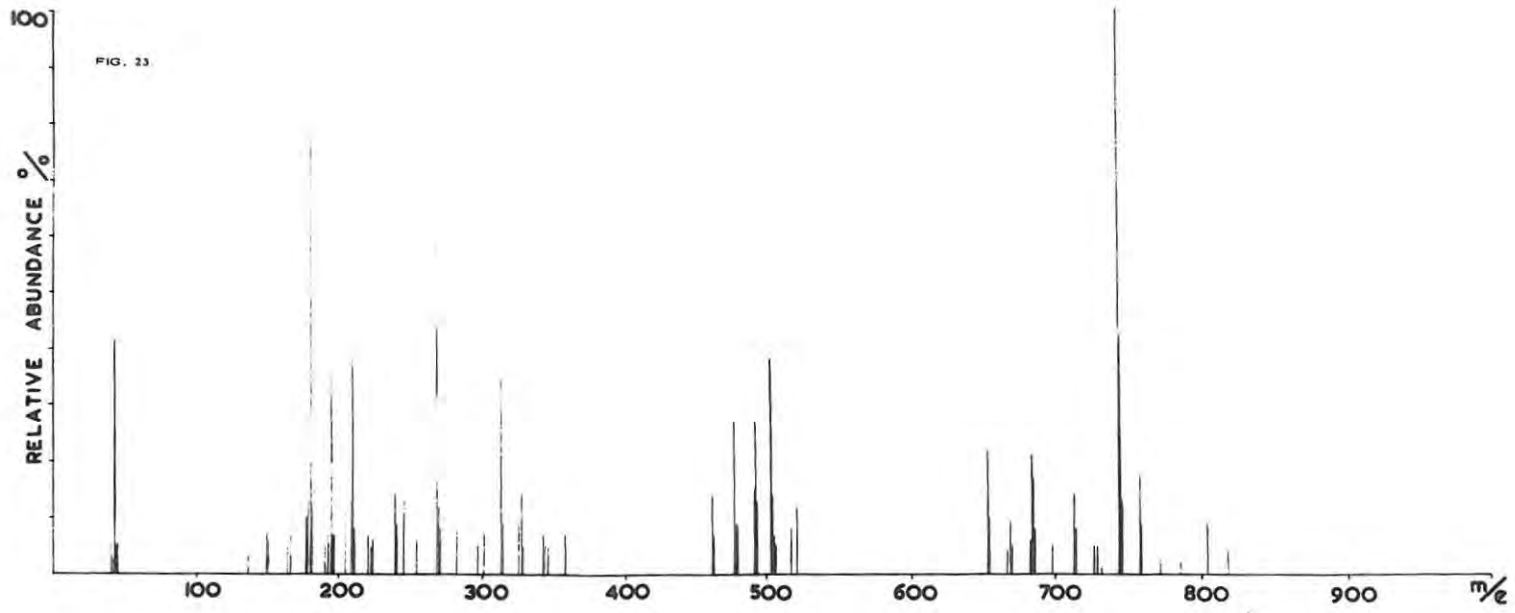


FIG. 23. Mass Spectrum of Nonamethyl Ether Diacetate of D.



PART III.

NON-PHENOLIC COMPONENTS ("NON-TANNINS") OF BLACK WATTLE (ACACIA MEARNSII) BARK.

Isolation of Amino and Imino Acids from Black Wattle Bark.

The bark was stripped from young (5-6 year-old) black wattle trees and cut across the grain into thin slivers. These were air-dried for 4 days. The dry bark (2 Kg.) was successively extracted with hot petroleum (b.p. 60-70°) (4 litres, solution A) to remove fats and waxes, with warm (60-70°) acetone (2 l.) to remove some of the polyphenolic tannins, and finally with warm (60°) ethanol (3 extractions, 6 l.).

The ethanolic solution was concentrated under vacuum to give a viscous brown residue. This was diluted with water (2 l.) and the brown precipitate filtered off. The aqueous solution was extracted five times with ethyl acetate and concentrated to a small volume (200 ml.). The residual tannins were removed by shaking the solution with pre-chromed hide powder (100g.)[201].

The clear, tannin-free solution was passed through two sulphonated polystyrene cation-exchange resin columns (Permutit Zeo-Karb 225, 16 x 2.5 cm.). The amino acids and imino acids were adsorbed on the resin, while the carbohydrates (solution B) were removed by elution of the columns with water. The nitrogenous acid fraction was obtained by elution with dilute (6N) NH_4OH. The ammoniacal eluates were concentrated to dryness under reduced pressure to give a dark-brown residue.

The residue was dissolved in water (100 ml.) and the mixture resolved by chromatography on 20 sheets of Whatman no. 3 paper in n-butanol : acetic acid : water (6:1:2, by vol.) for 24 hr. Several bands were detected by spraying strips along the sides of the chromatograms with ninhydrin and isatin spray reagents (cf. Table 13). Bands at R_F 0.08, 0.16, 0.24 and 0.33 were cut. Due to the overlapping of bands at R_F 0.16 and 0.24, these were re-run under similar conditions for a period of 48 hr.

The products were stripped from the paper by elution with 20% aqueous ethanol and the fractions concentrated to dryness under reduced pressure.

(-)-Pipecolic Acid.

The product from the band at R_F 0.33, when spotted on paper and sprayed with ninhydrin, gave a characteristic mauve and exhibited a red-purple fluorescence under ultraviolet light. It gave a pale, blue-green with isatin spray. The residue recovered from the eluates was dissolved in boiling ethanol (50 ml.) and an insoluble residue filtered off. The solution was treated with charcoal and concentrated, when (-)-pipecolic acid crystallized in white micro-crystals (250 mg.), m.p. 260-264°. Three recrystallizations from aqueous ethanol gave m.p. 277° (decomp.). $[\alpha]_D^{25}$ - 27.4° (c, 0.98 in water). Mixed m.p. with authentic (-)-pipecolic acid from Acacia oswaldii leaves, kindly supplied by Professor J.W. Clark-Lewis, gave no depression (m.p. 275-276°).

Clark-Lewis and Mortimer[119] recorded a m.p. 273-275° and $[\alpha]_D$ -25° for (-)-pipecolic acid.

(-)-L-Proline.

The residue of the eluates of the band at R_F 0.24 was dissolved in boiling aqueous ethanol (95%, 10 ml.) and filtered from an insoluble residue. The solution was treated with charcoal, concentrated to a small volume (10 ml.), cooled to 0° and ether (3 ml.) added. After 3 days at 0° a white amorphous mass separated. This was filtered off and re-precipitated from ethanol with ether, yielding a white powder (110 mg.) which settled at 225° (decomp.) $[\alpha]_D^{26}$ -83.1° (c, 1.66 in ethanol). With ninhydrin spray reagent the compound gave a yellow which changed to grey on ageing. Isatin spray gave a bright blue.

The picrate of proline was prepared by the method of Signaigo and Adkins[123]. Proline (20 mg.) and picric acid (40 mg.) were dissolved in the minimum (1 ml.) of hot glacial acetic acid. The mixture was cooled to room temperature, ether (4 ml.) added and the solution cooled to 0°. After 2 days at this temperature long yellow needles of the picrate crystallized. These were recrystallized from ethanol (3.2 mg.), m.p. 153-4°. The mixed m.p. with the picrate of authentic (-)-proline prepared by the same method was 152-154°.

Kapfhammer and Eck[122] recorded $[\alpha]_D$ -84.9° for (-)-proline and m.p. 152-153° for the picrate.

4-Hydroxy-trans-(-)-L-pipecolic Acid.

The solids recovered from the eluates of the band at R_F 0.16 were dissolved in aqueous ethanol (95%, 10 ml.) and an insoluble brown precipitate filtered off. The solution was treated with charcoal and concentrated to small volume (2 ml.). After a week at room temperature

a crystalline precipitate (245 mg.) appeared. This was recrystallized three times from aqueous ethanol, m.p. 292°, $[\alpha]_D^{25}$ -12.0° (c, 0.78 in water).

The product gave a characteristic green with ninhydrin spray that faded through khaki to grey, showing a brick-red fluorescence under ultraviolet light. Isatin spray reagent gave no colour. Mixed m.p. with authentic 4-hydroxy-*trans*-(-)-L-pipecolic acid from *Acacia oswaldii*, kindly supplied by Professor J.W. Clark-Lewis, was 292-3°.

Virtanen *et al.*[116,117] recorded m.p. 270°, $[\alpha]_D$ -12.5°, while Clark-Lewis and Mortimer[119] found m.p. 294°, $[\alpha]_D$ -13.0°.

α-Alanine, Arginine, Aspartic Acid, Glutamic Acid and Serine.

The band at low R_F (0.08) consisted of a mixture of amino acids which were all present in fairly low concentration. These amino acids were not fully resolved in *n*-butanol : acetic acid : water (6:1:2, by vol.) and therefore the solvent system developed by Levy and Chung[202] was used. This consisted of first dipping the Whatman no. 52 paper in borate buffer (pH 9.3), drying, and then developing the chromatogram downward using phenol : cresol : borate buffer (pH 9.3) (25:25:7, by vol.) for 24-27 hr.

By using this solvent system, and comparing with the R_F values of a large number of reference amino acids, *α*-alanine, arginine, aspartic acid and glutamic acid could be readily identified in the mixture. An additional component, corresponding to serine, was found in very low concentration.

A number of solvent systems designed to separate complex mixtures of amino acids by one-dimensional paper chromatography was developed by Hardy, Holland and Nayler[203]. Of these a selected number were used to separate the mixture of amino acids (cf. Table 12).

The colours produced by the amino acids and imino acids when sprayed with ninhydrin and isatin reagents are summarized in Table 13.

TABLE 12.

R_F Values of Amino Acids in Various Solvent Systems.

Solvent System	α-Alanine	Arginine	Aspartic Acid	Glutamic Acid	Serine
Phenol:Cresol:Borate Buffer (25:25:7)	0.26	0.51	0.03	0.06	0.10
n-Butanol:Acetone:water (2:2:1).	0.24	0.04	0.07	0.09	0.19
n-Butanol:Ethanol:water (2:2:1).	0.26	0.09	0.07	0.14	0.20
n-Butanol:Ethanol:water Propionic Acid (10:10:5:2).	0.41	0.17	0.16	0.29	0.23
n-Butanol:Methyl Ethyl Ketone:water:Cyclohexanol (10:10:5:2)	0.27	0.03	0.06	0.05	0.35

Petroleum-soluble Components.

Solution A, the petroleum-ether extract of fresh black wattle bark, was concentrated to a small volume (200 ml.), when a white, waxy precipitate (4g.) formed. This was filtered off, dried over $CaCl_2$ and examined by thin-layer chromatography on Kieselgel G using benzene : acetone (17:3, v./v.). On spraying the chromatoplate with a mixture of

concentrated H_2SO_4 and 40% aqueous formaldehyde (20:1, v./v.) it was noted that the precipitate consisted of six components, shown as grey-black spots, of which two at R_F 0.97 (a long-chain β-diketone) and 0.63 (a "steroid" alcohol) predominated. The remaining 4 minor components were present in low concentration and were not investigated.

TABLE 13.

Colours Produced by Amino and Imino Acids with Ninhydrin and Isatin.

Acid	Spray Reagent.	
	Ninhydrin	Isatin
(-)-Pipecolic Acid	Mauve	Pale, blue-green
L-Proline	Yellow→brown→grey	Bright blue
4-OH-(-)-L-pipecolic Acid	Green→khaki→grey	—
α-Alanine	Purple	Pink
Arginine	Purple	Pink
Aspartic Acid	Blue→purple	Blue-grey
Glutamic Acid	Purple	Red-pink
Serine	Purple	Pink→blue-grey

Long-chain β-Diketone.

The crude extract (4g.) was dissolved in petroleum ether (b.p. 40-60°) (250 ml.) and shaken with 5% aqueous (w./v.) NaOH solution and then with water. The petroleum solution was dried over anhydrous Na_2SO_4 for 2 hr. and evaporated to dryness under reduced pressure at 60°. The white residue was triturated with fresh petroleum ether and the remaining solids crystallized from a mixture of ethanol : benzene (2:1, v./v.). Recrystallization from the same mixture yielded white micro-crystals, m.p. 80-82°. (Found: C, 80.0, 80.1 ; H, 13.4, 13.5. $C_{32}H_{64}O_2$ requires: C, 79.9 ; H, 13.4%). λ_{max} 278 mμ (log ϵ, 2.99 in ethanol).

The infrared spectrum of the compound (cf. Fig. 24) was determined in Nujol and showed maxima at 3350 $cm.^{-1}$ (rounded, O-H stretching), 3100 $cm.^{-1}$ (inflection, O-H stretching chelate H-bonds), 2780 $cm.^{-1}$ (weak, C-H stretching), 1730 $cm.^{-1}$ (very strong, ketone C=O stretching vibrations) 1400 $cm.^{-1}$ (weak C-H deformation), 1175, 1100 and 1060 $cm.^{-1}$ (strong, C-O stretching and O-H deformation) and 730 and 720 $cm.^{-1}$ (strong, C-H deformations of alkenes).

The nuclear-magnetic-resonance spectrum (cf. Fig. 25) showed a multiplet at τ = 9.12 p.p.m. (protons of terminal methyl groups), a sharp signal at τ = 8.73 p.p.m. and a multiplet at τ = 7.70 p.p.m. (methylene proton resonances), and two triplets centred at τ = 5.98 and 6.40 p.p.m. The downfield triplet is probably due to C-H protons, while the one at τ = 6.40 p.p.m. may represent a vinyl proton, split by methylene protons.

The β-diketone was also obtained by treating the crude petroleum soluble fraction (1g.) in warm petroleum (b.p. 40-60°) with warm aqueous cuprous acetate solution. Just sufficient copper acetate solution was added to ensure that the aqueous layer remained blue after shaking. The copper complex formed more readily on addition of a small volume of ethanol (1-2 ml.). After filtration and washing with light petroleum, the complex was dried. The β-diketone was readily regenerated from the copper complex by shaking a hot petroleum solution, with added ethanol (2 ml.), with 6N hydrochloric acid. The petroleum solution, after separation from the aqueous fraction, was evaporated to give colourless crystals of the β-diketone. Recrystallization from ethanol :

FIG. 24. Infrared Spectrum of β-diketone.

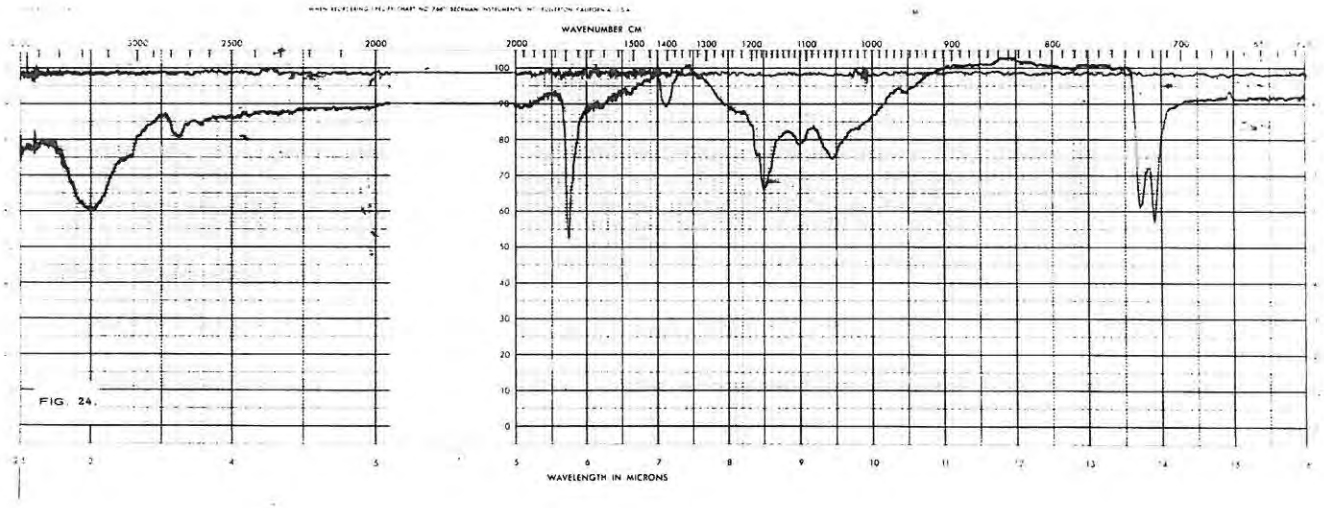
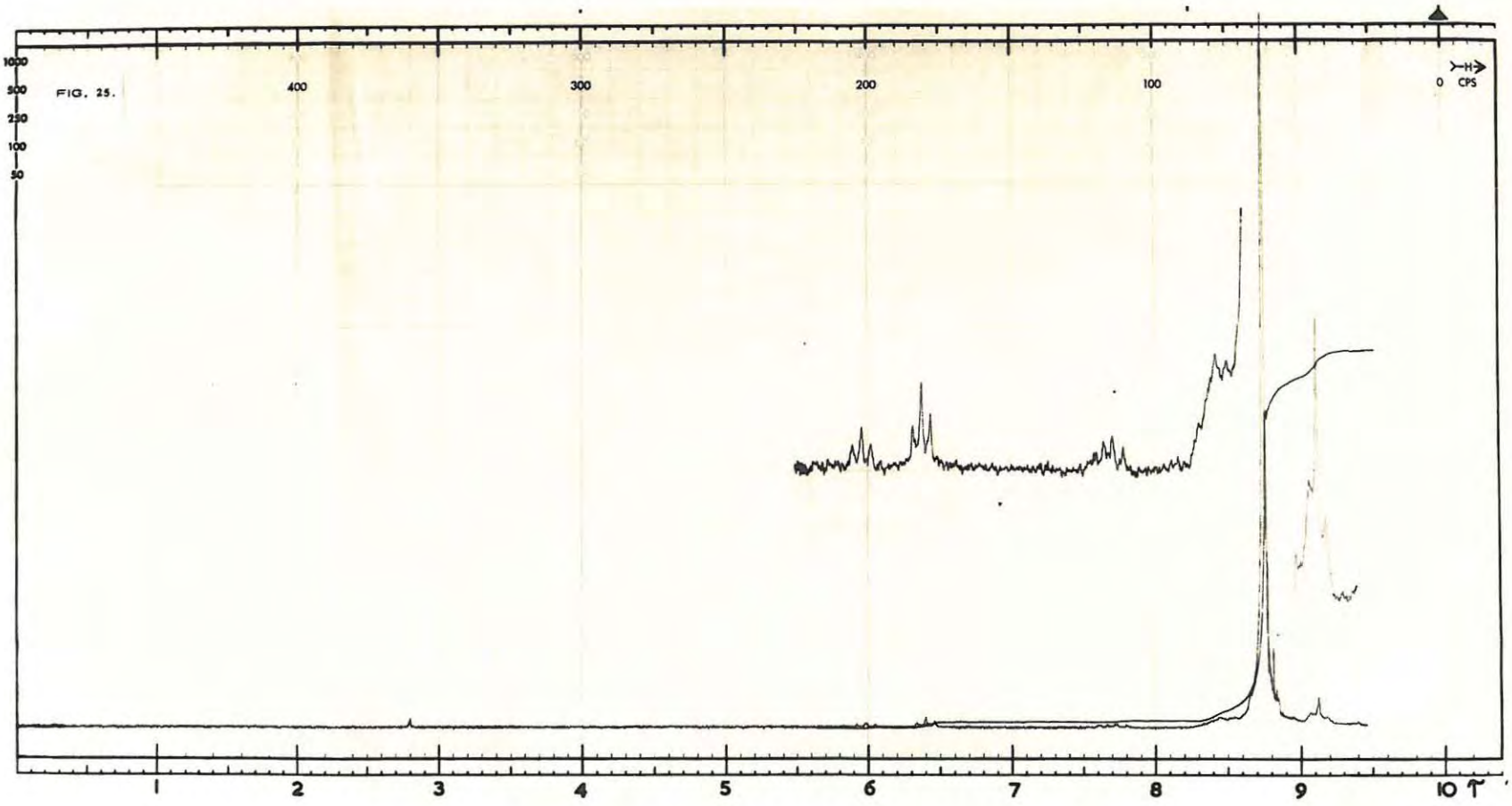


FIG. 25. N.M.R. Spectrum of β -diketone.



benzene (2:1, v./v.) gave colourless micro-crystals, m.p. 80-82°. A mixed m.p. with the original β-diketone was undepressed.

The copper complex showed infrared absorption maxima in the carbonyl region at 1725 cm.$^{-1}$ (medium) and 1600 cm.$^{-1}$ (strong) in a Nujol mull (cf. Fig. 26).

An attempt was made to determine the molecular weight of the β-diketone by mass spectrometry, but due to the inherent instability of this class of compounds[204] this was not possible. The peak of largest mass reflected a value of 448, which contrasted with the molecular weight of 480 calculated from the empirical formula. This suggests that the peak at M = 448 is not a true molecular ion peak. The main peaks in the mass spectrum (cf. Fig. 27) occurred at: 420, 392, 364, 125, 111, 97, 83, 69, 57, 55, 43 and 41.

The m.p. (80-82°) of the black wattle bark β-diketone is higher than that of analogous long-chain β-diketones extracted from Acacia and Eucalyptus waxes by Horn and Lamberton[152]. Their diketones melted in the range 40-65°.

The Steroid Alcohols.

The residue (1g.) obtained by concentrating the whole crude petroleum extract of fresh, air-dried black wattle bark was resolved on ten preparative thin-layer chromatoplates (20 x 20 cm.) on Kieselgel G (1 mm. thick) with benzene : acetone (17:3, v./v.) as solvent. The plates were developed for 2 hr. giving a migration of 18 cm. The steroid fraction was located by spraying a narrow strip along the edge of the plate with 20% (v./v.) phosphomolybdic acid in ethanol[205]. A band at R_F

FIG. 26. Infrared Spectrum of Copper Complex of $\underline{\beta}$-diketone.

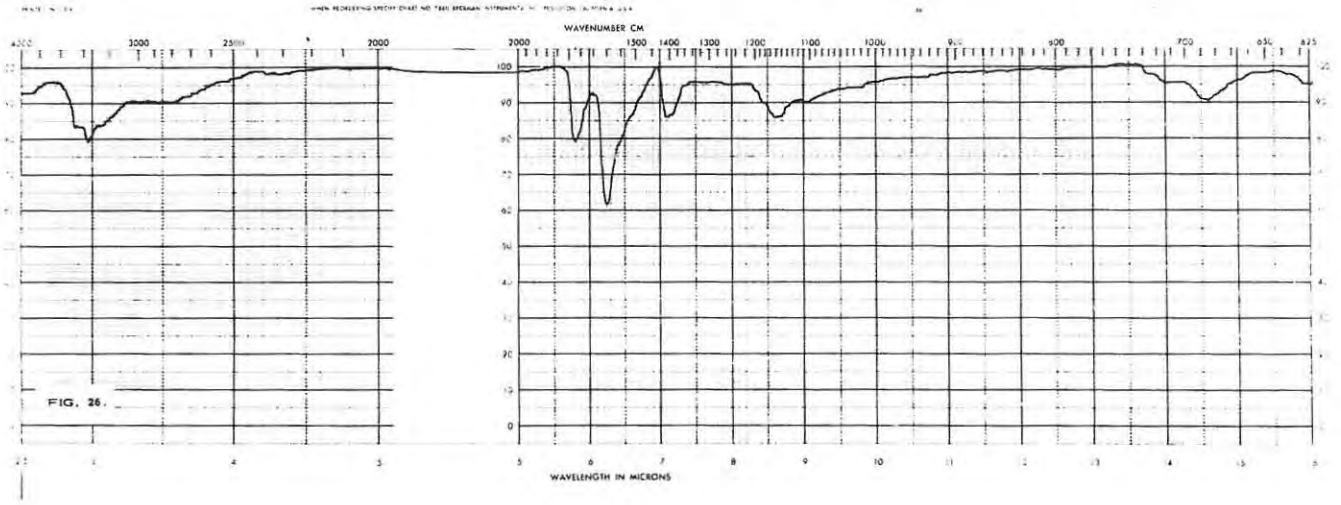
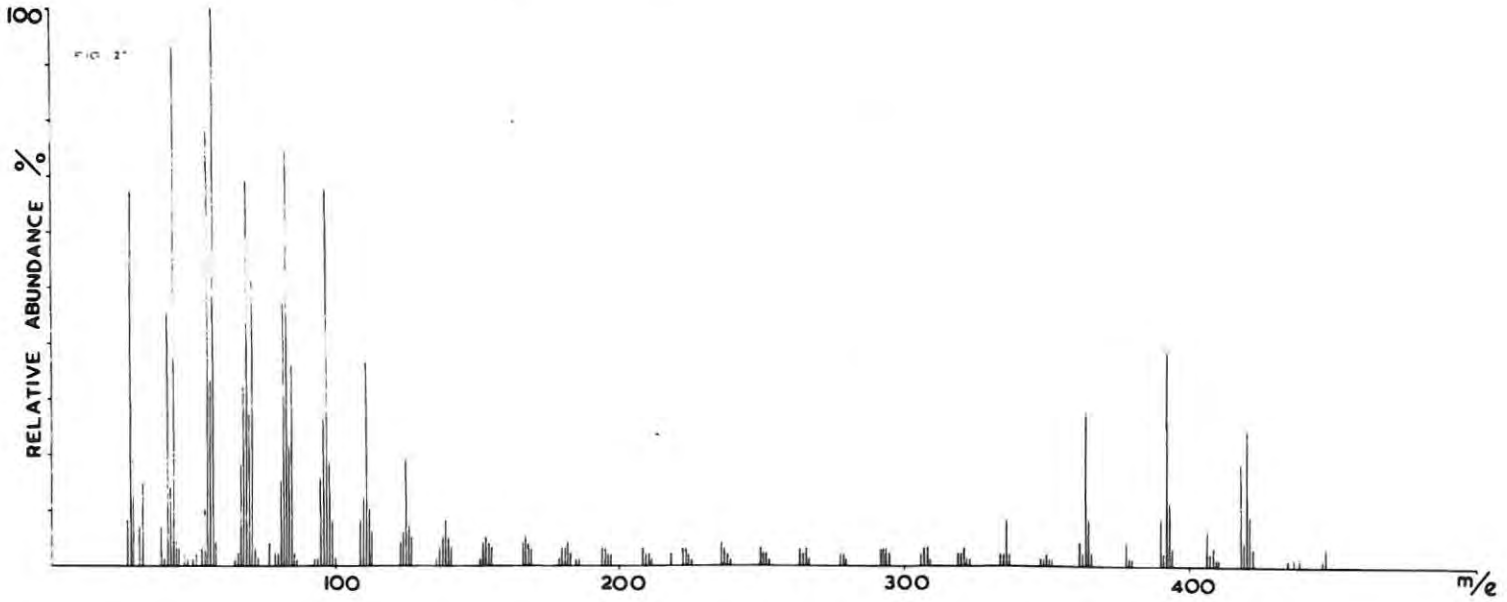


FIG. 27. Mass Spectrum of β-diketone.



0.60-0.65 gave a blue on a yellow background, and was removed by scraping the silica gel off the plate and eluting the product with petroleum b.p. (60-70°). A solid white residue was obtained on concentrating the petroleum solution. This was crystallized from ethanol as colourless needles (98 mg.), m.p. 160-162°. (Found: C, 82.5 ; H, 11.6. Calculated for $C_{29}H_{48}O$: C, 84.4 ; H, 11.7 and for $C_{29}H_{50}O$: C, 83.9 ; H, 12.2%). The Liebermann-Burchard[206] reaction gave a violet-to-green colour characteristic of the common phytosterols.

The nuclear-magnetic-resonance spectrum of the steroid (cf. Fig. 28) showed the following characteristics: τ = 9.45 and 9.21 p.p.m. (singlets, tertiary methyl resonances), 8.98 p.p.m. (doublet, possibly secondary methyl), 7.5-9.2 p.p.m. (methylene proton resonances), 5.5-6.7 p.p.m. (multiplet), 4.87 p.p.m. (multiplet, possibly two vinyl protons).

The infrared spectrum (cf. Fig. 29) of the product in chloroform solution showed the following signals: 3600 $cm.^{-1}$ (weak, O-H stretching), 3420 $cm.^{-1}$ (weak, rounded, O-H stretching), 2970 and 2870 $cm.^{-1}$ (strong, C-H stretching), 1710 $cm.^{-1}$ (weak, ketonic C=O stretching), 1450 and 1380 $cm.^{-1}$ (medium, O-H deformations), 1090 $cm.^{-1}$ (weak), 1035 $cm.^{-1}$ (medium) and 972 $cm.^{-1}$ (medium).

The n.m.r. and infrared spectra of the steroid and those of a "steroid alcohol", m.p. 160-161°, obtained from black wattle wood by Stephen[141], were superimposable. A mixed m.p. of these two steroid alcohols from wattle wood and bark gave no depression, m.p. 161-162°.

FIG. 28. N.M.R. Spectrum of Steroid Mixture.

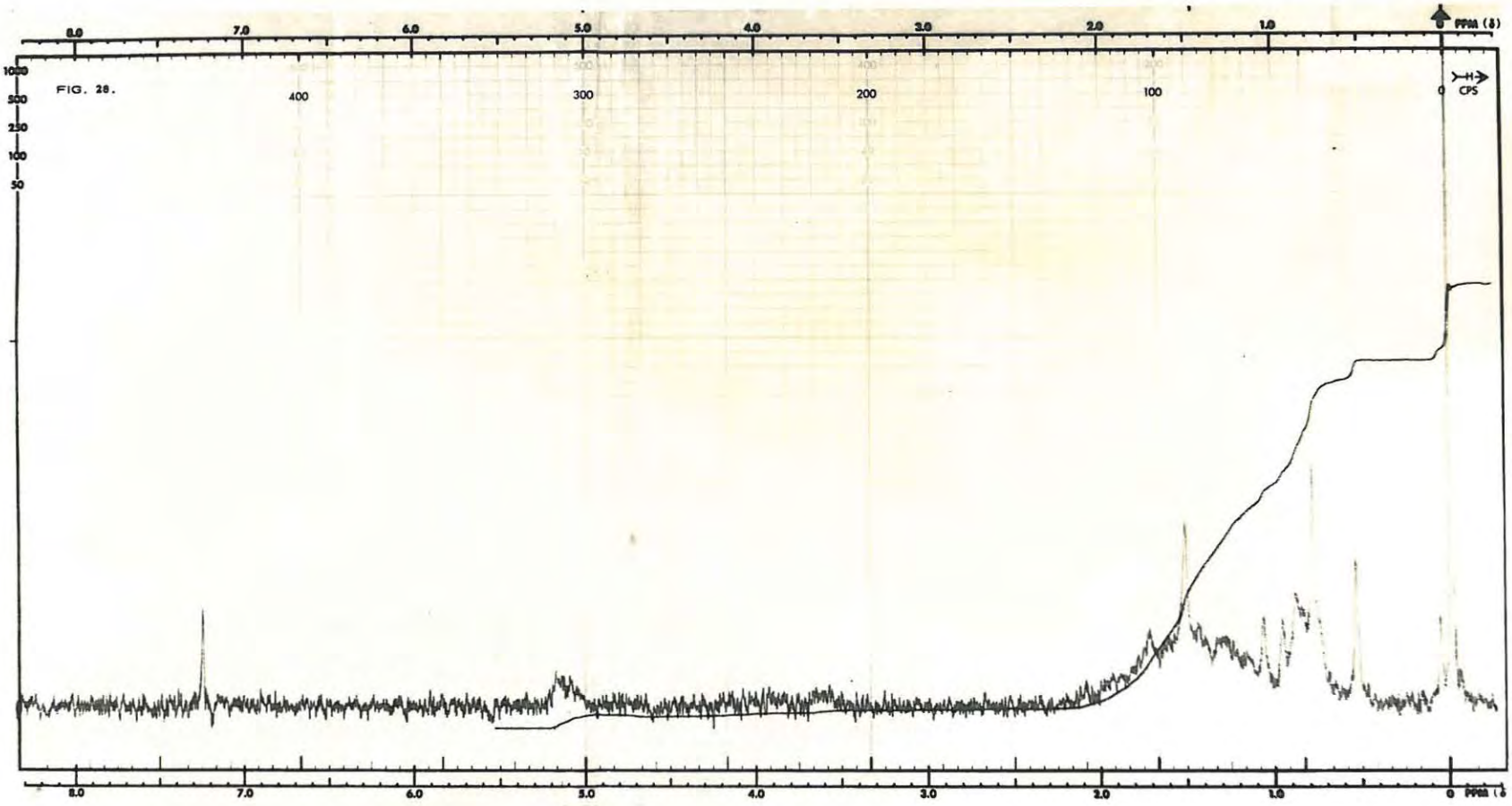
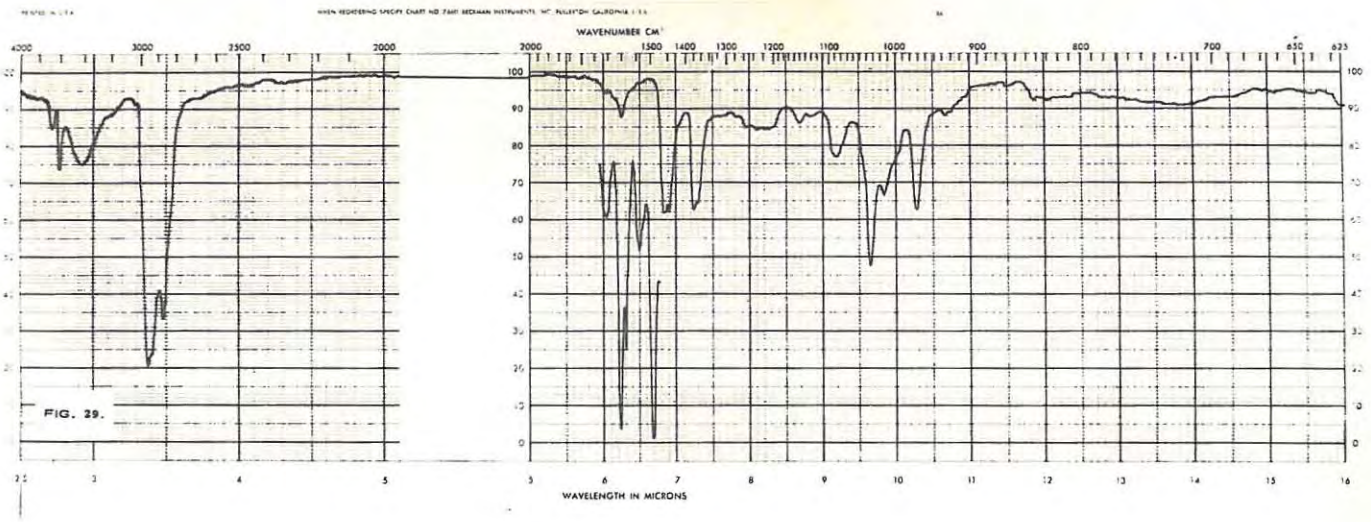


FIG. 29. Infrared Spectrum of Steroid Mixture.



Steroid Acetate.

The steroid (51 mg.) was dissolved in pyridine (0.3 ml.) and acetic anhydride (0.4 ml.) added. After 30 minutes at room temperature the acetate precipitated from solution and was recovered from water. Crystallization from a mixture of ethanol : petroleum ether (b.p. 60-70°) (9:1, v./v.) yielded colourless plates (45 mg.), m.p. 175-176°. (Found: C, 80.7 ; H, 10.8 ; $CO.CH_3$, 8.8. Calculated for $C_{31}H_{50}O_2$: C, 81.9 ; H, 11.1 ; $CO.CH_3$, 9.5 and for $C_{31}H_{52}O_2$: C, 81.5 ; H, 11.5 ; $CO.CH_3$, 9.4%).

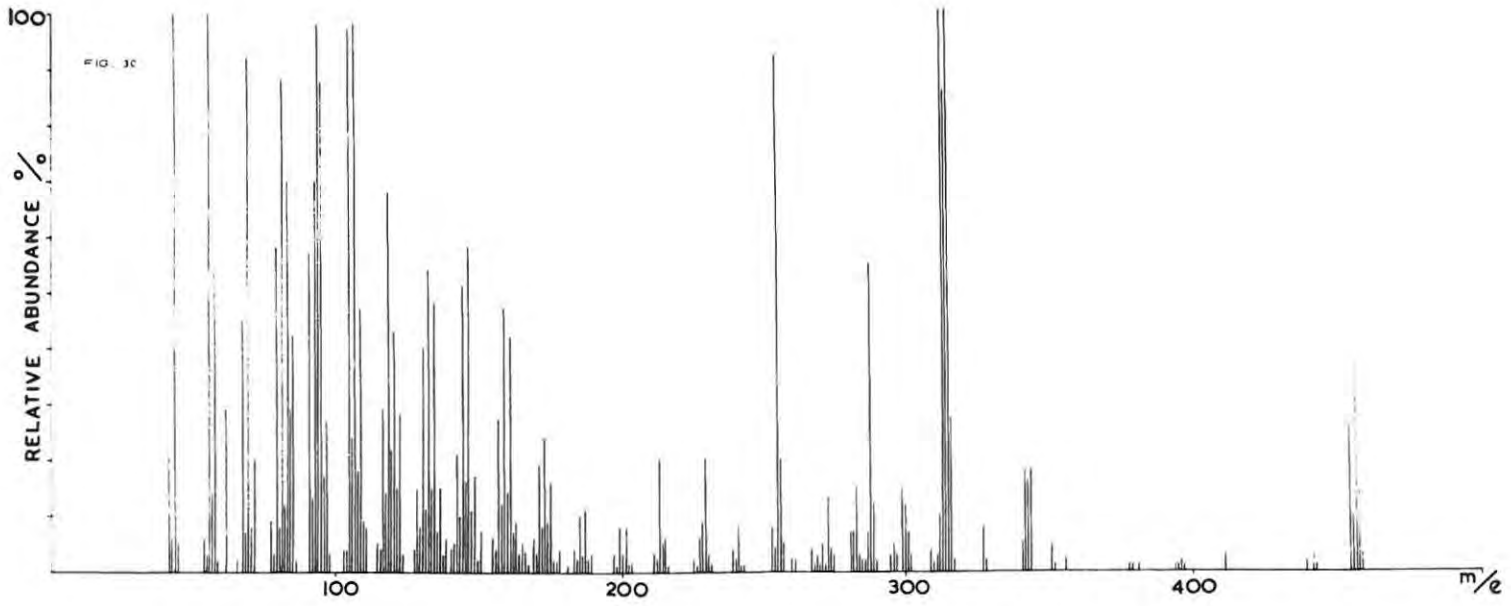
The mixed m.p. of the steroid acetate with the acetate of the black wattle heartwood steroid[141] showed no depression, 173-175°. Keppler[66] recorded a m.p. 160-161° for the steroid from wattle wood, and m.p. 167-169° for its acetate.

The n.m.r. spectrum of the steroid acetate was very similar to that of the alcohol, except that acetyl proton signals at τ = 7.97 p.p.m. in the acetate replaced the hydroxyl proton signal at 8.48 p.p.m. in the alcohol. The signal of the single proton at 5.5.-6.7 p.p.m. of the steroid alcohol was also shifted to 5.0-5.6 p.p.m. in the acetate.

The steroid acetate was subjected to mass spectrometric analysis which showed that the product was a mixture of two components. Molecular ion peaks showed the molecular weights of the two components to be 454 and 456 which corresponded to molecular formulae $C_{31}H_{50}O_2$ and $C_{31}H_{52}O_2$ (cf. Fig. 30).

From the mass spectrometric data it appeared likely that the mixture consisted of stigmasterol ($C_{31}H_{50}O_2$), a less common plant sterol,

FIG. 30. Mass Spectrum of Steroid Acetate Mixture.



and β-sitosterol ($C_{31}H_{52}O_2$), an ubiquitous phytosterol. These two sterols are commonly associated in a variety of plants.

Selective Hydrogenation of the Steroid Mixture.

In order to ascertain whether the mixture consisted of stigmasterol and β-sitosterol, the selective hydrogenation of the double bond in the side-chain of the one component was attempted by the method of Bernstein and Wallis[207]. This consisted of mild reduction of the mixture of acetates with a palladium catalyst.

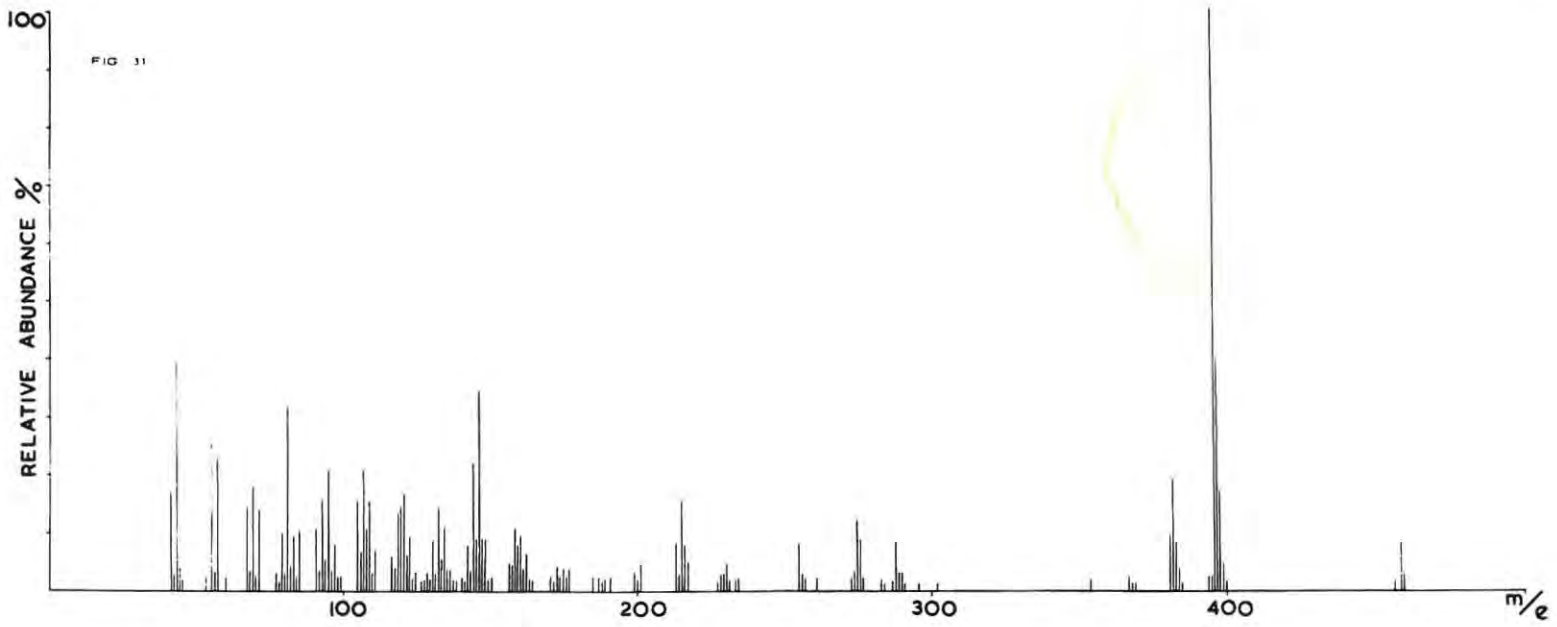
The steroid acetate mixture (145 mg.) was hydrogenated over $PdCl_2$ (80 mg.) catalyst in anhydrous ethyl acetate (25 ml.) at room temperature and atmospheric pressure for 5 hr. The reduction was terminated after one equivalent of hydrogen had been absorbed, the catalyst was filtered off and the ethyl acetate solution evaporated to dryness under reduced pressure. The residue crystallized from ethanol : petroleum (b.p. 60-70°) (9:1, v./v.) as colourless plates (73 mg.), m.p. 128-129°. A mixed m.p. with β-sitosteryl acetate, prepared from authentic β-sitosterol (Mann Assayed Chemicals), was undepressed.

The mass spectrum of the product of selective hydrogenation of the mixture of steroid acetates gave a parent peak at mass 456 and four main peaks at masses 396, 147, 81 and 43. Other prominent peaks occurred at: 459, 458, 398, 397, 382, 381, 275, 216, 215, 213, 145, 133, 121, 120, 109, 107, 105, 95, 93, 71, 69, 67, 57, 55 and 41 (cf. Fig. 31).

Carbohydrate Components.

Solution B, the aqueous solution of carbohydrates obtained by elution of the cation-exchange resin with water, was concentrated to

FIG. 31. Mass Spectrum of Product of Selective Hydrogenation of Steroid Mixture.



dryness under reduced pressure. Descending chromatography of the residue on Whatman no. 1 paper in a mixture of n-butanol : acetic acid : water (6:1:2, by vol.) with reference sugars, indicated the presence of traces of glucose and fructose, with sucrose and an unknown component as main constituents (cf. Stephen[141]).

A 24 hr. downward migration indicated the presence of sucrose (3.3 cm. migration, red), together with traces of glucose (5.6 cm., blue) and fructose (8.9 cm., red), with naphthoresorcinol-H_3PO_4 spray reagent[208]. The unknown component was identified as the cyclitol, (+)-pinitol, (7.5 cm., buff on a brown background) with lead tetra-acetate spray reagent[209]. The main components were thus sucrose and (+)-pinitol.

Isolation of (+)-pinitol was attempted on a larger scale. Fresh black wattle bark (2 kg.) was cut into thin slivers and dried at room temperature for 4 days. The dry bark was extracted with boiling 90% aqueous (v./v.) acetone (3 l.) for 2 hr. After evaporation of the acetone solution the solid extract (500g.) was dissolved in warm water (250 ml.) at 60°. An insoluble residue was filtered off and the filtrate evaporated to dryness under reduced pressure. The dry, powdered residue was refluxed with dry acetone for 2 hr. when the carbohydrates were precipitated as white semi-crystalline lumps, and the tannins slowly dissolved in the acetone.

Sucrose.

Sucrose (500 mg.) crystallized first from a methanol : acetone (4:1, v./v.) solution (10 ml.) of the white lumps. The sucrose was re-crystallized from aqueous ethanol as large cubic crystals, characteristic

of sucrose, m.p. 189-190°, $[\alpha]_D^{20}$ + 66.4° (c, 0.92 in water). A mixed m.p. with authentic sucrose showed no depression, m.p. 190-192°. Hydrolysis of the natural sucrose with 6N hydrochloric acid, by boiling for 10 minutes, gave only glucose and fructose (paper chromatography).

(+)-Pinitol.

The mother-liquor of the sucrose crystallization, on concentration, gave a white crystalline precipitate. This was recrystallized from methanol : acetone (4:1, v./v.) to give white micro-crystals (420 mg.), m.p. 185-186°. $[\alpha]_D^{25}$ + 67.9° (c, 0.67 in water). (Found: C, 43.0 ; H, 7.3 ; OCH_3, 16.2. Calculated for $C_7H_{14}O_6$: C, 43.3 ; H, 7.3 ; OCH_3, 16.0%). A mixed m.p. with authentic (+)-pinitol from Adesmia spp.[144], kindly supplied by Dr. H.H. Appel, was undepressed at 186-188°. Anderson, MacDonald and Fischer[147] found m.p. 185-186°, $[\alpha]_D^{23}$ + 66.8° (c, 2.5 in water) for (+)-pinitol. Appel[144] found m.p. 186-187°, $[\alpha]_D^{18}$ + 64.8°.

The di-isopropylidene derivative of (+)-pinitol from wattle bark was prepared according to the method of Anderson et al.[147], as modified by Adhikari, Bell and Harvey[210]. To (+)-pinitol (15 mg.) was added acetone (12 ml.) and concentrated hydrochloric acid (0.15 ml.). The mixture was vigourously stirred in a stoppered flask for 20 hr. After 1 hr. all the (+)-pinitol had dissolved. The solution was left standing for 60 hr., and then neutralized with triethylamine (3-4 drops, litmus). Long, needle-shaped crystals of triethylamine hydrochloride formed first. These were filtered off and from the mother-liquor the di-isopropylidene derivative crystallized as white needles (4 mg.), m.p.

104-106°. Anderson et al.[147] found m.p. 104.5-106.0° for this derivative.

The Amino Acid and Imino Acid Contents of the Seeds, Seed Pods, Flowers, Leaves, Twig Bark, Stem Bark, Root Bark, Stem Heartwood and Root Heartwood of Black Wattle.

The above portions of the black wattle tree (Acacia mearnsii) were extracted with aqueous 80% (v./v.) methanol and examined by two-dimensional chromatography with n-butanol : acetic acid : water (6:1:2, by vol.) (24 hr. downward migration), and then n-butanol : methyl ethyl ketone : water (2:2:1) under the same conditions. Duplicate chromatograms were run on Whatman no. 1 paper, one of which was sprayed with ninhydrin and the other with isatin, and heated for 5-10 minutes at 120°. With the exception of variations in the root and stem heartwoods (cf. Table 14), pipecolic acid, 4-hydroxy-pipecolic acid and proline were present in all the portions of the black wattle tree. In the flowers and seeds these compounds were accompanied by very high concentrations of a complex mixture of amino acids.

Paper chromatography of ethanolic extracts using ninhydrin and isatin sprays showed the presence of pipecolic acids and amino acids in the leaves of the green wattle (Acacia decurrens) and silver wattle (Acacia dealbata)[129].

Semi-quantitative Estimation of Imino Acids in Black Wattle Heartwoods, Barks and Leaf Extracts.

Stem and root heartwood drillings, root bark, stem bark and twig bark which had been cut across the grain into thin slices, and leaves which had been macerated in a "Waring Blendor" (1g. of each) were extracted with aqueous 80% (v./v.) ethanol (5 ml.) for 24 hr. Samples

(0.05 ml.) of each solution were run on Whatman no. 1 paper in the same solvents as before, and sprayed with the same spray reagents.

TABLE 14.

Percentage Concentration of Imino Acids in Fresh Black Wattle Barks, Heartwoods and Leaves.

Portion of Tree	(-)-Pipecolic Acid	4-OH-(-)-Pipecolic Acid	(-)-Proline
Leaves	1.0 (1.8)	0.6 (1.1)	0.2 (0.4)
Twig bark	0.8 (1.3)	0.3 (0.5)	0.2 (0.3)
Stem bark	0.2 (0.3)	0.2 (0.3)	0.1 (0.2)
Root bark	0.6 (1.0)	0.3 (0.5)	0.2 (0.3)
Stem heartwood	0.04 (0.04)	0.05 (0.05)	N.D.*
Root heartwood	0.02 (0.03)	N.D.*	N.D.*

The values in parentheses are on a dry-weight basis.
N.D.* = not detectable.

Standard solutions of L-(-)proline, trans-4-hydroxy-L-(-)-pipecolic acid and L-(-)-pipecolic acid were prepared by dissolving 1.0 mg. of each in water (5 ml.) and spotting suitable volumes (0.1, 0.2, 0.3, etc.) on two-dimensional chromatograms. Comparison of these sprayed chromatograms with those obtained from the various extracts of black wattle bark, also run at suitable dilutions, enabled semi-quantitative estimations of these three imino acids and their average nitrogen content (cf. Tables 14 and 15).

The Carbohydrate Content of Flowers, Leaves, Stem Bark, Stem Sapwood and Stem Heartwood of Black Wattle.

The aqueous ethanolic extracts (as above for the imino acids) of the flowers, leaves, stem bark, sapwood and heartwood of the black

TABLE 15.

The Average Nitrogen Content of Amino and Imino Acids.

Compound	% of Nitrogen Fraction	% Nitrogen		Average % Nitrogen
Pipecolic acid	25	10.9		12.7
4-Hydroxy-pipecolic acid	25	9.7		
Proline	25	12.2		
Arginine	± 25	24.1	14.75 average	
∝ -Alanine		15.7		
Aspartic acid		9.7		
Glutamic acid		9.5		
Serine	Trace	13.3		

wattle tree were examined. In the heartwood fructose, glucose and sucrose were absent, but (+)-pinitol was present. The sapwood contained mainly fructose, accompanied by low concentrations of sucrose and glucose. Both the bark and leaves contained mainly sucrose and (+)-pinitol, with a low admixture of glucose and fructose. The flowers contained fructose in fairly high proportion, with low concentrations of sucrose, glucose and (+)-pinitol.

The Leucoanthocyanidin Content of the Leaves, Twig Bark, Stem Bark and Root Bark of Black Wattle.

Root bark, stem bark (sampled at various positions), twig bark and leaves were extracted with methanol for 24 hr. The extracts were evaporated to dryness under reduced pressure at 60° and the anthocyanidins were generated from the solids (1-2 mg.) of each by the method of Pigman et al.[198]. This consists of heating a solution of the material

in isopropanol : 3N hydrochloric acid (4:1, v./v.) for 1 hr. in a water-bath using a pressure vessel. The resultant red solutions of anthocyanidins were streaked on Whatman no. 1 paper and run in a mixture of 90% formic acid : 3N HCl (1:1, v./v.)[194] for 2 hr. The R_F values of the resultant bands were calculated and the colours were noted. Details are contained in Table 16.

Detection of Shikimic Acid and Quinic Acid in Black Wattle Bark.

The tannins were precipitated from an aqueous solution of fresh black wattle bark extract using basic lead acetate. The lead tannate was precipitated by centrifuging for 20 min. at 3000 r.p.m. The clear non-tannin solution was passed slowly through an anion-exchange-column ("Permutit De-acidite F F") in the CO_3^{2-} form. After washing the column with water, the acids were eluted from the column using a 10% aqueous ammonium carbonate solution. Evaporation to dryness on a water-bath removed the ammonium carbonate and left an off-white residue of organic acids.

The residue was chromatographed on Whatman no. 1 paper in n-butanol : acetic acid : water (6:1:2, by vol.). In addition to the amino acids and imino acids, detected by ninhydrin and isatin sprays, the presence of small quantities of other acids were shown by bromocresol green[211] which gave a yellow on a blue-green background.

Chromatography with reference acids showed the presence of shikimic acid, when sprayed with sodium periodate-aniline reagent[212], and quinic acid, using the sodium periodate-sodium nitroprusside-piperazine spray[213].

TABLE 16.

The Anthocyanidins Generated from Black Wattle Barks and Leaves.

Anthocyanidin	Colour	Root Bark, 6-12" below Ground Level	Bark, Ground Level	Bark, 5ft. above Ground Level	Bark, Base of Main Branch	Twig Bark	Leaves
Delphinidin	Red-purple	-	+	+	+	+	+
Cyanidin	Red-blue	-	-	-	-	-	-
Robinetinidin	Violet-pink	+++	+++	+++	+++	+++	-
Fisetinidin	Red-pink	+++	+	+	+	+	-

- = Absent, + = present, +++ = present in high concentration.

Sodium periodate (160 mg.) was dissolved in an acetate buffer consisting of a mixture of equal volumes of 1N acetic acid and 1N sodium acetate, prepared fresh daily. The chromatogram was sprayed with this solution, allowed to dry and finally sprayed with a 3% ethanolic aniline solution, when shikimic acid produced a cherry-red spot on a pale-yellow background.

Quinic acid was detected by spraying the chromatogram with a saturated solution of sodium periodate diluted with two volumes of water. After allowing the paper to dry, it was sprayed with a solution consisting of sodium nitroprusside (50 mg.) and piperazine (50 mg.) in water (2 ml.) and ethanol (10 ml.). Heating the chromatogram for 5 minutes at 100° showed quinic acid as an orange-yellow spot. The R_F of quinic acid (0.30) was fractionally lower than that of shikimic acid (0.49).

DISCUSSION.

The structural complexity of the natural condensed tannins is well known. They are considered to be comprised of C_{15}-flavan nuclei having 3,4-diol or 3-ol type substitution. Considerable attention has been paid to their chemistry since the 1920's, but significant progress in this field has only been possible in very recent years.

Problems associated with the structural investigation of the tannins are essentially three-fold: their isolation in stereochemically pure form from extremely complex mixtures, their ease of denaturation, and difficulties encountered in obtaining significant degradation products. The advent of physical techniques, such as nuclear-magnetic-resonance (n.m.r.) spectroscopy, paper ionophoresis, mass spectrometry and optical rotatory dispersion, have provided new methods of approach also in this field.

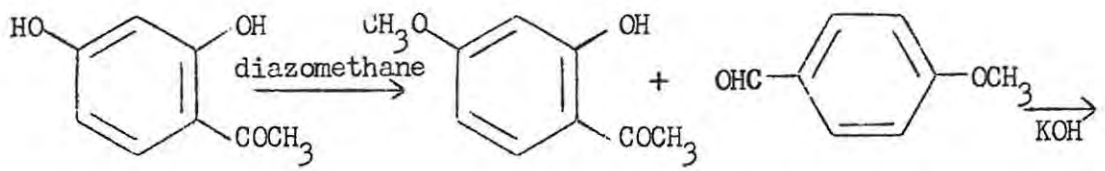
Condensed tannins from black wattle bark yield anthocyanidins on treatment with acids, and are therefore proanthocyanidin in character. High resolution n.m.r. spectroscopy has proved to be a particularly powerful physical aid, providing data regarding the chemical shifts and splitting patterns of heterocyclic and benzenoid protons of component flavandiol and flavanol units. Since the link between flavan-3,4-diol or catechin moieties was suspected to exist between heterocyclic and benzenoid carbon atoms, detailed knowledge of the chemical shifts and coupling constants of protons in model compounds was essential for determining the structures of tannins.

In order to establish accurate criteria for subsequent work on the biflavanols a complete series of the four possible racemates of a related flavan-3,4-diol was required. Since diols with a high degree of hydroxyl substitution on benzenoid nuclei are known to present synthetic problems, the synthesis of the four racemates of 7,4'-dimethoxyflavan-3,4-diol was attempted - the methyl ethers exhibiting greater stability than the corresponding free-phenolic forms. The synthetic routes leading to these racemates were abbreviated by preparing the two readily available isomers, and attempting selective epimerization of these to give the remaining desired racemates.

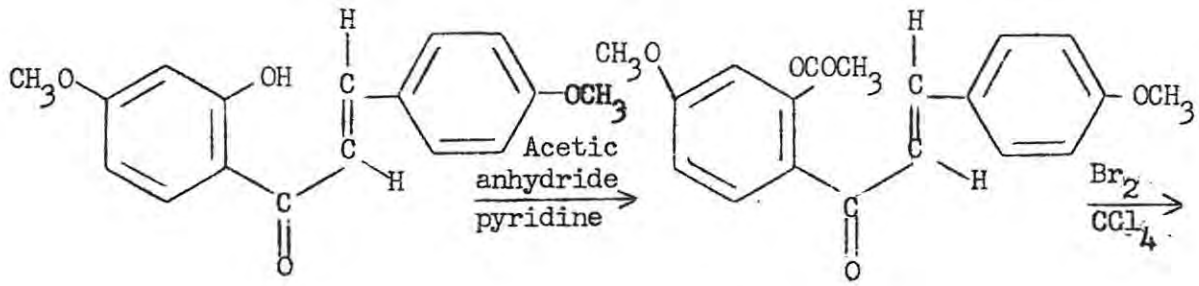
Syntheses of the Racemates of 7,4'-Dimethoxyflavan-3,4-diol.

The 2,3-cis-3,4-cis and 2,3-trans-3,4-trans diol racemates were readily synthesized by stereospecific hydrogenations. These were selectively epimerized at C-4 to yield the required 2,3-cis-3,4-trans and 2,3-trans-3,4-cis isomers, respectively.

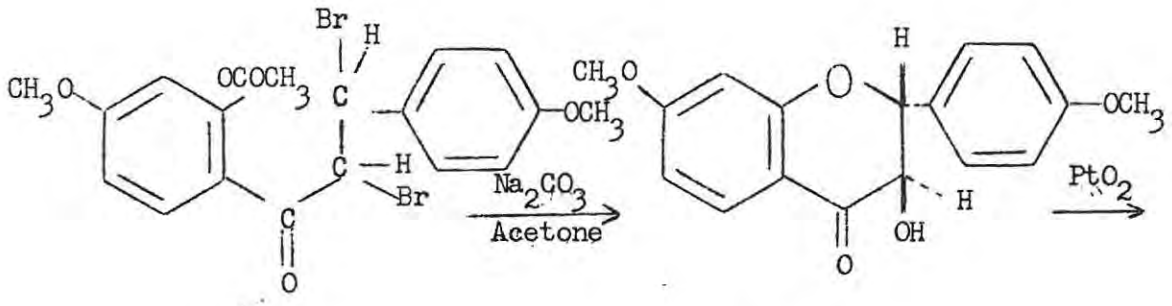
All the syntheses depend on the preparation of the key intermediate, 2'-hydroxy-4,4'-dimethoxychalcone IV, obtained by condensing 2-hydroxy-4-methoxyacetophenone (peonol) II with anisaldehyde III in an inert atmosphere. Oxidative ring-closure of 2'-hydroxy-4,4'-dimethoxychalcone with hydrogen peroxide in alkaline solution yielded 7,4'-dimethoxyflavonol X, while successive acetylation and bromination of the chalcone gave 2'-acetoxy-4,4'-dimethoxychalcone dibromide VI. Ring closure of the latter using an aqueous sodium carbonate-acetone mixture gave 7,4'-dimethoxy-2,3-trans-dihydroflavonol VII.



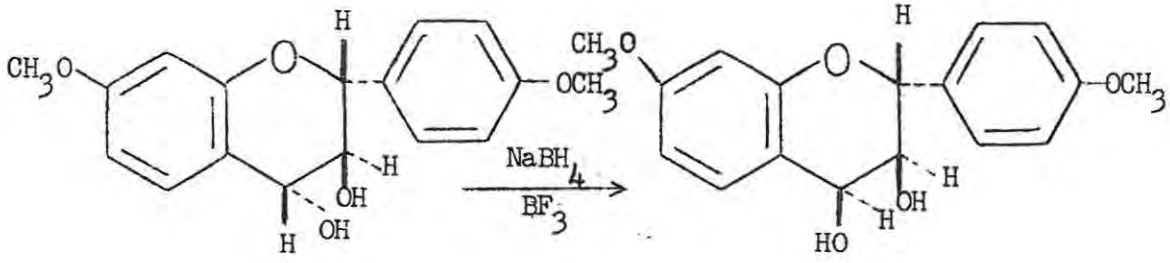
(I) (II) (III)



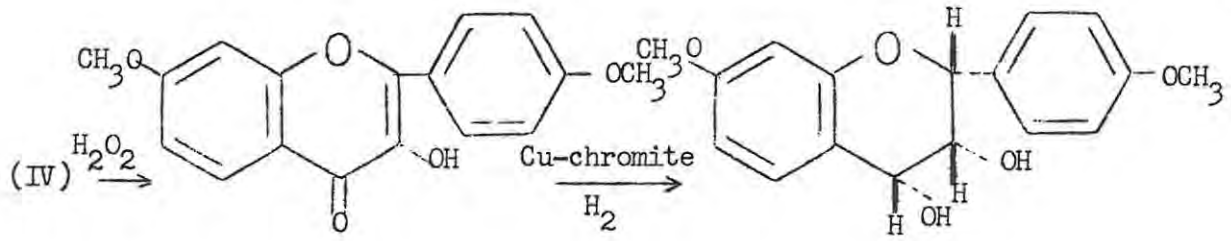
(IV) (V)



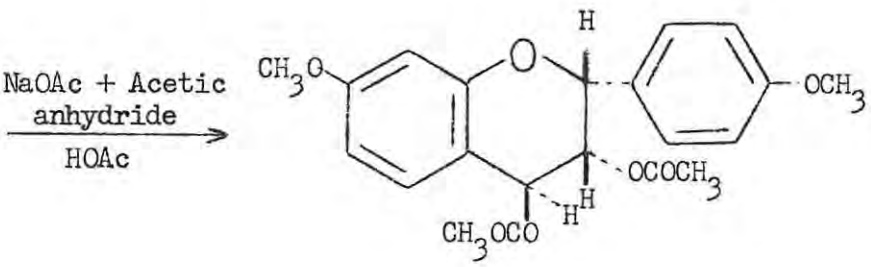
(VI) (VII)



(VIII) (<u>trans</u>, <u>trans</u>-diol). (IX) (<u>trans</u>, <u>cis</u>-diol).



(X) (XI) (cis, cis-diol)



(XII) (cis, trans-diol-diacetate)

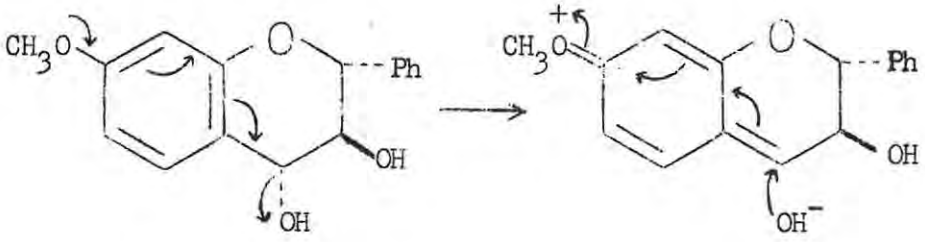
Reduction of this dihydroflavonol VII with Adams catalyst under conditions first used by Roux and Freudenberg[61] yielded the 7,4'-dimethoxy-2,3-trans-flavan-3,4-trans-diol VIII. Assuming a half-chair configuration with the 2-phenyl group in the equatorial position, this synthesis therefore gives the 2,3-trans-3,4-trans ($J_{2,3}$ = 9.0 ; $J_{3,4}$ = 6.9 c./sec.) racemate with a preferred 2(eq), 3(eq), 4(eq) arrangement of substituents.

The epimerization method of Kashikar and Kulkarni[82] was used for obtaining the 2,3-trans-3,4-cis isomer of 7,4'-dimethoxyflavan-3,4-diol IX. This involved the selective epimerization of the 2,3-trans-3,4-trans racemate at C-4 with sodium boronhydride and boron trifluoride in diglyme, giving only the 2,3-trans-3,4-cis diol in good yield. This racemate ($J_{2,3}$ = 10.3 ; $J_{3,4}$ = 3.1 c./sec.) has the preferred 2(eq),

3(eq), 4(ax) configuration on the above assumptions.

7,4'-Dimethoxy-2,3-cis-flavan-3,4-cis-diol XI was prepared by catalytic reduction of 7,4'-dimethoxyflavonol X using copper chromite at high temperature and pressure. Raney nickel afforded the same racemate but in lower yield. Catalytic hydrogenation of the carbonyl and ethylenic double bonds of planar flavonols results in cis addition[214]. The cis, cis diol ($J_{2,3}$ = 1.2 ; $J_{3,4}$ = 4.2 c./sec.) has the preferred 2(eq), 3(ax), 4(eq) arrangement.

Synthesis of the 2,3-cis-3,4-trans racemate proved the most difficult of the four, as anticipated. Attempted synthesis of this racemate, using methods previously employed for flavan-3,4-diols having 6-methyl substituted A-rings, gave the now anticipated negative result. This may be due to electron release from the 7-methoxyl group, allowing the formation of a quinone-methide intermediate which would create an active electrophilic centre at C-4[215] (cf. XIII). Side reactions arising from such a situation would be : facile inversion at this point, and the possibility of polymerization as a result of linkage with nuclei having nucleophilic centres.



(XIII)

In relation to this difficulty it is interesting to note that Rao and Venkateswarlu[216] showed how an ethyl group at C-6 of a 7-hydroxyflavan has a stabilizing effect on the molecule - no explanation was given for this observation.

A possible method of epimerization of 2,3-cis-3,4-cis diols by acetylation to give the diacetates of 2,3-cis-3,4-trans diols was evident from the work of Fujise et al.[68,69]. They found that acetylation with acetic anhydride and pyridine of two flavan-3,4-diols, stereoisomeric at C-4, gave their respective diacetates, but that drastic acetylation with acetic anhydride and sodium acetate yielded the same diacetate. This suggested the epimerization of one of the diols under these conditions. Their method was successfully used to convert the 7,4'-dimethoxy-2,3-cis-flavan-3,4-cis-diol XI to the desired 7,4'-dimethoxy-3,4-trans-diacetoxy-2,3-cis-flavan XII. At present this represents the only route whereby this racemate may be synthesized; the reaction probably proceeding via an intermediate acetoxonium ion[217]. Concurrent epimerizations by Clark-Lewis et al.[75] of 6-methyl-flavan-3,4-diols served to confirm these results. The 2,3-cis-3,4-trans product XII ($J_{2,3}$ = 0.9 ; $J_{3,4}$ = 2.8 c./sec.) has the preferred 2(eq), 3(ax), 4(ax) arrangement of substituents.

Mechanism of Epimerization of 2,3-trans-3,4-trans to 2,3-trans-3,4-cis Racemate with $NaBH_4$ and BF_3.

The epimerization of flavan-3,4-diols with $NaBH_4$ and BF_3 was initiated by Kulkarni et al.[218] who proposed no mechanism for the inversion. Their stereochemical conclusions have also been shown to be

inaccurate[48,53]. The reaction mechanism of the mixed reagent is apparently analogous to the $LiAlH_4$ + $AlCl_3$ epimerizations of alcohols[219].

2,3-trans-3,4-trans-7,4'-Dimethoxyflavan-3,4-diol XIV, having the preferred 2(eq), 3(eq), 4(eq) arrangement of phenyl and hydroxyl substituents, was selectively epimerized at C-4 with $NaBH_4$ + BF_3 in diglyme to give a quantitative conversion to the 2,3-trans-3,4-cis racemate. In this inversion the H^+ ions of the hydroxyl groups substituted at C-3 and C-4 are presumably replaced by bulky BF_2^+ ions, resulting in steric crowding of two vicinal and equatorial $-OBF_2$ groups XV. This unstable situation promotes a strong steric acceleration to inversion at C-4, causing the 4(eq) $-OBF_2$ substituent to swing to the less crowded 4(ax) position XVI. Treatment with acetic acid yields the epimeric 2,3-trans-3,4-cis diol XVII.

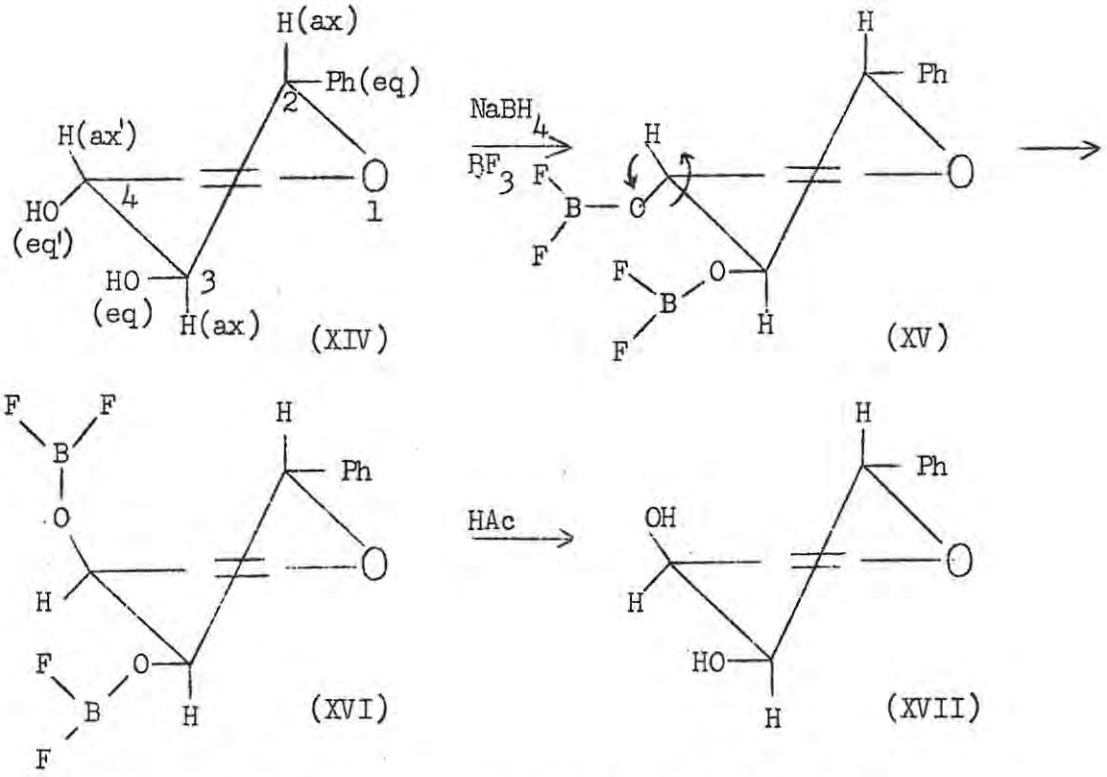
The correct stoichiometric proportions of $NaBH_4$: BF_3 is essential for the epimerization to be quantitative[219] {cf. (a) below}.

On this basis selective epimerization of 2,3-cis-3,4-cis flavandiols, with the mixed reagent, to the 2,3-cis-3,4-trans isomers was unsuccessful, although the mechanism involves a similar inversion of a 4(eq) to a 4(ax) hydroxyl as above. Its failure is predictable when the equatorial and axial arrangements of these two racemates are considered - entailing the rearrangement of 3(ax), 4(eq)- to the more crowded 3(ax), 4(ax)- configuration.

$$NaBH_4 + 3BF_3 \longrightarrow NaF + {}_4BHF_2$$

$$R\text{-}OH + BHF_2 \longrightarrow R\text{-}OBF_2 + H_2$$

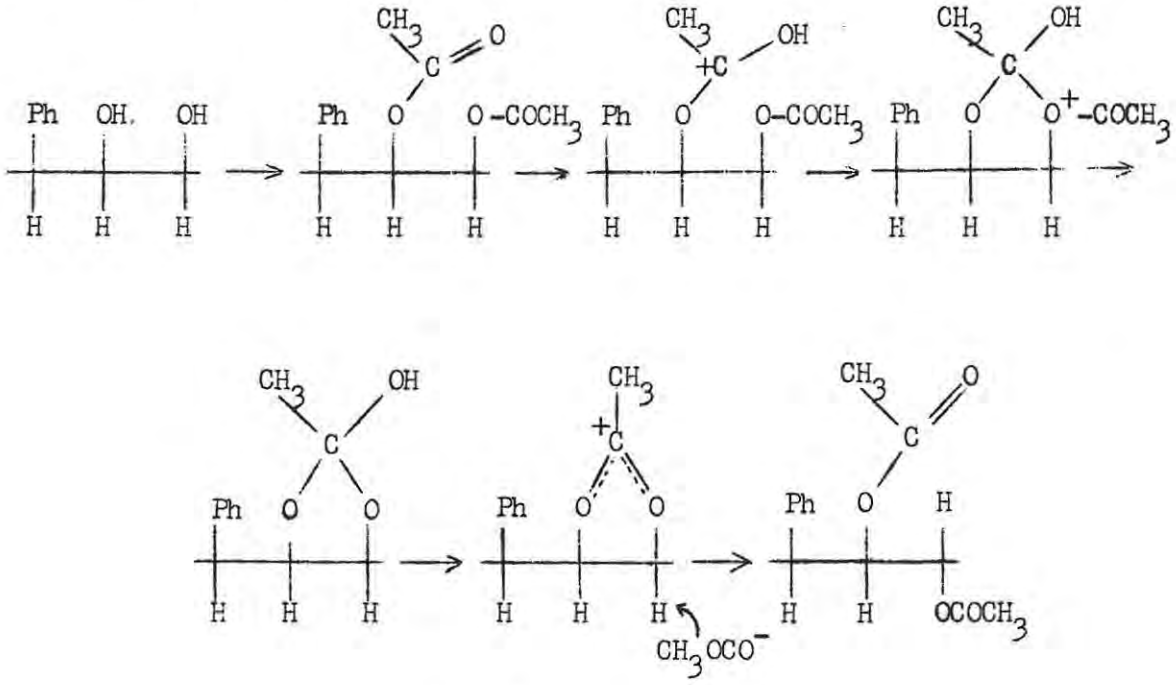
(a)



Mechanism of Epimerization of 2,3-cis-3,4-cis-Flavan-3,4-diols to 2,3-cis-3,4-trans-diacetylflavans by Acetylation.

Saayman and Roux[83] suggested that selective epimerization at C-4 of 2,3-cis-3,4-cis diols to 2,3-cis-3,4-trans diacetates by acetylation proceeded via a cyclic intermediate. The mechanism is visualized to be analogous to that proposed by Angyal, Gorin and Pitman[217] for the stereospecific epimerization of cis-glycol arrangements in cyclitols. This involves the formation of a cyclic acetoxonium ion intermediate. The 2,3-cis-3,4-cis diol initially yields the 3,4-cis diacetate, leading to the formation of a positively charged acetoxonium ion through the loss of acetylium ions (cf. XVIII). Nucleophilic attack on the acetoxonium ion by an acetoxy group involves the shift of an electron-pair to the positive centre. Steric effects cause the acetate ion to approach C-4 from the less hindered trans-position, giving the 2,3-cis-3,4-trans-

diacetoxyflavan. A small amount of the cis, cis diacetoxy flavan was also formed - probably due to inversion occurring at C-4 caused by the 7-methoxyl substitution, as shown before.



(XVIII)

Clark-Lewis and Williams[84] came to similar conclusions regarding the mechanism of this epimerization. In their case the reaction proceeded more readily due to the fact that only the 6-position of the A-ring was substituted by a methyl group.

Nuclear-Magnetic-Resonance Spectra of 7,4'-Dimethoxyflavan-3,4-diol Racemates.

The relative configurations of the methyl ether diacetates of the synthetic racemates were confirmed by n.m.r. spectroscopy (later correlated with paper ionophoresis of the methyl ethers) by comparison with data from analogous compounds.

Chemical shifts of the heterocyclic and benzenoid protons and coupling constants of the 2-, 3- and 4-protons of the racemates of 7,4'-dimethoxyflavan-3,4-diacetate are summarized in Table 1 (cf. Figs. 1, 3, 4 and 6).

Integral curves of these spectra allow the allocation of seven protons in the benzenoid region, three in the heterocyclic area and the remainder in the methoxyl and acetyl regions.

The six protons of the 3- and 4-acetyl groups, situated furthest upfield at τ = 7.87 - 8.16 p.p.m., show two sharp three-proton signals which are unsplit by spin-spin coupling. Chemical shifts of these acetyl protons may be correlated with their stereochemistry in that axial acetoxy-groups are shifted downfield in each case, when compared with equatorial acetoxy-groups which always occur higher upfield (cf. Bokadia, Brown et al.[48]).

At τ = 6.19 - 6.25 p.p.m. the six protons of the 7- and 4'-methoxyl groups occur as two singlets, the 7-methoxyl signal being at a higher field than the 4'-methoxyl peak.

The 5-, 6- and 8-benzenoid protons of the A-ring are discernable as a doublet (5-proton), centred at τ = 2.7 - 2.9 p.p.m. ; a quartet (6-proton), centred at τ = 3.43 p.p.m. ; and a doublet (8-proton), centred at τ = 3.50 - 3.54 p.p.m. Magnetic equivalence of the 2' and 6', and 3' and 5' benzenoid protons in the C-ring produces an A_2B_2 system, both pairs giving ortho-coupled (J = 8.5 c./sec.) doublets.

Heterocyclic C-ring protons form an ABX system with the 2- and 4-protons represented as doublets and the 3-proton as a quartet. The spectrum of the 2,3-*cis*-3,4-*trans* isomer is differentiated from those of the other three racemates by the relative upfield positions of both 3- and 4-protons (τ = 4.77 and 4.15 p.p.m., respectively) and by the far downfield position of the 5-proton (τ = 2.65 p.p.m.) (*cf.* Table 1). The $J_{2,3}$ coupling constant of this racemate could be calculated from analysis of the 3-proton quartet at 100 Mc/sec.

Presuming a half-chair conformation for the heterocyclic ring and a predominantly 2(*eq*) arrangement of the 2-phenyl group, the correlation between the vicinal coupling constants and dihedral angles, as measured from Dreiding models on the basis of Karplus' valence bond equation[55], is good. These values for the 2,3-*trans*-3,4-*trans* ($J_{2,3}$ = 9.0 ; $J_{3,4}$ = 6.9 c./sec. ; $\theta_{2,3}$ = 190°, $\theta_{3,4}$ = 160°), 2,3-*trans*-3,4-*cis* ($J_{2,3}$ = 10.3, $J_{3,4}$ = 3.1 c./sec. ; $\theta_{2,3}$ = 180°, $\theta_{3,4}$ = 50°), 2,3-*cis*-3,4-*trans* ($J_{2,3}$ = 0.9, $J_{3,4}$ = 2.8 c./sec. ; $\theta_{2,3}$ = 70°, $\theta_{3,4}$ = 50°), and 2,3-*cis*-3,4-*cis* racemates ($J_{2,3}$ = 1.2, $J_{3,4}$ = 4.2 c./sec. ; $\theta_{2,3}$ = 70°, $\theta_{3,4}$ = 70°) are in general agreement with values obtained for analogous flavan-3,4-diol racemates by Drewes and Roux[47] and Clark-Lewis *et al.*[31].

The relative configurations of substituents of the racemates are as follows: 2,3-*trans*-3,4-*trans* [2(*eq*), 3(*eq*), 4(*eq*)], 2,3-*trans*-3,4-*cis* [2(*eq*), 3(*eq*), 4(*ax*)], 2,3-*cis*-3,4-*trans* [2(*eq*), 3(*ax*), 4(*ax*)] and 2,3-*cis*-3,4-*cis* [2(*eq*), 3(*ax*), 4(*eq*)].

Paper Ionophoresis of 7,4'-Dimethoxyflavan-3,4-diol Racemates.

The paper ionophoretic behaviour of the methyl ethers of flavan-3,4-diols, on correlation with their stereochemistry as established by n.m.r. techniques, offers a simple but reliable criterion for differentiating between certain 3,4-cis and 3,4-trans diol arrangements.

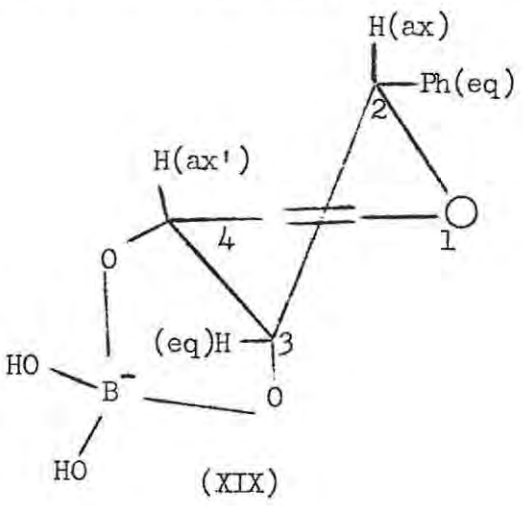
Examination of the ionophoretic mobilities of 7,4'-dimethoxyflavan-3,4-diol racemates (cf. Table 5) in sodium borate solution showed variable positive mobilities for the 3,4-cis diols, the 2,3-trans-3,4-cis isomer having a higher positive mobility than the 2,3-cis-3,4-cis compound. This may be due to an increase of affinity of the 2,3-cis isomer for cellulose in comparison with the 2,3-trans racemate, and may be related to molecular shape[196]. Parallel affinity effects are shown by paper chromatography in water or dilute acetic acid solutions[220].

The 2,3-trans-3,4-trans isomer shows a negative migration possibly caused by electro-endosmotic flow.

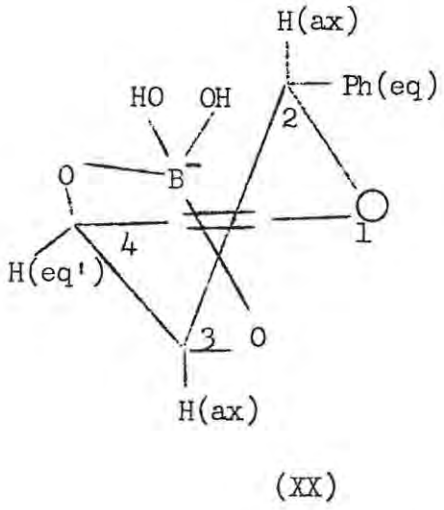
Paper ionophoresis in borate solution under standard conditions apparently affords a method for distinguishing between methylated flavan-3,4-cis- and 3,4-trans-diols without resorting to the use of reference compounds (cf. Drewes and Roux[56]).

Raman spectral studies and X-ray diffraction data on boron minerals[221] have indicated that the borate ion has tetrahedral symmetry and may be represented by $B(OH)_4^-$. Boric acid therefore does not act as a proton donor, but rather as a Lewis acid which may accept an electron pair of a base (e.g. OH^-) to form the tetrahedral anion $B(OH_4)^-$ [222].

The complex formed between borate ions and the hydroxyl groups of 2,3-cis-3,4-cis and 2,3-trans-3,4-cis diol systems may be represented by XIX and XX, respectively.



(XIX)



(XX)

The Relative Configuration of Guibourtacacidin from Guibourtia coleosperma.

Synthesis of the four racemates of 7,4'-dimethoxyflavan-3,4-diol, and their n.m.r. spectral analysis, enabled the structural elucidation of the natural guibourtacacidins[223]. Comparison of the n.m.r. spectrum of the diacetate of this product (cf. Fig. 7) with those of the synthetic racemates (cf. Figs. 1,3,4 and 6) showed it to be a mixture of three isomers. The 2,3-cis-3,4-trans diastereoisomer was predominant, with low concentrations of 2,3-trans-3,4-trans and 2,3-trans-3,4-cis isomers, these three occurring in the approximate ratio of 5:1:1.

For example, the n.m.r. spectrum of guibourtacacidin shows a doublet at τ = 4.13 p.p.m. which corresponds to the uniquely high upfield (τ = 4.15 p.p.m.) 4-proton of the 2,3-cis-3,4-trans synthetic isomer. Smaller signals at τ = 3.68-3.88 p.p.m. in the spectrum of

guibourtacacidin agree with the 4-proton allocations of the 2,3-trans-3,4-trans and 2,3-trans-3,4-cis synthetic racemates. The same applies to the positions of the 2-proton signals. Protons due to the acetyl groups (τ = 7.89 and 8.12 p.p.m.) of guibourtacacidin show close identity with corresponding peaks of the 2,3-cis-3,4-trans isomer (τ = 7.92 and 8.08 p.p.m.), while the minor signals in this region (τ = 8.00, 8.09 and 8.15 p.p.m.) in the spectrum of guibourtacacidin reflect the corresponding signals of the acetyl protons of the two minor isomers.

Guibourtacacidin was initially assigned the 2,3-trans-3,4-trans configuration[224]. This prompted Phatak and Kulkarni[186] to attempt the synthesis of (±)-7,4'-dimethoxy-3,4-diols related to guibourtacacidin. Reduction of 7,4'-dimethoxy-2,3-trans-dihydroflavonol (m.p. 133°) with $LiAlH_4$ gave them a mixture of 2,3-trans diols melting over the range 113-121°. Resolution by acetylation and subsequent hydrolysis yielded diols, m.p. 114-115° and 135-136°, to which they assigned the 2,3-trans-3,4-cis and 2,3-trans-3,4-trans configurations, respectively.

The latter compound (m.p. 135-136°), kindly made available by Professor A.B. Kulkarni, is now shown to be a mixture of two geometrical isomers by n.m.r. spectrometry[53] (cf. Fig. 5). The structural assignments of Phatak and Kulkarni for the diols, m.p. 135-136° (presumed 3,4-trans) and m.p. 114-115° (presumed 3,4-cis) were also shown to be erroneous and had to be reversed according to the revised configurations proposed by Bokadia, Brown et al.[48].

Epimerization of Optically Active Free-Phenolic Natural Flavan-3,4-diols.

Structural and stereochemical elucidation of the 7,4'-dimethoxy-flavan-3,4-diol racemates, having a low degree of benzenoid hydroxylation,

paved the way to the investigation of members in the series which have higher hydroxyl substitution. Phenolic hydroxyl substitution confers higher reactivity to the flavonoid molecule. Hydroxyl groups at the 3',4' and 5' positions of the C-ring, for instance, enhance the sensitivity of the diol to oxidative condensation. This is probably due to the para-directing effect of the 3' and 5' hydroxyl groups, creating additional nucleophilic sites at the 2' and 6' positions.

The investigation of the stereochemistry of the 7,4'-dimethoxyflavan-3,4-diol racemates enabled the structural and stereochemical study of the biflavanol components B and D. These contain leucorobinetinidin nuclei[226], and hence the epimerization of (+)-leucorobinetinidin [(+)-7,3',4',5'-tetrahydroxy-2,3-trans-flavan-3,4-trans-diol] was first investigated. The epimerization procedure developed by Drewes and Roux[86] for (+)- and (-)-7,3',4'-trihydroxy-flavan-3,4-diols of the same relative configuration was followed. This entailed autoclaving aqueous solutions of the optically pure diols and separation of the four diastereoisomers by paper chromatography in 2% acetic acid, followed by water-saturated sec.-butanol. (+)-2,3-trans-3,4-trans-Leucorobinetinidin under these conditions gave rise to its remaining three isomers. Limited amounts of the natural product and the ease of oxidation of its isomers, due to the pyrogallol nucleus, precluded their isolation. Results of the paper chromatographic investigation of these diastereoisomers were in agreement with those of Drewes and Roux[86] for the corresponding trihydroxyflavan-3,4-diols. R_F comparison of the diastereoisomers generated from (+)-leucorobinetinidin with the leucorobinetinidin biflavanols B and D showed the latter com-

pounds to be distinct from these isomers.

The epimerization of these monomeric natural products, having a high degree of hydroxyl substitution of the B-ring, coupled with the syntheses of the 7,4'-dimethoxyflavan racemates by total synthetic and epimeric methods, facilitated the approach to the structural determination of the more complex condensed tannins.

Between the monomeric flavanoid components and the highly condensed tannins of black wattle bark extract there is distributed an entire series of low molecular weight condensed products - the so-called "phenolic half-tannins". Of these the leucorobinetinidin biflavanols B and D were studied in some detail.

Biflavanol Components B and D.

Biflavanols B and D were isolated from the bark of young black wattle trees by Craig countercurrent separation and paper chromatography of enriched low molecular weight fractions obtained by ethyl acetate extraction. They had close R_F values by paper chromatography. The amorphous products B and D showed instability on handling in the free-phenolic forms due to oxidation, and their final purification was achieved by thin-layer chromatography of their more stable methyl ethers.

Methylation with diazomethane resulted in the formation of an octamethyl ether of B, $C_{38}H_{42}O_{12}$, m.p. 130-135°, and a nonamethyl ether of D, $C_{39}H_{44}O_{13}$, m.p. 161°. Acetylation of these with pyridine and acetic anhydride yielded a diacetyl octamethyl ether of B, $C_{42}H_{46}O_{14}$, m.p. 120-124°, and the corresponding diacetyl nonamethyl ether of D, $C_{43}H_{48}O_{15}$, m.p. 140°. Acetylation of the free-phenolic

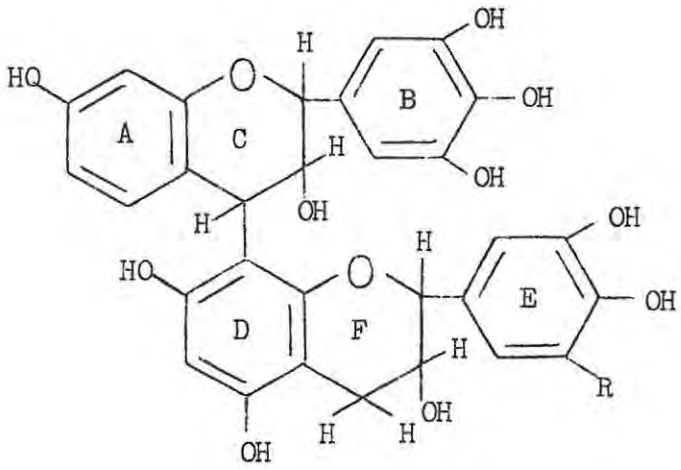
forms gave a deca-acetate of B, $C_{50}H_{46}O_{22}$, m.p. 178-185°, and an undeca-acetate of D, $C_{52}H_{48}O_{24}$, m.p. 148°. These analyses account for eight phenolic and two aliphatic hydroxyl groups in the empirical formula $C_{30}H_{26}O_{12}$ of the parent compound B. Product D similarly reflects an empirical formula $C_{30}H_{26}O_{13}$ with nine phenolic hydroxyl groups.

The free-phenolic form of B, which had been purified by preparative paper ionophoresis in borate buffer, on fusion with solid potassium hydroxide gave the degradation products resorcinol, phloroglucinol, protocatechinic acid, gallic acid and β-resorcylic acid. Product D on similar treatment yields the same products with the exception of protocatechuic acid. This shows the presence of both resorcinol and phloroglucinol nuclei in the A- and D-rings of the products B and D, with 3',4' and 3',4',5' hydroxyl substitution in the B- and E-rings of component B and 3',4',5' substitution in both these rings of component D (cf. XXI).

Products B and D gave relatively low yields of anthocyanidins on treatment with acid - individual treatment of these with 3N HCl and isopropanol under pressure[198] (for the generation of anthocyanidins) giving both robinetinidin chloride (R_F = 0.30) and an orange-coloured component (R_F = 0.65). Acid-induced fission of B with 3N HCl-ethanol[96] (for the generation of catechins) gave, amongst other minor products, (+)-catechin, the orange pigment, resorcinol and phloroglucinol.

Similar hydrolytic fission of D yielded (+)-gallocatechin, the same orange pigment, resorcinol and phloroglucinol. The action of acids on B and D therefore appears to cleave the molecule into an antho-

cyanidin fraction and a catechin fraction under different conditions. Products B and D both yield the same anthocyanidin, indicating identically substituted A- and B-rings. The observation that product B yields (+)-gallocatechin, whereas D gives (+)-catechin, serves to indicate that in the latter case an additional hydroxyl group is substituted at the 5'- position of the E-ring.

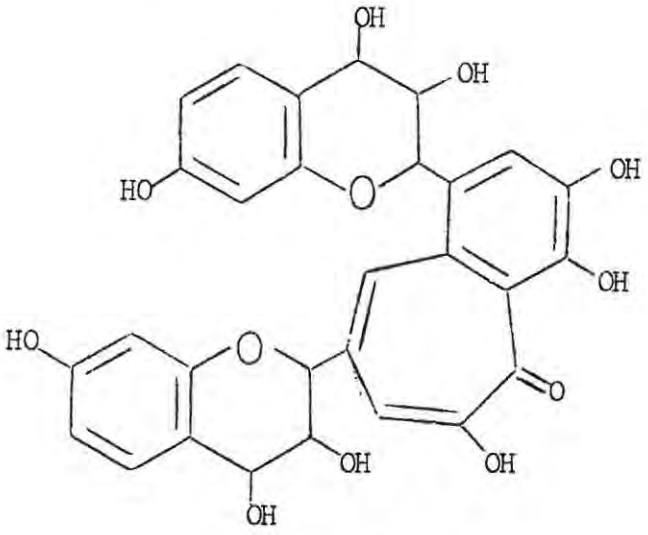


B : R = H
D : R = OH

(XXI)

Condensation of (+)-catechin and (+)-gallocatechin with (+)-7,3',4',5'-tetrahydroxy-2,3-trans-flavan-3,4-trans-diol under conditions developed by Creasy and Swain[106] yielded amongst others products which were identical by chromatography with B and D, respectively. These condensations therefore indicate that the natural biflavanols B and D may be derived from a (+)-2,3-trans-3,4-trans-leucorobinetinidin nucleus linked to a (+)-2,3-trans-catechin or (+)-2,3-trans-gallocatechin unit.

Paper chromatography of the condensation products of B and D, obtained by this treatment with 0.4N HCl, showed the presence of the same orange pigment as generated by degradative methods. The pigment is therefore a prominent degradation product of both biflavanols, and an attempt was made to isolate it from fresh black wattle bark extract. The marked instability of the pigment and its derivatives, however, complicated its isolation and structural determination. It is suggested that the pigment is formed from the degradation products by a process of ring expansion of the leucorobinetinidin moieties to give a benzotropolone structure XXII similar to the products isolated by Takino et al.[227], and Ollis et al.[228] from the fermentation products of tea leaf extracts.



(XXII)

These authors proposed the formation of an orange pigment from (-)-epicatechin nuclei during these fermentation processes. Saayman and Roux[229], however, showed that neither (+)-catechin nor

(+)-gallocatechin generated the orange pigment under the conditions of Pigman et al.[198], while (+)-2,3-trans-3,4-trans-leucorobinetinidin readily yields it. The generation of the orange pigment has been correlated with the presence of (+)-2,3-trans-3,4-trans-leucorobinetinidin or its polymers also in Robinia pseudacacia[62].

Nuclear-Magnetic-Resonance Spectra of Derivatives of B and D.

The n.m.r. spectrum of the octamethyl ether of B (cf. Fig. 15) reflects the presence of seven methoxyl signals at τ = 6.16-6.35 p.p.m., the 7-methoxyl signal of the A-ring being slightly upfield at τ = 6.43 p.p.m.

A heavily split multiplet at τ = 7.92-8.45 p.p.m. shows appreciable line-broadening and is attributable to two protons of the free-hydroxyl groups at the C-3 positions of the C- and F-rings.

Five heterocyclic protons occur as a complex multiplet at τ = 5.13-5.80 p.p.m., with a further two heterocyclic protons as a methylene multiplet at τ = 6.76-7.65 p.p.m. consistent with a catechin structure (cf. Figs. 15 and 16 for allocations of heterocyclic protons).

The integral curve shows the presence of nine benzenoid protons in a complex multiplet at τ = 3.23-3.95 p.p.m. Meta-coupled 2' + 6' protons of the B- and E-rings are distinguishable as broadened singlets at τ = 3.63 and 3.81 p.p.m.

The n.m.r. spectrum of the octamethyl ether diacetate of B (cf. Fig. 17) confirms the analyses and shows two aliphatic acetyl signals at τ = 8.43 and 8.18 p.p.m., each resulting from three protons.

The former signal is further upfield than usual in the corresponding catechin derivatives, possibly due to the diamagnetic anisotropy of the adjacent benzene ring D.

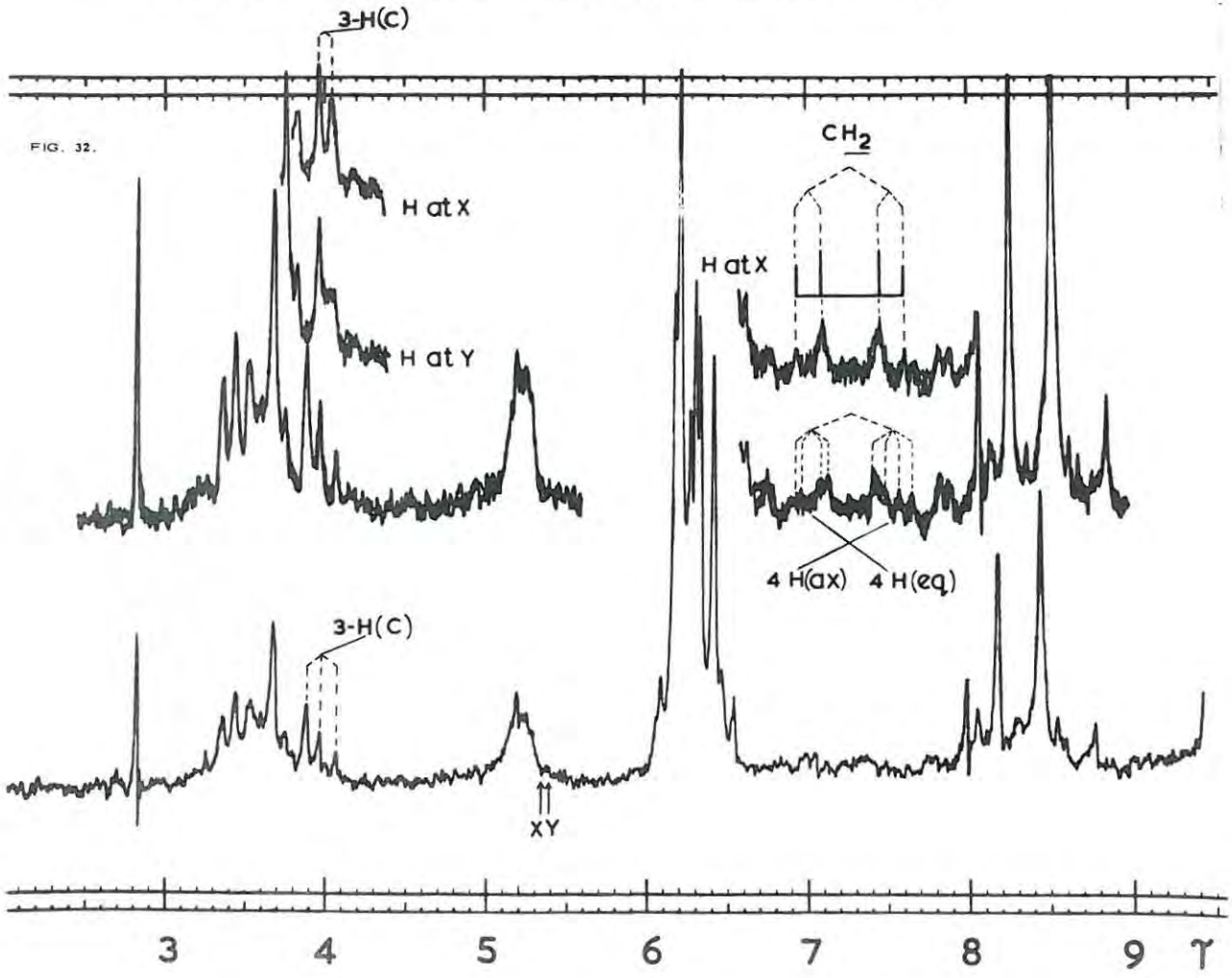
A 3-proton triplet of the heterocyclic C-ring occurs far downfield in the benzenoid proton region and partially overlaps a broadened singlet (τ = 3.89 p.p.m.) of the meta-coupled 2' + 6' protons of the B- and E-rings.

Four heterocyclic protons occur as a multiplet at τ = 5.07-5.33 p.p.m., while the remaining two protons of the heterocyclic F-ring yield a methylene multiplet at τ = 7.17 p.p.m. Spin-spin decoupling by irradiation of the four-proton multiplet above, accompanied by frequency-sweeps, resolved the methylene multiplet into an AB quartet exhibiting geminal coupling (J = 16.0 c./sec.) (cf. Fig. 32 and p.173).

The spectrum of the deca-acetate of B reflects the presence of eight phenolic acetyl groups occurring downfield (τ = 7.74-7.81 p.p.m.) from the aliphatic acetyl signals.

Heterocyclic protons of the deca-acetate of B are characterized by the upfield position of a doublet (τ = 5.57 p.p.m.), a three-proton multiplet (τ = 4.94-5.21 p.p.m.) showing a superimposed doublet (τ = 5.24 p.p.m.), and a triplet downfield at τ = 4.29 p.p.m. These two doublets and the triplet are spin-coupled as evidenced by the spin-decoupling technique (cf. Fig. 33). Decoupling experiments accompanied by frequency sweeps resulted in a simplification of the multiplet due to the collapse of the upfield doublet (τ = 5.57 p.p.m.) to give a singlet. The triplet therefore represents a 3-proton, the upfield

FIG. 32. N.M.R. Spectrum of Octamethyl Ether Diacetate of B Showing Geminal Coupling of Methylene Protons by Spin-decoupling.



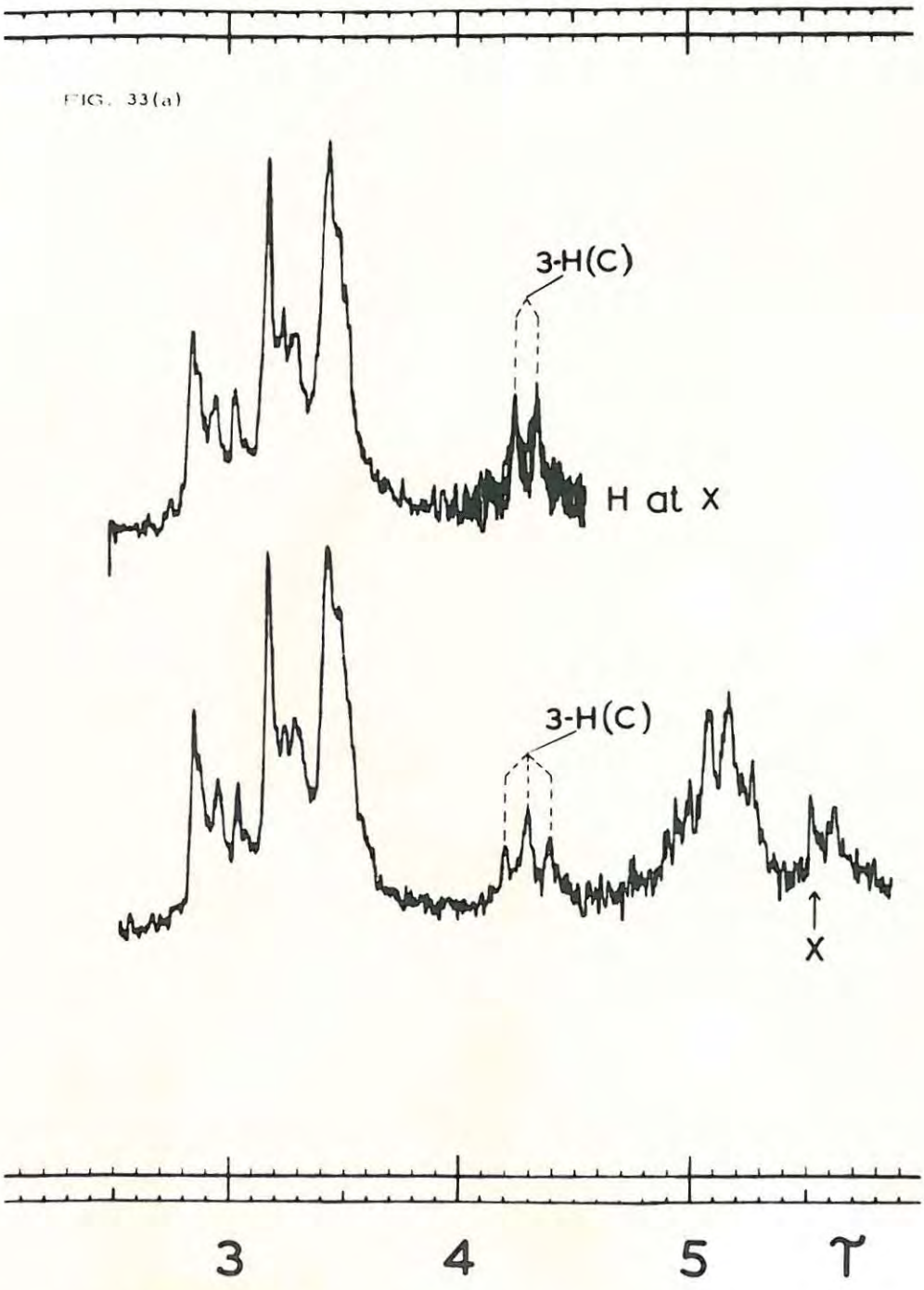
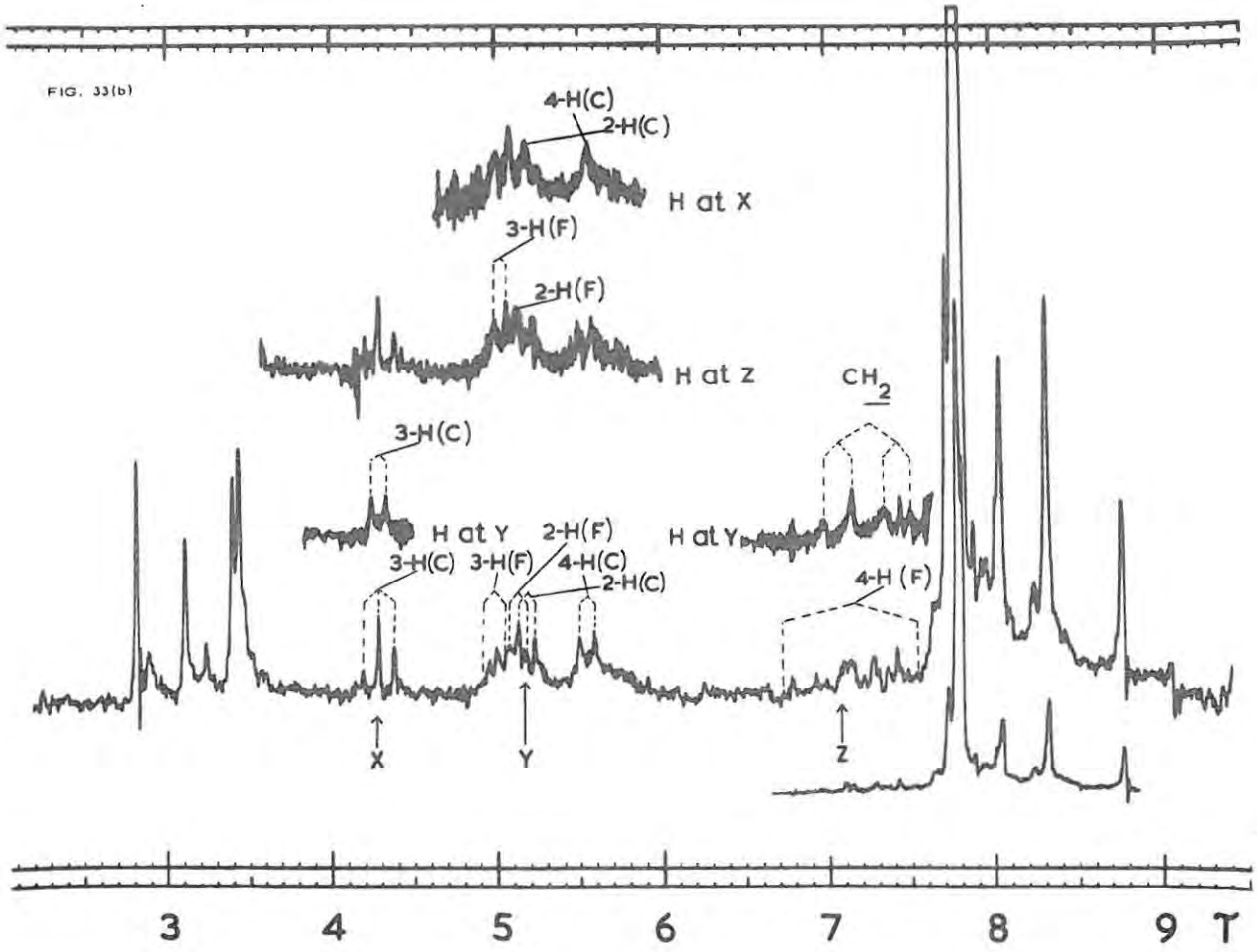


FIG. 33(a). N.M.R. Spectrum of Deca-acetate of B. Spin-decoupling Experiment.

FIG. 33(b). N.M.R. Spectrum of Undeca-acetate of D. Spin-decoupling Experiments.



doublet a 4-proton, and the doublet superimposed on the multiplet, a 2-proton - all being attributable to the C-ring. The remaining F-ring protons in the multiplet are : a 2-proton (τ = 5.12 p.p.m.) and a collapsed multiplet (τ = $\pm$ 4.94 p.p.m.) due to the 3-proton.

N.m.r. spectra of the corresponding derivatives of biflavanol D are essentially similar to those of B, except for a simplification of the multiplet resulting from the benzenoid protons. Here the 5'-position of the E-ring is hydroxylated, giving rise to a superimposed AB system of meta-coupled 2' + 6' protons of the C- and E-rings. This is reflected by doublets at τ = 3.58 and 3.75 p.p.m.

The 2-, 3- and 4-protons of the C-rings of components B and D form an ABX system with large coupling constants ($J_{2,3}$ = 9.5-10.0 c./sec. ; $J_{3,4}$ = 9.0-10.0 c./sec.) indicating trans-diaxial arrangements in each case and a 2,3-trans-3,4-trans [2(eq), 3(eq), 4(eq)] arrangement of substituents for the leucorobinetinidin portion of these biflavanols. The 2-, 3- and 4-protons of the heterocyclic F-rings of B and D form an ABX_2 system ($J_{2,3}$ = $\pm$7.0 c./sec.), consistent with a 2,3-trans [2(eq), 3(eq)] arrangement of phenyl and hydroxyl substituent groups. Both products B and D therefore have 2,3-trans-3,4-trans (leucoanthocyanidin) and 2,3-trans (catechin) relative configurations.

The clearly-defined uncoupled benzenoid proton (singlet, τ = 3.11 p.p.m.) in the deca-acetate of B shows that the link between these moieties is from C-4 of the C-ring to the 6- or 8- position of the phloroglucinol D-ring as in XXIII and XXIV, respectively. The corresponding uncoupled benzenoid proton in the undeca-acetate of D

occurs as a singlet at τ = 3.17 p.p.m. Insufficient data regarding the chemical shifts of similar protons in 6- and 8- substituted flavans is available to enable distinction, at present, between the alternative structures XXIII and XXIV. (cf. p.173).

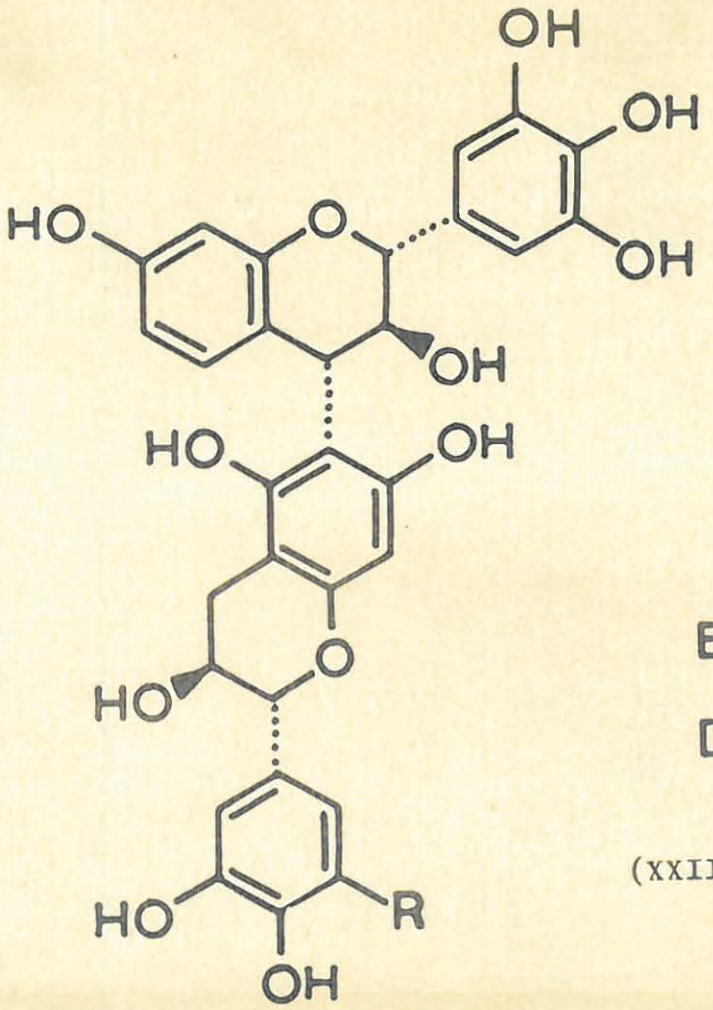
Mass Spectra of Derivatives of B and D.

Mass spectra of the octamethyl ether diacetate of B (M = 774) and the nonamethyl ether diacetate of D (M = 804) were compared with the mass spectrum of (+)-leucorobinetinidin tetramethyl ether diacetate [(+)-7,3',4',5'-tetramethoxy-3,4-trans-diacetyl-2,3-trans-flavan] (M = 446) (cf. Figs. 21, 22 and 23). The fragmentation pattern of the latter was used as a basis for the study of the mass spectra of derivatives of B and D. Fragmentation schemes for these three products are given by (a) and (b) below.

Molecular ion peaks of the derivatives of B and D allowed the accurate assessment of their molecular weights. Analytical data, used in conjunction with these molecular weights, enabled the determination of molecular formulae of B and D.

Acetyl groups are readily lost in each case, followed by the loss of a number of methoxyl groups. Significant ions are formed by the operation of two retro Diels-Alder fragmentations during the fission of the heterocyclic C- and F-rings.

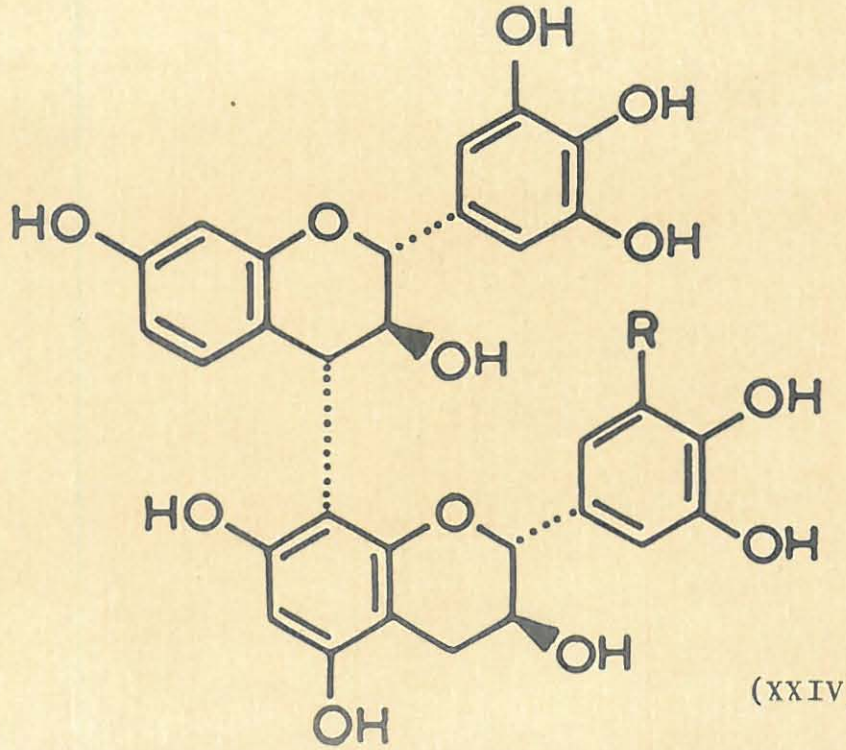
Elimination of acetic acid from the molecular ions leads to ions of masses 714 and 744 for B and D, respectively. Rupture of the linkage between the diol and catechin moieties results in ions of mass 327 for both B and D, indicating that their 3,4-diol units are identical.



B : R=H

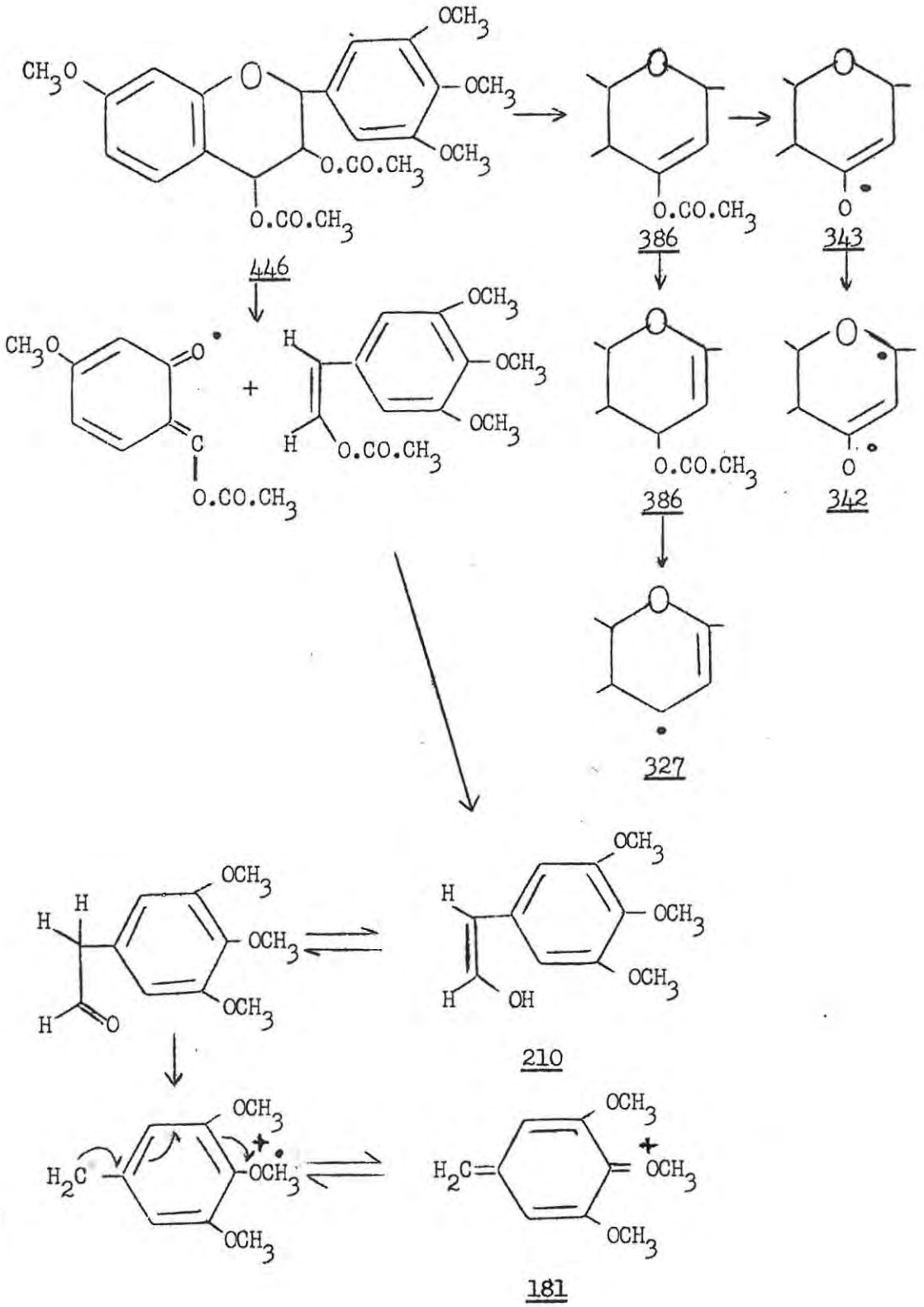
D : R=OH

(XXIII)



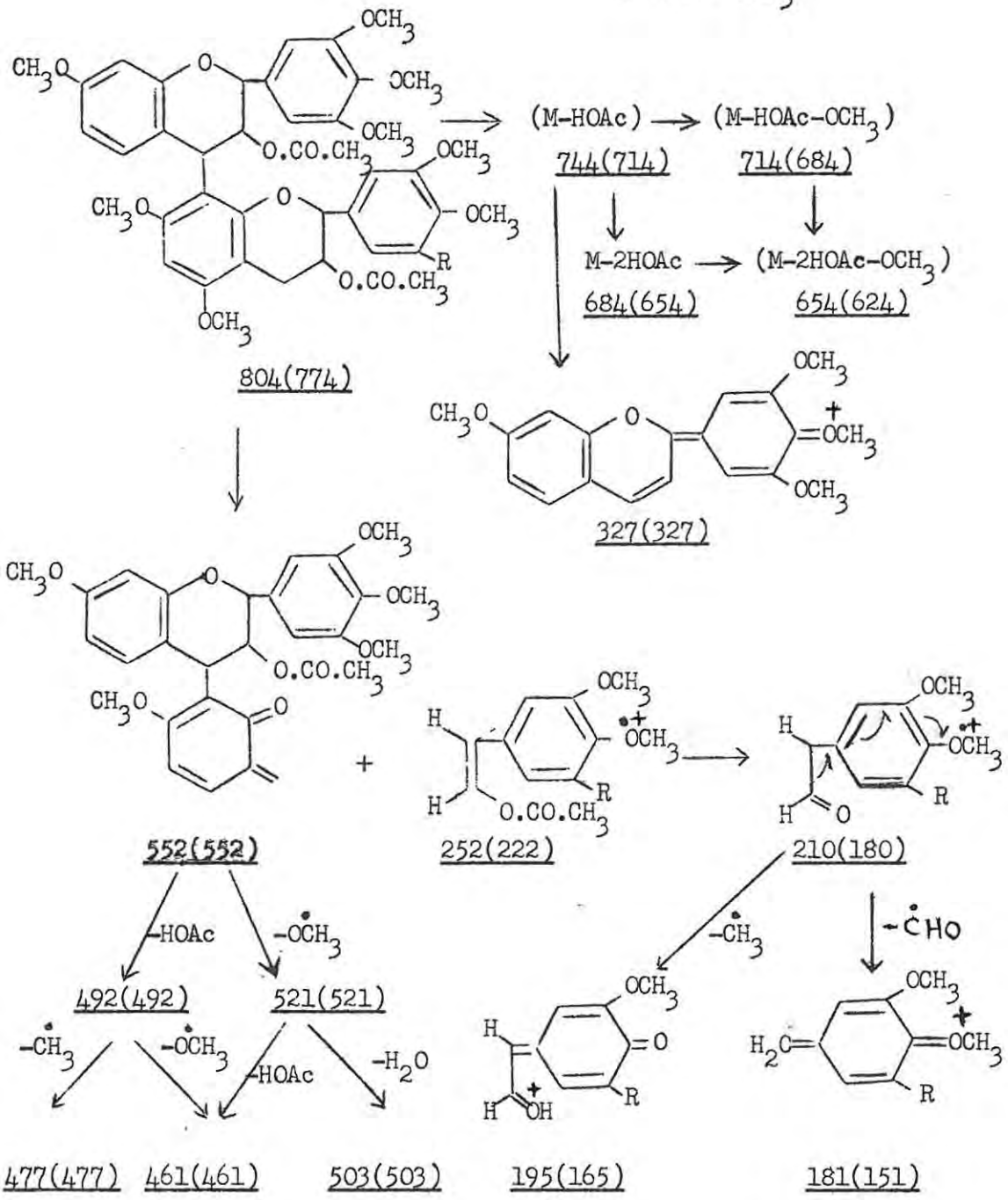
(XXIV)

Scheme (a).


OCH3
CH3O
O
OCH3
OCH3
O.CO.CH3
O.CO.CH3
446
386
343
342
327
210
181
H
OH

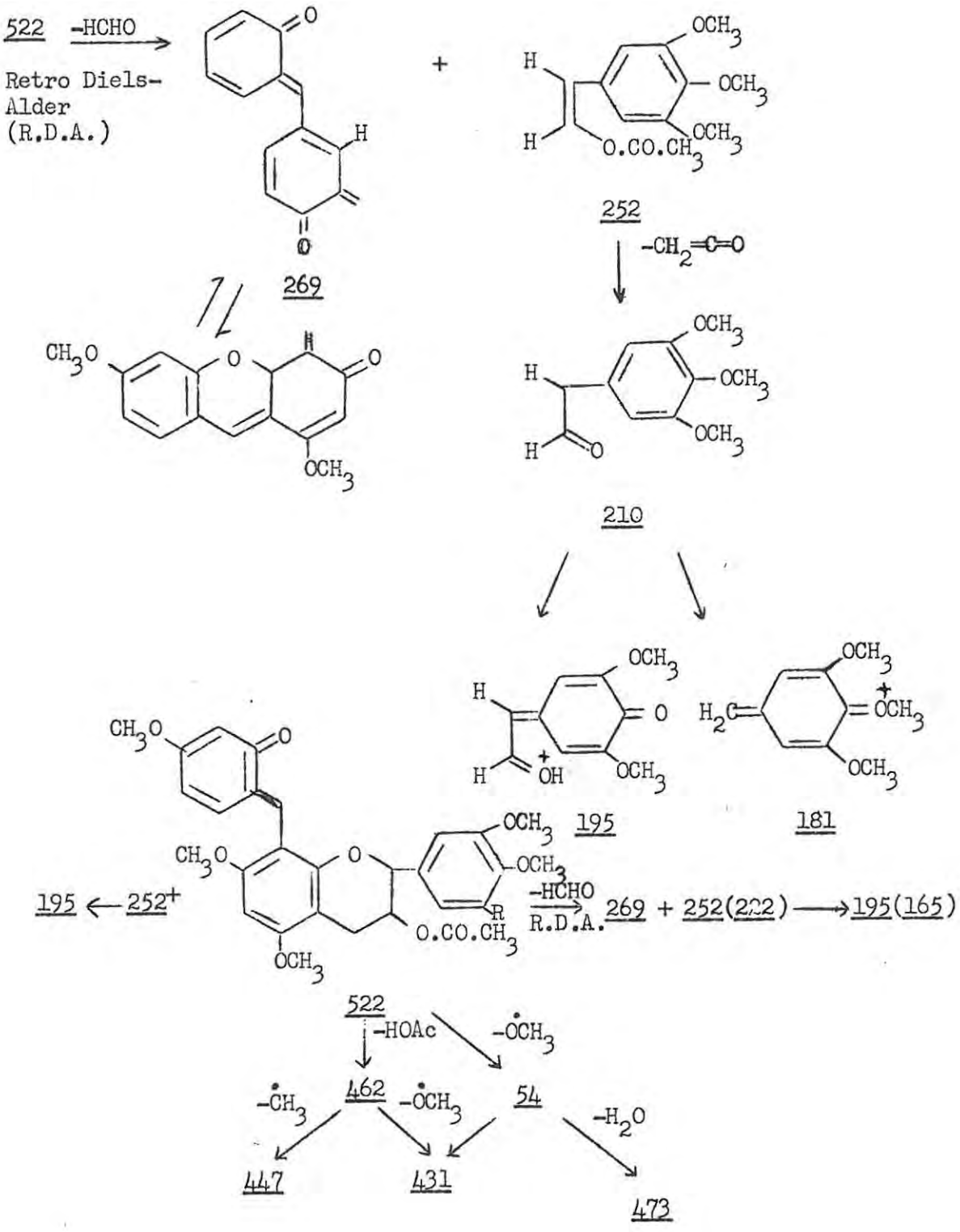
Scheme (b).

B : R = H*
D : R = OCH_3



*Values in brackets refer to fragments of Product B.

Scheme (b) (continued).



Fission of the F-ring by the first retro Diels-Alder reaction gives rise to ions of masses 522, 491, 473, 462, 447, 431, 252, 210, 195 and 181. The ion of mass 522, on suffering a second retro Diels-Alder fission through the cleavage of the C-ring and simultaneous loss of formaldehyde, gives rise to ions of masses 252, 210 and 195. In the case of D it is impossible to distinguish between these two retro Diels-Alder processes, since the same ions are formed, whichever one occurs first. In B, however, the fragments are different due to the difference in methoxyl substitution of the B- and E-rings of this product. Since both sets of ions are represented in the spectra, it appears that there is no preference as to which of the C- and F-rings is fissioned first.

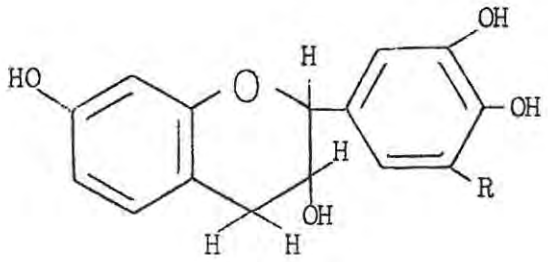
Fragments of mass 269, resulting from the double retro Diels-Alder processes, and which still retain the linkage between the A- and D-nuclei, are particularly significant and occur in high relative abundance.

Fragmentation patterns of these derivatives of B and D can only be rationalized on the basis of a linkage of the 4-position of the 3,4-diol moiety to the 6- or 8-position of the catechin unit, since alternate linkages would have resulted in ions of different masses. The 6- and 8-positions of the D-ring are equally amenable to linkage with the C-4 position of the C-ring, no distinction being possible between these.

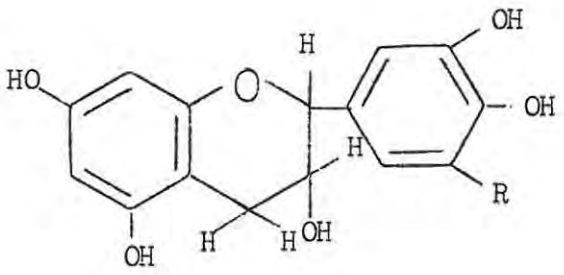
Aspects of Biogenesis of Biflavananols B and D.

Products B and D are found in association with the 2,3-trans-

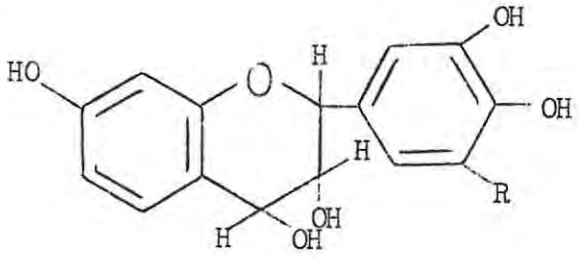
flavan-3-ols, (-)-robinetinidol XXV (R = OH), (-)-fisetinidol XXV (R = H), (+)-catechin XXVI (R = H), (+)-gallocatechin XXVI (R = OH), (all having the 2R ; 3S absolute configuration) and traces of 2,3-trans-3,4-trans-flavan-3,4-diols, (+)-leucorobinetinidin XXVII (R = OH) and (+)-leucofisetinidin XXVII (R = H) (both having the 2R, 3S, 4R absolute configuration) in the bark of black wattle.



(XXV)



(XXVI)



(XXVII)

These monomeric flavans represent the basic flavonoid units present in the biflavanols. The fact that the monomers occur with the biflavanols, suggests that the former compounds serve as biogenetic precursors of the associated constituents B and D.

Biflavanols may be formed by dehydrogenation of two catechin nuclei[105], but more likely through the reaction of a benzyl-carbonium ion at C-4 of a flavan-3,4-diol with a nucleophilic centre at C-6 or

C-8 of a catechin. The latter mechanism is preferred on the grounds of the condensation of (+)-leucorobinetinidin with (+)-catechin or (+)-gallocatechin, *in vitro*, to yield products showing chromatographic identity with B and D, respectively.

Biflavanols B and D therefore belong to a new class of compounds in which "resorcinol-type" flavanoids are carbon-linked to those of the "phloroglucinol series". Products B and D have very limited tanning properties[230], as evidenced by their low affinity for hide-powder (collagen) or gelatin, and fall into the category of "phenolic half-tannins".

Non-tannins of Black Wattle Bark.

The significance of the non-tannins in the biogenesis of heartwood tannins of black wattle (*Acacia mearnsii*) was discussed recently by Saayman and Roux[129]. Black wattle bark non-tannins were shown to consist of the carbohydrates : sucrose, (+)-pinitol, glucose and fructose; the imino acids : (-)-L-pipecolic acid, *trans*-4-hydroxy-(-)-L-pipecolic acid and (-)-L-proline; the amino acids : α-alanine, arginine, aspartic acid, glutamic acid and serine; a novel β-diketone; and two closely related steroid alcohols : β-sitosterol and stigmasterol.

Some non-tannins are undesirable constituents of tanning liquors in that the nitrogenous acids and carbohydrates often act as nutrients for bacterial and mould growth. Research into the fermentative aspects of these solutions may be of value in the field of industrial brewing.

Carbohydrate Constituents.

The hexose sugars sucrose, glucose and fructose are present in relatively high concentrations in the bark and sapwood of A. mearnsii. Sucrose was identified, and isolated for the first time from the bark, after initial paper chromatographic detection of these related sugars. Fructose is the predominant sugar in the sapwood of black wattle, with lower concentrations of sucrose and glucose. Black wattle heartwood contains no sucrose, glucose or fructose, indicating that these may be consumed or transformed during the process of tannin-formation at the sapwood-heartwood transition zone.

The cyclitol (+)-pinitol accompanies the hexose sugars in the bark and sapwood and survives in the heartwood of black wattle. This indicates that (+)-pinitol is not utilized in the synthesis of tannins in the heartwood. Its simultaneous presence in the leaves, bark, sapwood and heartwood suggests a possible origin in the leaves, after which it is translocated vertically along the vascular tissues of the plant, as well as radially to the sapwood and heartwood. Due to the poor response of (+)-pinitol to spray reagents and its concurrence with sucrose on paper chromatograms, its presence as a major constituent of black wattle bark was previously overlooked.

(+)-Pinitol is a common constituent of the Leguminosae[139], having been detected in or isolated from several Acacia spp.[140,141]. Biogenesis of (+)-pinitol is apparently from glucose, without fragmentation of the glucose molecule, via meso-inositol[231]. The role of (+)-pinitol in the metabolism of the plant is not clearly under-

stood; it may represent a stable end-product of carbohydrate biosynthesis.

Nitrogenous Acid Components.

The imino-acids (-)-L-pipecolic acid, trans-4-hydroxy-(-)-L-pipecolic acid and (-)-L-proline, isolated from the non-tannin fraction of black wattle bark extract by ion exchange and paper chromatography, are present in all parts of the tree. The stereochemistry of these acids is known[119,120], and they were identified by comparison with authentic samples kindly donated by Professor J.W. Clark-Lewis. Paper chromatography shows that the concentrations of these acids decline gradationally from leaf to twig bark and stem bark. Root bark, on the other hand, shows higher concentrations than stem bark. The high concentrations of these acids in the leaves and green twig bark possibly indicates their origins in these portions of the tree.

The α-amino-acids ~~α-alanine~~ alanine, arginine, aspartic acid, glutamic acid and serine are present in low concentrations in the bark and heartwood compared with the imino acids, but are prominent in the flowers and seeds of black wattle. These amino acids, amongst others, have been detected in Armeria maritima[125] where they are associated with 4-hydroxy-pipecolic acid, and in the green bean (Phaesolus vulgaris)[112,113] with pipecolic acid. Grobbelaar and Steward[114] showed by radioactive tracer techniques that lysine is the precursor of pipecolic acid in the developing green bean, and that it may be converted into 4-hydroxy-pipecolic acid by direct

hydroxylation[232,233].

The concurrence of these amino- and imino-acids in the leaves and other parts of A. mearnsii may indicate a similar biogenetic sequence in this and other related Acacia spp.[234]. The pipecolic acids have been isolated from Acacia oswaldii leaves[119] and A. excelsa heartwood[118], and are now shown present in the leaves of A. decurrens and A. dealbata. They are therefore prominent constituents of certain of the Leguminosae.

These pipecolic acids may be functional in metabolic processes in the plant or may be end-products of the biosynthesis of amino-acids.

Commercial Implications of the Nitrogen Content of Wattle Bark.

Wattle bark extract has been shown to have a relatively high (± 0.3%) nitrogen content, reflecting the presence of approximately 2.5% of the above nitrogenous acids[235]. Bennett[236], however, noted in 1916 that commercial wattle bark has even higher (0.87%) nitrogen content. Holmes and Wollenberg[237] showed that the nitrogen content of wattle extract has important implications in leather analysis where hide-substance is estimated by the Kjeldahl method. The error involved, according to them, is considerable - amounting to 1 part in 27 of hide substance. This leads to an error of 7 units for a leather of 100 degree of tannage. Recently Heidemann and Kroll[238] reported a 0.35% nitrogen content for mimosa extract.

Analysis of current production of wattle extract shows 0.25% nitrogen, respesenting 0.28% on a dry-weight basis[235]. This is shown

to make a small but significant contribution to the degree of tannage of 4 units for tannages in closed systems.

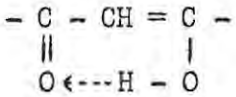
β-Diketone.

β-Diketones are prominent components of cuticle waxes of plants. Horn *et al.*[152,153] showed their presence in *Acacia spp.*, the carnation (*Dianthus caryophyllus*), and a high content in the waxes from many highly glaucous species of eucalyptus. *E. globulus* leaf waxes were found by Hall *et al.*[239] to consist of 70-75% long-chain *β*-diketones. Of these the principal member is n-tritriacontan-16, 18-dione ($n\text{-}C_{15}H_{31}COCH_2COC_{15}H_{31}$), m.p. 68°.

Acacia mearnsii bark contains a novel *β*-diketone, m.p. 80-82°, which occurs with a mixture of steroid alcohols in the petroleum-ether extract of the bark. The *β*-diketone was separated from the mixture by thin-layer chromatography on Kieselgel G. Its molecular weight from the empirical formula, $C_{32}H_{64}O_2$ (based on analyses), was anticipated to be 480. The melting point was somewhat higher than those of natural *β*-diketones (40-70°) isolated before.

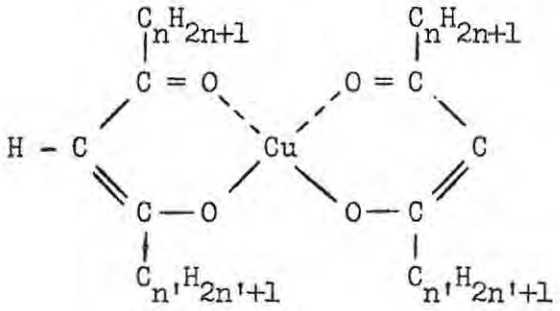
Infrared spectral analysis (*cf.* Fig.24) shows the enolic character of the black wattle diketone by the presence at 3350 $cm.^{-1}$ of O-H stretching frequencies. The nuclear-magnetic-resonance spectrum (*cf.* Fig. 25) shows the protons of terminal methyl groups occurring as a multiplet centred at τ = 9.12 p.p.m., methylene proton resonances at τ = 8.73 and 7.70 p.p.m. and a vinyl proton, split by methylene protons, occurring as a triplet centred at τ = 6.40 p.p.m. A triplet centred at τ = 5.98 p.p.m. may also be due to a methine proton. The signal

of the proton associated with the hydroxyl group (enolic form of the diketone) is probably shifted far downfield due to hydrogen bonding as in XXVIII (cf. Allen and Dwek[240], and Tulloch and Weenink[241]).



(XXVIII)

The β-diketone from wattle bark forms a blue copper complex, the structure of which may be represented by XXIX. Acid treatment readily regenerates the diketone.



(XXIX)

Although Bowie, Williams, et al.[242] reported that simple β-diketones break down in a regular manner under electron impact in mass spectrometry, the long-chain members appear to be relatively unstable[243]. Thus the mass spectrum of the β-diketone from wattle bark (cf. Fig.27) shows a peak at mass 448 which is considerably lower than the estimated molecular weight of 480 from the empirical formula. Since there is a significant "M-2" ion at 446, this suggests that 448

does not represent a molecular ion peak. Decomposition of the fragments (M = 446, 448) is obviously by successive eliminations of ethylene molecules. The fragmentation pattern cannot be readily correlated, at present, with obvious structural features[243].

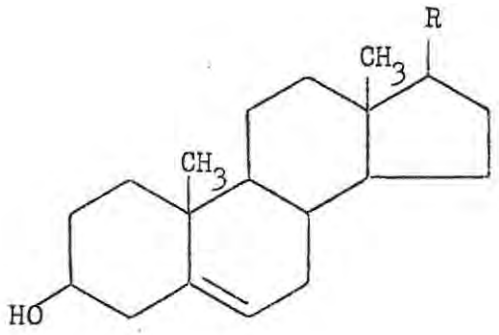
Steroid Alcohols.

From an ethereal extract of black wattle heartwood Keppler[66] isolated a "steroid", m.p. 160-161°, for which he proposed the empirical formulae $C_{26}H_{46}O$ or $C_{27}H_{48}O$. Previously Stephen[141] had separated the constituents of the isohexane extract of Acacia mearnsii heartwood into three fractions, ranging in melting points from 160-169°. He could not correlate the properties of these sterols with those of known phytosterols, and was not able to propose a definite empirical formula.

The petroleum ether extract of black wattle bark yields a steroid fraction obtained by preparative thin-layer chromatography on Kieselgel G of the extract[129]. Repeated recrystallization gives colourless needles, m.p. 160-162°, which show a violet-to-green Liebermann-Burchard reaction.

Mass spectrometric analysis of the acetate of the steroid, m.p. 175-176°, clearly shows that the product is a mixture of two components (cf. Fig.30). Molecular ion peaks show the masses of these two constituents to be 454 and 456, corresponding to molecular formulae $C_{31}H_{50}O_2$ and $C_{31}H_{52}O_2$. Signals at masses 313 and 315 indicate cleavage of side-chains, while the peak at 255 represents the subsequent loss of an acetate ion from the nucleus.

The fragmentation pattern of the mixture shows the close similarity between the two components, suggesting that the compound with mass 456 differs from the one of mass 454 only through the presence of an additional double bond, possibly located in the side-chain. These considerations lead to the assumption that the mixture consists of β-sitosterol XXX, an ubiquitous phytosterol, and stigmasterol XXXI, a less common plant sterol. These are commonly associated naturally.



(XXX) R= $-CH(CH_3)-CH_2-CH_2-CH(CH_3)-CH(CH_3)-CH_2-CH_2-CH_3$

(XXXI) R= $-CH(CH_3)-CH=CH-CH(CH_3)-CH(CH_3)-CH_2-CH_2-CH_3$

Selective hydrogenation of the steroid acetate mixture using the method of Bernstein and Wallis[207] gave a homogeneous product, β-sitosterol, which is fully hydrogenated in the side-chain. These mild reducing conditions leave the nuclear double bond intact. The mass spectrum of the product, m.p. 128-129° (cf. Fig.31) shows a molecular ion peak at 456, confirming the conversion of stigmasterol into β-sitosterol.

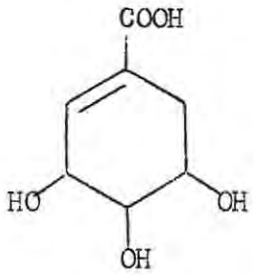
Biosynthesis and Functions of Steroid Alcohols.

Animal sterols, such as cholesterol, are formed by the condensation of isoprene units via the intermediates farnesene and squalene, with the introduction of a hydroxyl group by oxidation at a later stage in the synthesis[244,245]. Although the biosynthesis of plant sterols has not been investigated in such detail, it appears that a similar scheme operates in this case. Isoprene moieties have been shown to be derived from acetic acid, via the amino acids[246].

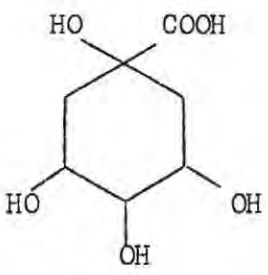
The function of sterols appears to be closely connected with the growth of younger portions of the plant. Duperon et al.[247] showed that a modification in the concentration of sterols occurs during the germination of Phaeseolus vulgaris seeds.

Shikimic Acid and Quinic Acid.

Traces of shikimic XXXII and quinic XXXIII acids were detected in the non-tannin fraction of Acacia mearnsii bark. Ion-exchange chromatography of this fraction gave an acidic portion which showed the presence of these acids in comparison with reference acids, using selective spray reagents.



(XXXII)



(XXXIII)

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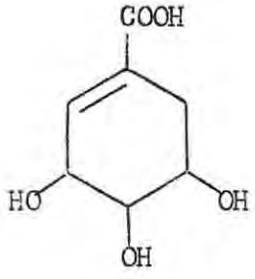
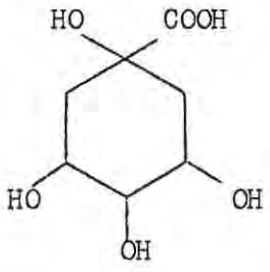


(XXXII)



(XXXIII)

The presence of traces of both shikimic and quinic acids in the bark is of considerable significance, since these acids are known to be precursors of aromatic compounds in plants along the two well-established biosynthetic pathways - one based on the shikimic-prephenic acid pathway[248], and the other on acetate synthesis[249].

Biosynthesis of Condensed Tannins.

Flavonoid moieties are derived from simple non-tannin molecules. The C_6 unit of the A-ring is probably acetate derived, while the $C_6 - C_3$ unit of the B- and C-rings most likely originate from shikimic acid via the amino-acids. Condensed tannins appear to be built up of flavonoid monomers, amongst which the catechins and leucoanthocyanidins are the most prominent precursors. These condensed tannins, which are responsible for tanning, may be composed of from five to ten such monomeric units linked together by C-C bonds.

Low molecular weight condensed tannins, which have limited tanning properties, contain two to five such units. Examples of this class of compounds are the biflavanols B and D, consisting of (+)-leucorobinetinidin linked to (+)-catechin and (+)-gallocatechin, respectively. An analogous compound F, derived from (+)-leucofisetinidin and (+)-catechin accompanies these in wattle bark[226]. In these products the catechin moieties may be responsible for termination of condensation with further flavonoid units because of the presence of a methylene group, at C-4 of the heterocyclic ring, which is less amenable to linkage than an hydroxyl group.

The condensation of two flavan-3,4-diol moieties, as in bimolecular leucofisetinidin[250], is not subject to the above phenomenon, and the product has a free-hydroxyl group, at C-4 of one heterocyclic ring, available for condensation with a further flavan-3,4-diol or catechin unit. In this manner polymeric molecules may be formed biosynthetically, giving rise to the highly condensed tannins.

ADDENDUM.

Nuclear-magnetic-resonance Spectra of Derivatives of B and D.

Analysis of the fine structure of the methylene protons of the methyl ether diacetates of B and D ($J_{3(\underline{ax}),4(\underline{ax})}$=8.5; $J_{3(\underline{ax}),4(\underline{eq})}$= 5.5 c./sec.) confirms the presence of 3(ax) protons. This evidence of an ABX_2 system in the heterocyclic E-ring correlates with the degradative evidence and confirms the presence of a catechin moiety in each component.

The additional hydroxyl group on the phloroglucinol D-ring of B and D restricts rotation about the 4 - 6(8) link and, therefore, rotational isomers could exist amongst the methyl ether and acetyl derivatives. Considerations of the number of asymmetric centres as well as optical isomerism leads to the possibility of 64 isomeric forms, of which only one is represented in these compounds.

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