PROTOTYPES OF BLACK WATTLE TANNINS AND THEIR STEREOCHEMISTRY

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 was carried out at the Leather Industries' Research Institute, Grahamstown, and is wholly original except where due reference is made in the text. It has not been submitted in whole, or in part, for any degree at any other University.

The author expresses his thanks to:

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SUMMARY.

A number of interrelated flavonoid compounds, present in trace quantities in mature black wattle (Acacia mearnsii De Wild) bark, have been isolated and identified for the first time. They include a) the resorcinol-pyrogallol analogues, (+)-leuco-robinetinidin (7,3',4',5'-tetrahydroxyflavan-3,4-diol), dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one) and robtein (2',4',3,4,5-pentahydroxychalcone) and b) the resorcinol-catechol analogues, (+)-leuco-fisetinidin (7,3',4'-trihydroxyflavan-3,4-diol), fustin (7,3',4'-trihydroxyflavan-3-ol-4-one), (-)-fisetinidol (7,3',4'-trihydroxyflavan-3-ol), butein (2',4',3,4-tetrahydroxychalcone) and butin (7,3',4'-trihydroxyflavan-4-one). In addition, two flavonol glycosides, myricitrin (3,5,7,3',4',5'-hexahydroxyflavone-3-rhamnoside) and quercitrin (3,5,7,3',4'-pentahydroxyflavone-3-rhamnoside), which belong to the phloroglucinol-pyrogallol and phloroglucinol-catechol class of flavonoids respectively, were isolated from immature bark. These represent the only glycosides isolated hitherto from wattle bark or heartwood extracts.

Since only trace amounts of many of the above compounds were present in the bark extract, their isolation required a number of successive enrichment procedures. These included a preliminary large-scale countercurrent separation, further enrichment through countercurrent separation in a 160-tube Craig machine using a different

solvent system, and subsequent separation by means of preparative paper chromatography using both adsorptive and partitioning principles.

The existence of two major patterns of interrelated compounds in wattle bark based on a common resorcinol nucleus parallels the presence of similarly interrelated compounds in the heartwoods of Acacia mearnsii and Robinia pseudacacia. In the former heartwood they are based only on the resorcinol-catechol nucleus and in the latter, mainly on the resorcinol-pyrogallol nucleus. The isolation of myricitrin and quercitrin from wattle bark adds weight to the already known members of interrelated compounds belonging to the minor phloroglucinol-pyrogallol and phloroglucinol-catechol patterns.

Apart from the above compounds another component, F, of wattle bark was isolated. Data obtained from paper chromatography, infrared analysis, micro-degradation and acid hydrolysis suggest a "bis-flavonoid" compound consisting of a leuco-fisetinidin nucleus linked with a catechin nucleus. Limitations of material, however, prevented more detailed examination of the structure of the compound.

The relative configurations of (+)-leuco-robinetinidin and (+)-leuco-fisetinidin, regarded as the monomers of black wattle tannins, were established by synthesis of a number of racemic diastereoisomers, through a comparative study of their oxidation rates and by n.m.r. spectroscopy. The two leuco-anthocyanidins were shown to have the 2,3-trans-3,4-trans-configuration, as opposed to the previously

assigned 2,3-trans-3,4-cis-configuration, based on their ability to form isopropylidene derivatives in high yield. From earlier stereochemical correlations of the optically pure (+)-flavandiols with (+)-catechin, it was possible to establish the absolute configuration of the two naturally-occurring diols as 2R:3S:4R.

Racemic trans-trans-leuco-fisetinidin, apart from being obtained by the established method of catalytic hydrogenation of (±)-fustin in methanol, was also synthesized by catalytic hydrogenation of the fustin in glacial acetic acid and by its reduction with potassium borohydride. The latter proceeded smoothly without interference from side-reactions and the flavandiol was obtained in 75% yield. Reduction of racemic O-trimethylfustin with a mixture of lithium aluminium hydride and aluminium chloride yielded mainly the racemic 2,3-trans-3,4-cis-flavandiol. This methylated diol, obtained pure for the first time, was characterized through preparation of its diacetate and dibenzoate derivatives. The same trans-cis-isomer was also obtained in low yield from O-trimethylfustin by the oxime-amine method.

Reduction of racemic O-tetramethyldihydrorobinetin with the mixed reagent yielded, in addition to the known 2,3-trans-3,4-trans-, the new 2,3-trans-3,4-cis-O-tetramethyl-leuco-robinetinidin. The two isomers, present in approximately equal concentration, were separated by fractional crystallization. The new diol was characterized by

preparation of its diacetate.

Proposed future study of the mode of link in wattle tannins through oxidation rates and n.m.r. methods required the synthesis of other geometrical isomers of leuco-fisetinidin and leuco-robinetinidin. Thus, (±)-2,3-cis-3,4-cis-O-trimethyl-leuco-fisetinidin was obtained by hydrogenation of the corresponding O-trimethylflavonol with Raney nickel under pressure. Similar reduction of 7,3',4',5'-O-tetramethyl-flavonol yielded the new (±)-2,3-cis-3,4-cis-O-tetramethyl-leuco-robinetinidin. Slightly more drastic conditions of hydrogenation resulted in the formation of the corresponding 2,3-cis-flavan-3-ol. This was isolated readily and obtained pure for the first time.

A comparison of the oxidation rates with lead tetra-acetate and with periodic acid of the racemic trans-trans and trans-cis methylated leuco-anthocyanidins with the corresponding natural compound established unequivocally the 2,3-trans-3,4-trans-configuration of the naturally-occurring flavandiols. Trans-cis-diols were cleaved six to nine times faster than the corresponding trans-trans-diols. The evidence from the oxidation rates was substantiated by examination of the coupling constants of protons at C_2, C_3 and C_4 from the n.m.r. spectra of the diacetates of the various flavan-3,4-diol isomers. The coupling constants for the 3,4-trans- arrangements were large in comparison with the coupling constants for 3,4-cis-diols. The relative configurations of the two 2,3-cis-3,4-cis-diols, as well as that of the

2,3-cis-flavan-3-ol, were also proved by n.m.r. spectroscopy.

A study of the yields of isopropylidene and cyclic carbonate derivatives for the trans-trans- and trans-cis-diols showed that for the above highly substituted leuco-anthocyanidins, neither method may be used as criterion for establishing the cis- or trans-nature of the 3,4-diol grouping. Unambiguous evidence is presented that epimerization of the C_4-hydroxyl occurs in the 3,4-trans compounds during formation of these derivatives.

The chromatographic behaviour of the above trans-cis-, trans-trans- and cis-cis-racemates, as well as other methylated flavan-3,4-diols of known stereochemistry, was studied using borate impregnated paper and water-saturated butan-1-ol as solvent. Flavandiols with a 3,4-cis-configuration had relatively low R_F values whereas 3,4-trans-diols had higher R_F's. This suggests that the 3,4-cis-diols are capable of complexing with the borate. This simple method, based on a correlation between stereochemistry and paper chromatographic behaviour, thus makes possible differentiation between 3,4-cis and 3,4-trans methylated flavan-3,4-diols with known patterns of phenolic substitution, when suitable reference compounds are employed.

The evidence of complex formation of isomeric methylated flavan-3,4-diols on paper chromatograms prompted examination of their ionophoretic mobilities in the same borate buffer. All flavan-3,4-cis-diols showed positive mobilities, whereas flavan-3,4-trans-diols

exhibited either no mobility or negative mobility due to electro-endosmosis. Ionophoretic mobility in borate solution thus apparently affords an absolute method for distinguishing between methylated flavan-3,4-*cis*- and methylated flavan-3,4-*trans*-diols without the use of reference compounds.

Factors responsible for the reddening of wattle bark extracts were studied as a sequel to earlier work, which had shown that the 4-hydroxyl group in conjunction with either the 7- or 4'-hydroxyl or their simultaneous presence was responsible for this effect. A decision between these alternatives was made through the synthesis of two new flavan-4β-ols, 7-hydroxyflavan-4β-ol and 4'-hydroxy-flavan-4β-ol. Study of their chromogenic properties showed that the simultaneous presence of 7- and 4-hydroxyls was alone responsible for redness induced under neutral conditions.

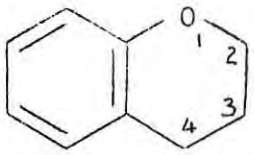
CONTENTS

REVIEW OF THE STEREOCHEMISTRY OF FLAVAN-3,4-DIOLS.

Introduction.

Possible conformations of chroman (I) and chromanone (II) derivatives.

The chromone nucleus III is planar and not capable of stereochemical variation. However, in chroman I and chromanone II



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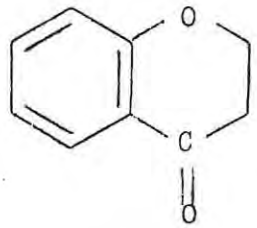

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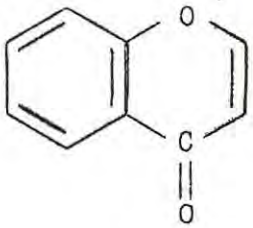
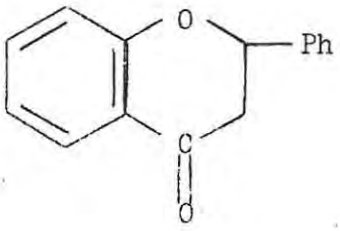


(III)

derivatives where complete substitution has occurred at C_2 and C_3,



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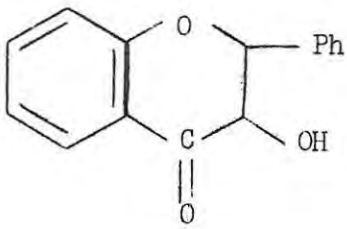

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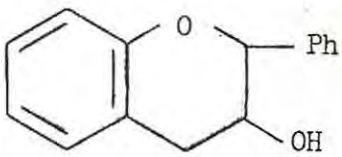

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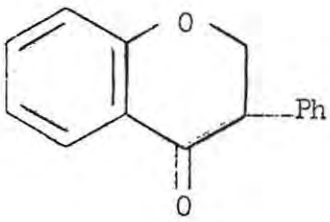

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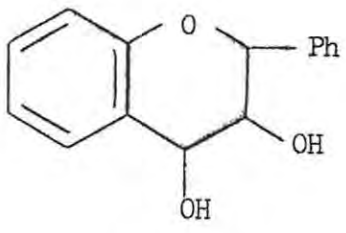


(IX)

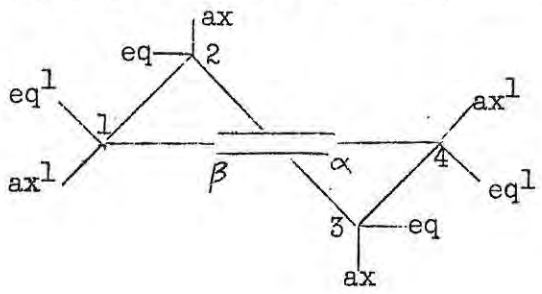
stereochemical differences become possible. To this class of compounds belong the flavanones IV, dihydroflavonols VI, catechins VII, isoflavanones VIII and flavan-3,4-diols IX. For these, three distinct conformations, the "half-chair" conformation of Barton, Cookson, Klyne and Shoppee [1], the "boat" conformation of Whalley [2] and the "sofa" conformation of Philbin and Wheeler [3], come into consideration.

The half-chair conformation.

This mode of representation, first proposed by Barton and co-workers [1] for cyclohexene was extended by Roberts [4] to include (+)-catechin and (-)-epicatechin. He regarded the conformation of the heterocyclic ring in these compounds to resemble that of cyclohexene. Subsequently, Mahesh and Seshadri [5] and King, Clark-Lewis and Forbes [6] adopted this conformation for the flavonoid compounds IV - IX, and it will be considered in some detail.

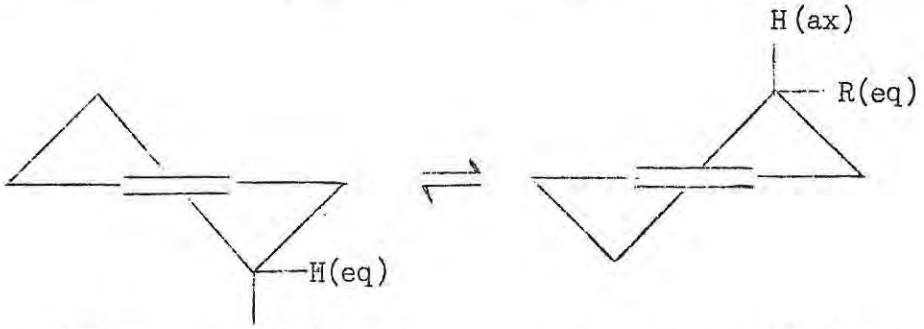
When a double bond is introduced into a six-membered ring such as cyclohexane, distortion of the ring occurs, and it may assume the half-chair conformation shown diagramatically in X for cyclohexene. Here the four carbon atoms associated with the olefenic system C_1-C_β-C_α-C_4 lie in a plane, with C_2 above and C_3 below it. Bonds extending to hydrogen (or other) atoms at C_2 and C_3 are normal axial (ax) or equatorial (eq) bonds but those at C_1 and C_4 differ and can

be described as pseudo-axial (ax ^{1}) or pseudo-equatorial (eq ^{1}).



(X)

For any substituted cyclohexene which is not made rigid by ring fusion, two interconvertible half-chair conformations are possible. These may conveniently be represented as XI and XII. For flavonoid



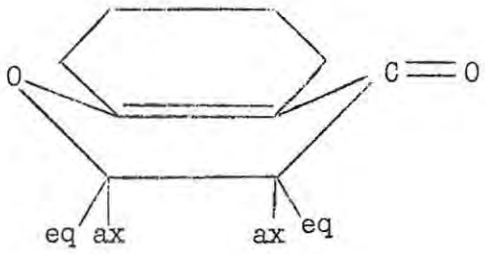
(XI) R(ax) (XII)

compounds it is generally considered [4,7] that the conformation of lowest energy (i.e. the most stable form) is that with the C_2-phenyl group in the equatorial position.

The boat conformation.

Molecular models indicate that the chroman and chromanone systems may also be represented by the boat conformation shown in XIII. This conformation is more strained [2] than the almost strainless half-

chair X so that at the outset it is rendered more unlikely. In addition, representation of 2-hydroxyisoflavanones and flavanones

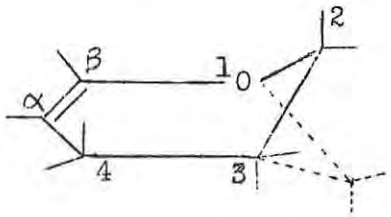


(XIII)

by the boat form, places the plane of the carbonyl group at an angle of approximately 35° to that of the benzene ring, a condition which would effectively destroy the observed conjugation [8] between the two systems in these groups of compounds. Thus, critical examination of the relevant factors, excludes the boat conformation.

The sofa conformation.

This third alternative conformation, advanced by Philbin and Wheeler [3] may be represented as in XIV. The heterocyclic ring



(XIV)

will, with the exception of C_2, be coplanar with the associated benzene nucleus. The exocyclic bonds at C_4 are regarded as being

of the pseudo-type. This modification of the half-chair to a "sofa" is regarded by Wheeler and co-workers as being more nearly in accord with the infrared absorption data obtained by Shaw and Simpson [8] for 2- and 3- substituted chromanones.

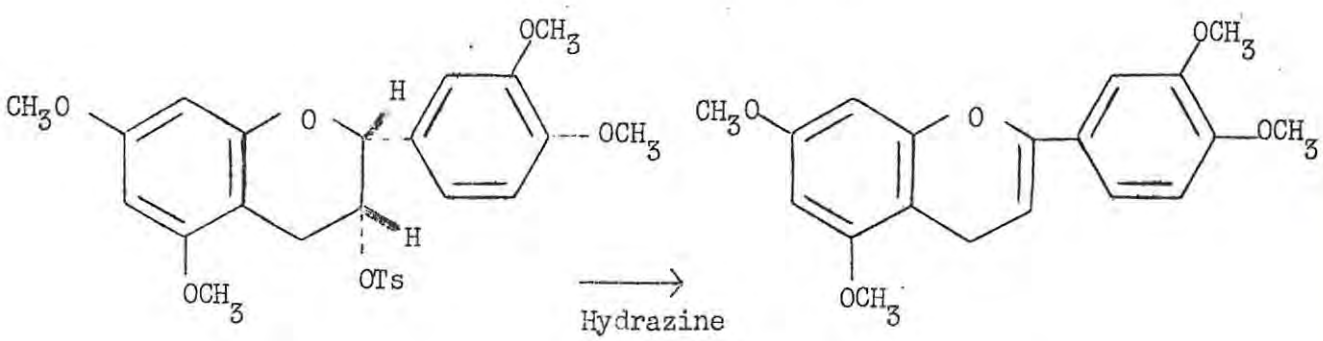
By studying likely interatomic distances and bond angles and constructing accurate rigid-wire models in which the angle units were joined by close-fitting metal sleeves, Philbin and Wheeler confirmed the sofa mode of representation. From these models it appears that no special steric hindrance will arise in structure XIV if C_3 and C_4 carry hydroxyl groups, as in flavan-3,4-diols with axial-equatorial combinations. The model readily undergoes conformational inversion whereby the axial and equatorial bonds at C_2 and C_3 are interchanged.

Conclusions. The energy barrier between the two possible half-chair conformations and that between the two sofa conformations is evidently small. Recent evidence, based on n.m.r. data and favouring the half-chair conformation for flavan-3,4-diols, has however been presented by Clark-Lewis, Jackman and Williams [9] and Drewes and Roux [10].

The Relative and Absolute Configurations of Catechins.

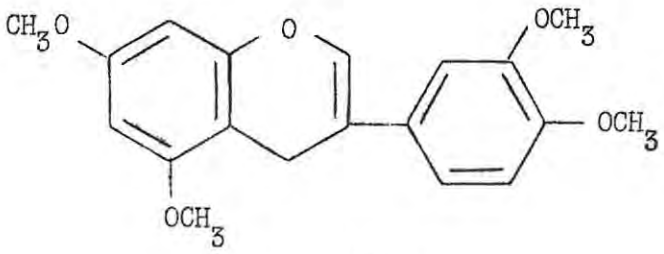
The stereochemistry of the leuco-anthocyanidins is directly related to that of the catechins, and a review of the relative and absolute configurations of these compounds is thus presented.

(+)-Catechin and (-)-epicatechin. Early work by Freudenberg, Fikentscher and Harder [11] had shown that catechin was likely to be the 2,3-trans isomer since it lost the elements of water only by rearrangement, whereas epicatechin, which was probably the 2,3-cis isomer lost water readily to form the flav-2-ene. Thus, when (-)-epicatechin tetramethyl ether-3-toluene-p-sulphonate XV is heated with hydrazine, water is eliminated and the flavene XVI is formed.



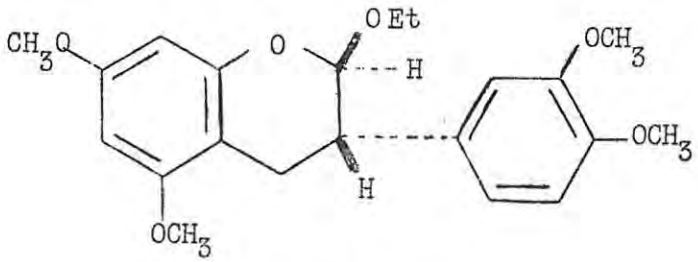
(XV) (XVI)

This trans-elimination of water appears consistent with a 2(ax)H and a 3(ax)-O-toluene-p-sulphonate which will permit coplanarity of the four participating centres [2,12]. Freudenberg, Carrara and Cohn [13] showed that the same reaction with catechin tetramethyl ether in boiling quinoline gave the isoflav-2-ene XVII. The flavene is thought to arise by separation of the toluene-p-sulphonate anion, 1,2-rearrangement of the resulting carbonium ion, and a loss of a proton.



(XVII)

The likely trans-nature of (+)-catechin was shown by the reaction of (+)-catechin tetramethyl ether with phosphorus pentachloride [13,14] which yields a reactive 2-chloroisoflavan. This compound reacts with ethanol to yield the same optically active 2-ethoxyisoflavan, XVIII. This type of molecular rearrangement is interpreted

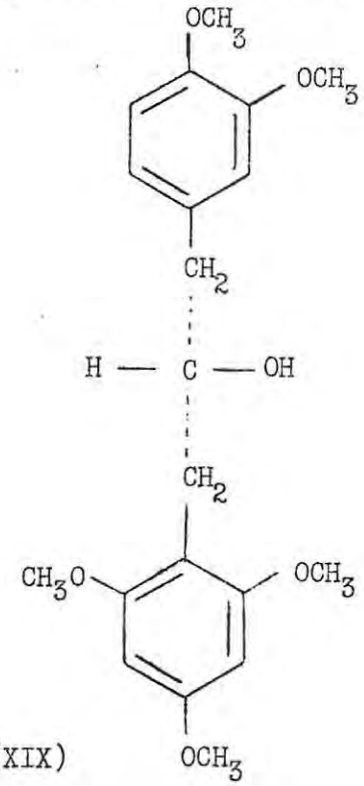


(XVIII)

as a 1,2-arrangement of trans-groups. Epicatechin does not undergo this rearrangement.

The relative configurations of (+)-catechin and (-)-epicatechin were first deduced by Freudenberg [15]. His conclusion was based on earlier findings [16] that (+)-catechin could be epimerized to (+)-epicatechin, and (-)-epicatechin epimerized to (-)-catechin. That

these reactions involve inversion of the bulky 2-aryl group and not the 3-hydroxyl group was proved conclusively by Birch, Clark-Lewis and Robertson [17]. (+)-Catechin tetramethyl ether and (-)-epicatechin tetramethyl ether were reduced with sodium in liquid ammonia. This resulted in ring opening (splitting of a benzyl ether link) to produce diphenyl propanols which were methylated. (-)-Epicatechin gave pentamethoxy-1,3-diarylpropane-2-ol XIX with an excess of the (+)-enantiomorph and (+)-catechin gave the diaryl propan-2-ol XX with an excess of the (-)-enantiomorph. This established that the 3-hydroxyl group was of



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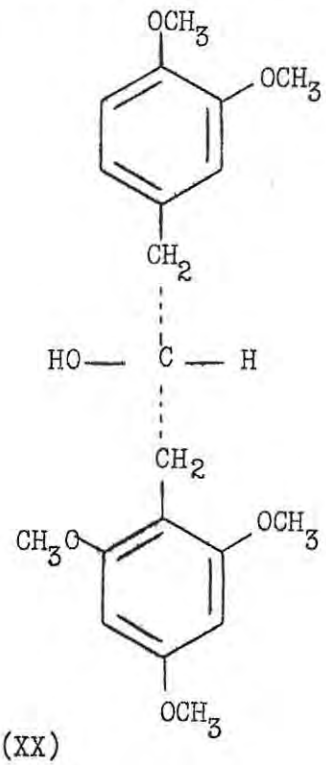
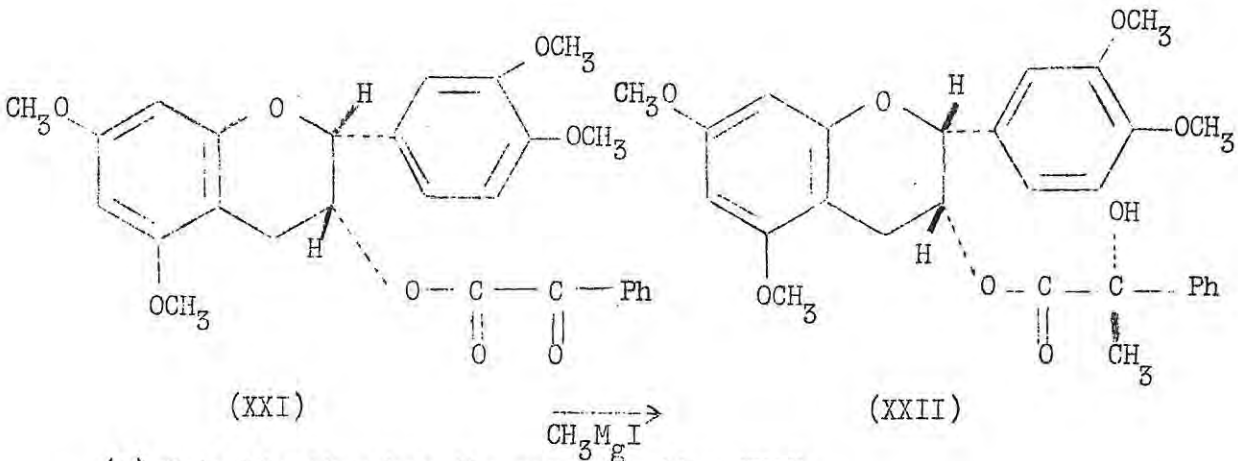


(XX)

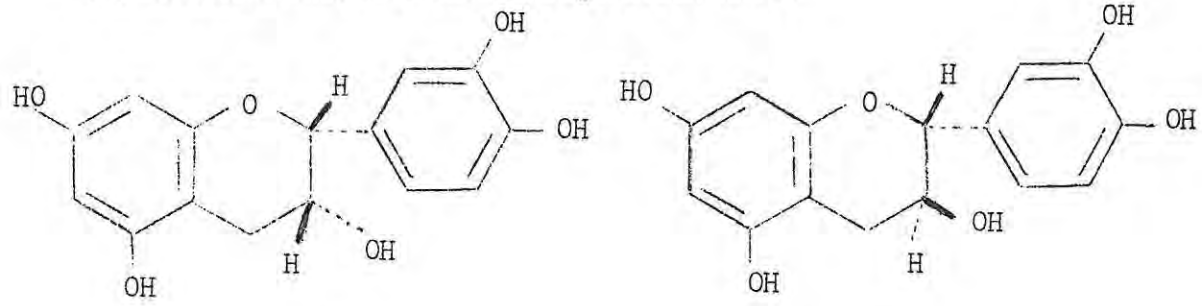
opposite configuration in (+)-catechin compared with (-)-epicatechin and the relative configuration of the two compounds could thus be deduced.

By applying Prelog's [18] atrolactic acid method, Birch and co-workers were able to deduce the absolute configuration of the catechins. This involved treating the phenyl glyoxylate of (-)-epicatechin XXI with methyl magnesium iodide and hydrolysing the resulting atrolactic ester XXII to atrolactic acid. A preponderance of (-)-isomer indicated that (-)-epicatechin had the configuration XXIII.



(XXI) $\xrightarrow{CH_3MgI}$ (XXII)

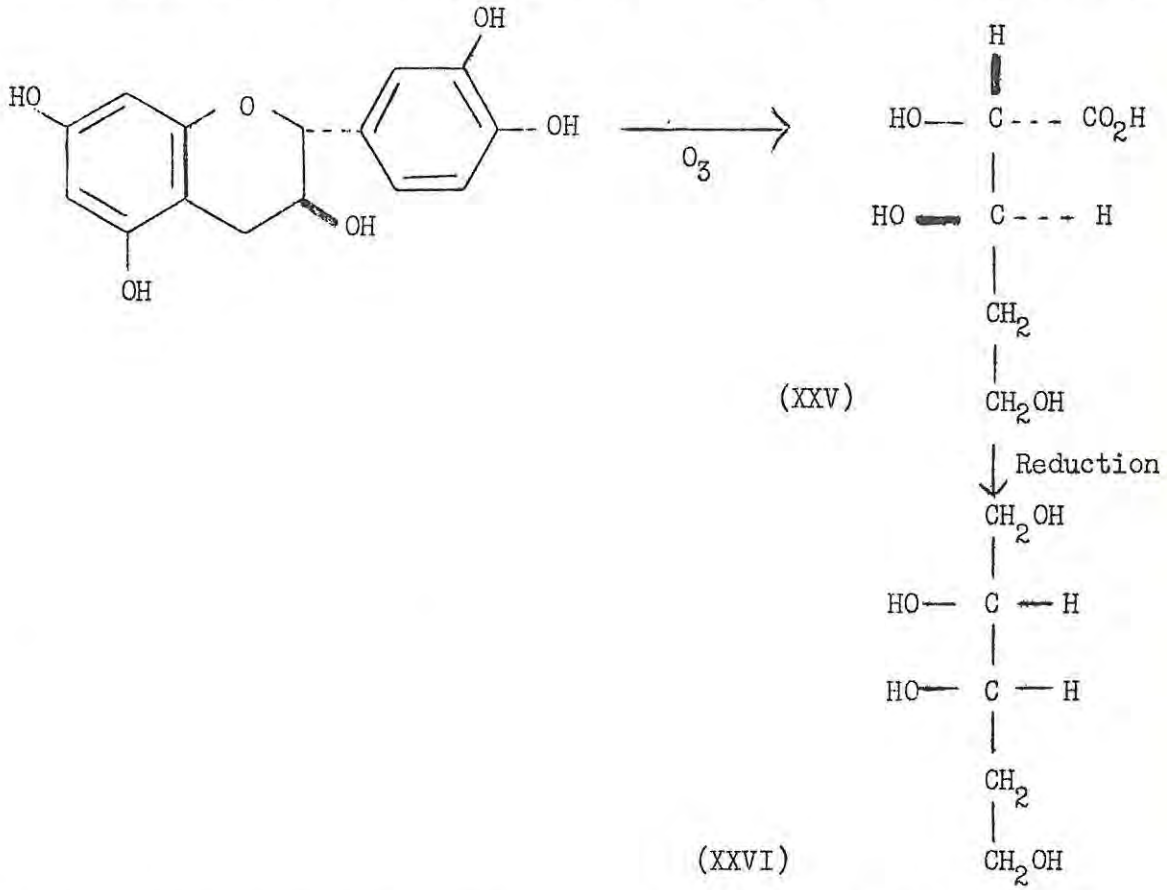
(+)-Catechin then has the configuration XXIV.



(XXIII) (XXIV)

The deductions of Birch and co-workers [17] were substantiated by Hardegger, Gempeler and Züst [19]. They showed that exhaustive ozonolysis of (+)-catechin yielded the dicarboxylic acid XXV. This acid was reduced to the 2-desoxy-D-adonital XXVI which was characterized as the tetraphenylurethane, identical with that prepared from 2-desoxy-D-

ribose. Hardegger, Züst and Lohse [20] subsequently found that (-)-epicatechin yielded the diastereoisomeric tetraphenylurethane identical with that prepared from 2-desoxy-D-xylose. In this way the configurations

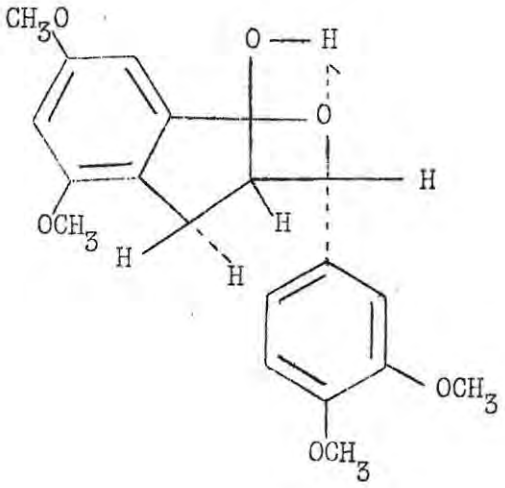


at the asymmetric centres C_2 and C_3 were established in both compounds, and their absolute configurations also established. In the R and S notation of Cahn, Ingold and Prelog [21] (+)-catechin is therefore defined as 2R:3S-5,7,3',4'-tetrahydroxyflavan-3-ol and (-)-epicatechin as 2R:3R-5,7,3',4'-tetrahydroxyflavan-3-ol.

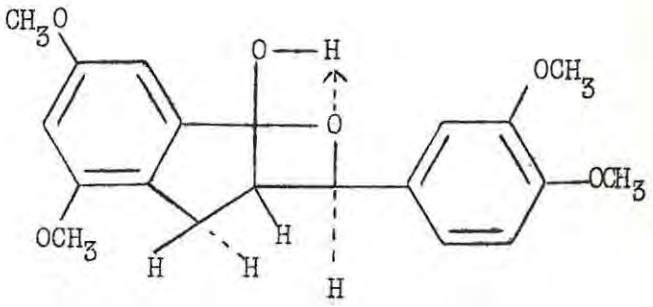
Conformations of catechins.

For every configuration which the flavan-3-ol molecule assumes there may be two possible conformations with the substituent

groups at C_2 and C_3 assuming either axial or equatorial positions. Early on, in order to explain the difference in R_F values between catechin and epicatechin Roberts [4,22] postulated the presence of 3(ax) and 3(eq) hydroxyls respectively. The hydrogen bonding between the 3(ax) hydroxyl and the heterocyclic oxygen would be tantamount to the loss of one hydroxyl group in catechin with a resultant higher R_F value. This view was criticized by Whalley [2] and Clark-Lewis [23], Whalley suggesting that the C_2-aryl and C_3-hydroxyl groups in catechin be axial and trans, XXVII. This conformation would meet with the steric requirements for the 1,2 shift in catechin. Epicatechin would then have C_2-aryl equatorial and C_3-hydroxyl axial as for catechin. XXVIII shows this conformation for (-)-epicatechin. In



(XXVII)



(XXVIII)

both instances Whalley regards the C_3-hydroxyl group to be hydrogen-bonded to the heterocyclic oxygen thus explaining the low hydroxyl stretching frequencies and the absence of a free

hydroxyl band near 3630 cm^{-1} in the infrared spectra of the two compounds [17]. Furthermore the higher R_F value of catechin could then be ascribed to an appreciable disparity in molecular shape with the near-planar epicatechin being more strongly adsorbed by the cellulose. Recent evidence has shown Whalley's view to have been incorrect.

Nuclear magnetic resonance spectra of catechins.

Only since the advent of n.m.r. has the exact conformation of substituents in flavan-3-ols been established with certainty, [9,24] and in the light of new evidence obtained by this method, many of the earlier theories required revision. The cis and trans stereochemistry at C_2 and C_3 is very clearly correlated by low spin-spin coupling constants ($J_{2,3} \sim 0$ c.p.s) for 2,3-cis (eq, ax) compounds and the much larger value ($J_{2,3} \sim 10$ c.p.s.) for 2,3-trans (eq. eq) compounds. Thus, accepting the half-chair conformation, in both cases the bulky C_2-aryl group assumes the equatorial conformation while the C_3-hydroxyl group is axial in compounds of the (-)-epicatechin (2,3-cis)-type and equatorial in compounds of the (+)-catechin (2,3-trans)-type.

The Absolute Configuration of Flavan-3,4-Diols.

Flavan-3,4-diols have three centres of asymmetry, compared with two in flavan-3-ols, so that four racemates and eight optically active forms are possible for each leuco-anthocyanidin.

The four racemates may be divided into two classes according to the cis- or trans-configuration of the 2,3- substituents. Both classes include 3,4-cis- and 3,4-trans-diol arrangements.

Absolute configuration at C_2 and C_3.

The stereochemistry at C_2 and C_3 of a particular flavandiol may be established by conversion of the flavandiol to the corresponding flavan-3-ol by the method of Weinges [25]. By a comparison of the molecular rotation differences [25] of derivatives of the flavan-3-ol with those of (+)-catechin(2,3-trans) and (-)-epicatechin(2,3-cis), it is established to which of the two series the compound belongs. Since the absolute configurations of (+)-catechin and (-)-epicatechin are now known, it is possible to assign absolute configurations at C_2 and C_3 to the new flavan-3-ol (and to the flavan-3,4-diol from which it is derived) by analogy.

By applying the above methods the configuration at C_2 and C_3 has been established for a number of flavan-3,4-diols. The 2,3-trans configurations of (+)-mollisacacidin [26] [(+)-7,3',4'-trihydroxyflavan-3,4-diol], (-)-leuco-fisetinidin [27] [(-)-7,3',4'-trihydroxyflavan-3,4-diol)] and (+)-leuco-robinetinidin [25] [(+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol] were established by their synthesis from the 2,3-trans-flavan-3-ol-4-ones, (+)-fustin (2R:3R), (-)-fustin (2S:3S), and (+)-dihydrorobinetin (2R:3R) respectively, and by their conversion into the 2,3-trans-flavan-3-ols,

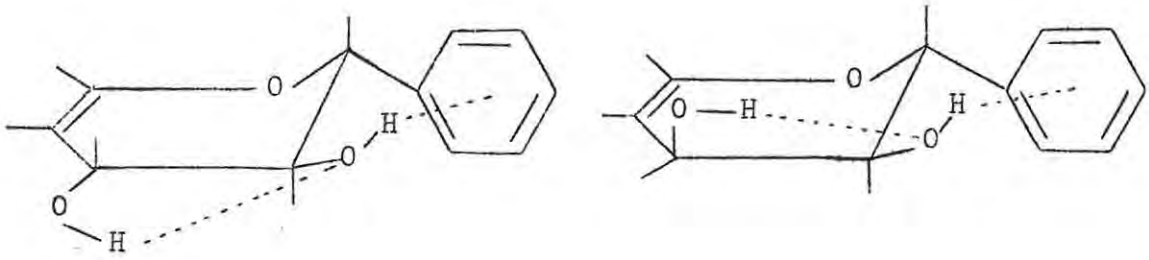
(-)-fisetinidol [28] (2R:3S), (+)-fisetinidol [25] (2S:3R) and (-)-robinetinidol [25] (2R:3S) respectively. Since the absolute configurations of these flavan-3-ols was known from molecular rotation differences in comparison with (+)-catechin [25], that of the flavan-3,4-diols could be assigned at C_2 and C_3. Clark-Lewis and Katekar [29] have recently confirmed (-)-fisetinidol as a stereochemical analogue of (+)-catechin by showing that its methyl ether underwent rearrangement to the (+)-2-ethoxy-7,3',4'-O-trimethyl-isoflavan. This reaction is considered to proceed by 1,2- arrangement of trans groups and is useful for characterizing members of the catechin (2,3-trans) series.

Absolute configuration at C_3 and C_4. The cis or trans nature of a flavan-3,4-diol may be reliably proved by studying the rate of cleavage of the 1,2-glycol unit with periodic acid [30,31] or with lead tetra-acetate [32,33,34,35]. In all instances the cis-diol grouping is fissioned more rapidly than the trans-diol group.

The formation of an isopropylidene derivative in high yield was the criterion used for the tentative 3,4-cis assignment of (+)-mollisacacidin [36], (-)-leuco-fisetinidin [37] and (+)-leuco-robinetinidin [38]. These assignments were only tentative since Angyal and Macdonald [39] had previously shown that in one instance bridging of a trans-diol by the isopropylidene group had occurred. Bokadia, Brown [34] et al., after examining the yields of

isopropylidene derivatives and correlating these with the results from lead tetra-acetate oxidation of flavandiols showed that with certain exceptions, a correlation between a high yield of isopropylidene derivative and the presence of a 3,4-cis-diol grouping appeared to exist. For highly substituted compounds such as the tetramethyl ether of leuco-robinetinidin the yield of isopropylidene has, however been shown to be an unreliable criterion for studying the configuration of the 3,4-diol grouping [10]. Also, contrary to the earlier assumptions by King and Bottomley, [40] (3,4-cis structure for melacacidin) and Kulkarni and Joshi [41], (3,4-cis structure for 6-methyl-4'-O-methylflavan-3,4-diol) the formation of a cyclic carbonate cannot be regarded as evidence for a 3,4-cis-diol group. Cyclic carbonates of 3,4-trans compounds have been prepared by Bokadia and co-workers [34], Corey, Philbin and Wheeler [42] and Drewes and Roux [10].

Philbin, Wheeler, Brutcher and Bauer [43] have employed infrared spectroscopy in their examination of the configuration of two isomeric unsubstituted flavan-3,4-diols. A study was made of the intramolecular hydrogen bonding exhibited by the two flavandiols. The 3,4-cis compound, in addition to giving a band due to the π-bonding of the hydrogen atom of the 3-hydroxyl group to the phenyl ring (an effect also shown by the 3,4-trans-diol), showed a strong absorption due to intramolecular OH.....O bonding at 3578 cm^{-1}. The two compounds were accordingly represented as XXIX and XXX.



(XXIX) 3,4-trans (Weak OH----O bond) (XXX) 3,4-cis (Strong OH----O bond)

Recently, n.m.r. methods have been applied to the study of the configuration of flavan-3,4-diols [9,24,42,10]. Assuming a half-chair conformation for the heterocyclic ring and a predominantly equatorial arrangement of the 2-phenyl group, then from the correlation between the dihedral angle of vicinal protons and their coupling constants, as outlined by Karplus [44] and by Conroy [45], an interpretation of the observed spin-spin coupling constants from the C_2-, C_3- and C_4-protons becomes possible.

Clark-Lewis, Jackman and Williams [9] have synthesized a complete set of the four possible racemates of 6-methyl-3',4'-dimethoxyflavan-3,4-diol and have reported nuclear magnetic resonance data for all the racemates. These results indicate the clear distinction between the 3,4-cis ($J_{3,4}$=4.3 c.p.s.) and 3,4-trans ($J_{3,4}\sim$1 c.p.s.) pair of 2,3-cis-flavan-3,4-diols. For the pair of 2,3-trans-flavan-3,4-diols the 3,4-cis compound has

$J_{3,4}$ = 3.3 c.p.s. and the 3,4-trans compound $J_{3,4}$ = 7.5 c.p.s. Determination of the spin-spin coupling constants for the 2-,3-, and 4-protons from nuclear magnetic resonance spectrometry thus provides a convenient and clear means for determining the stereochemistry of flavan-3,4-diols.

The methods outlined find application in the elucidation of the stereochemistry of flavan-3,4-diols obtained by means of stereospecific syntheses.

Stereospecific Syntheses of Flavan-3,4-Diols.

General Considerations.

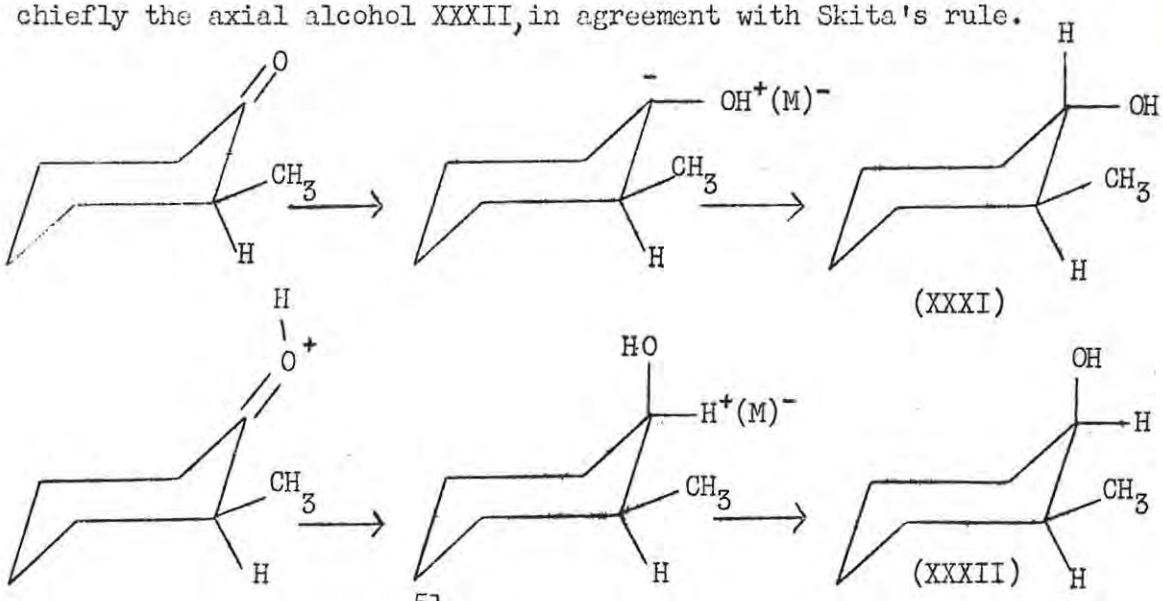
Stereospecific syntheses of leuco-anthocyanidins are potentially useful in determining the geometrical configuration of naturally-occurring flavan-3,4-diols. Attempts to synthesize all four possible racemates of a particular flavan-3,4-diol received new impetus with the advent of n.m.r. spectroscopy but even prior to this a complete set of four racemates of 6-methyl-4'-methoxyflavan-3,4-diol had been prepared by Kulkarni and Joshi.[41,46] For the syntheses of isomeric flavan-3,4-diols, a number of reagents were employed. These include sodium (or potassium) borohydride, lithium aluminium hydride, a mixture of lithium aluminium hydride with aluminium chloride, Raney nickel and platinum oxide. It should be noted that relatively few reducing agents are entirely stereo-

specific and usually mixtures with a preponderance of one stereoisomer, are obtained.

In a recent review Kamernitsky and Akhrem [47] have attempted to generalize the data on the stereochemistry of nucleophilic addition to the carbonyl group. The relationship between conditions of reaction and configuration of stereoisomers was first proposed by Skita [48]. According to the so-called Skita rule, catalytic hydrogenation in acid medium gives mainly the cis-form of the alcohol whereas hydrogenation in neutral medium gives mainly the trans-form. This rule was later extended by Skita and other workers such as Vavon [49] to:rapid hydrogenations yield more cis-isomer and slower hydrogenations yield more trans-isomer. The rule was generally regarded valid for the hydrogenation of hydrocarbons, amines, phenols and five- and six-membered cyclic ketones.

In agreement with the Skita rule are the findings of Brewster [50] on the reduction of cyclohexanones. He postulates the formation of a "surface hydride" and suggests that ketones might be able to accept a proton from the solvent and the equivalent of a hydride ion from the catalyst, in distinct steps. Attachment of the bulky metal surface M is likely to be in the equatorial position in the last-named step. The reduction of a ketone in neutral medium would produce chiefly the equatorial alcohol XXXI, while reduction of the reactive protonated form of a ketone produces

chiefly the axial alcohol XXXII, in agreement with Skita's rule.



In 1953 Barton [51] formulated rules regarding the spatial direction of the ionic reduction of ketones with sodium and alcohol, with complex metal hydrides, and with various metal catalysts. These rules state that:

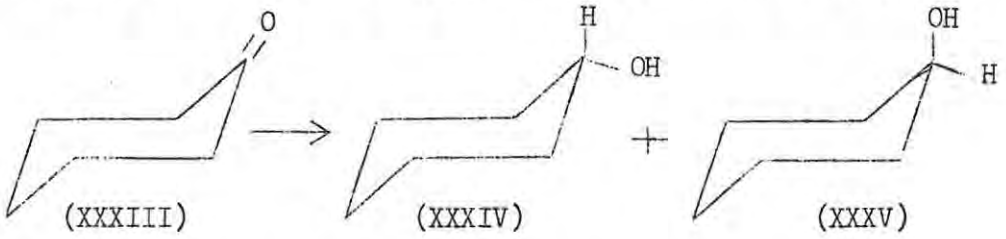
1) Catalytic hydrogenation of ketones in acid media (rapid hydrogenation) generally affords the axial alcohol.

2) Catalytic hydrogenation in neutral medium (slow) gives axial alcohols with sterically kindered ketones, but equatorial alcohols with sterically unhindered ketones.

3) Catalytic hydrogenation of oximes follows the same course as catalytic hydrogenation of ketones.

4) Reduction with sodium borohydride and with lithium aluminium hydride generally affords the equatorial epimer if the ketonic group is unhindered, but the axial form if it is hindered.

In the case of the non-hindered ketone, Barton regarded the directed reduction to go mainly towards producing the thermodynamically more stable isomer, with the equatorial arrangement of the hydroxyl group. Hückel [52] had pointed out earlier however, that the composition of the equilibrium mixtures formed in the reduction of ketones is determined, not by the relative thermodynamic stability of the isomers XXXIV and XXXV but, in the first instance, by the kinetics of the competing reactions XXXIII→XXXIV and XXXIII→XXXV. From his examination of the reduction of substituted cyclohexanones, menthone and related compounds,



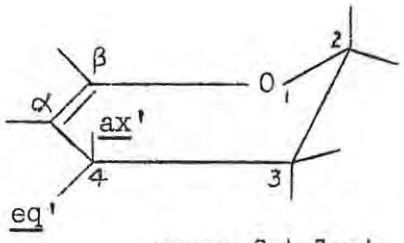
(XXXIII) (XXXIV) (XXXV)

Hückel [53] concludes that Barton's rules are accurate for some compounds but that there are also many exceptions. In his opinion lithium aluminium hydride cannot be regarded as a stereospecific reagent although in the reduction of certain terpene ketones containing a bridged ring system, it shows marked steric selectivity. Addition of aluminium bromide to lithium aluminium hydride may also completely change the direction of the reduction through the attack, not of the AlH_4^- ion but of the more bulky $AlBr_3H^-$.

The idea of spatial direction of the reduction of cyclic ketones with complex metal hydrides has been advanced by Dauben, Fonken and Noyce [54]. The two factors, "steric approach control"

comprising the screening of one side or other of the molecule and "product development control" comprising energetic effects depending on the relative stability of the reaction products, are thought to be operative.

The mechanism of catalytic hydrogenations has been studied in detail by Mitsui and Imaizumi [55]. Mitsui and Kasahara [56] have examined the n.m.r. spectra of flavan-4α-ol and flavan-4β-ol (catalytic reduction with Raney nickel) and their assignments are based on the conclusions which may be drawn from the manner in which spatial arrangements affect the adsorption of the molecule to the catalyst. In the synthesis of flavan-4-ols Bognar, Rakosi, Fletcher, Kehoe, Philbin and Wheeler [57] have suggested a possible mode of reduction: The molecule XXXVI (in



(XXXVI)

xxxxx Catalyst

the sofa conformation) may be visualised as sited on the catalyst at carbon atoms 1,3,4, α and β. The triangle formed by the atoms 1,2 and 3 points away from the surface and the catalyst is thus on the equatorial side of C_4. If reduction of the carbonyl double bond

(at C_4) is assumed to be effected by the previous non-catalytic attachment of a solvent proton to the C_4-oxygen atom (cf. Brewster [50]) followed by the deposition of a hydrogen anion on the C_4 atom, the C_4-hydroxyl bond will be released into the pseudo-axial conformation at an early stage of the reduction and the bond attached to the hydrogen atom at C_4 will subsequently be in the equatorial position. This mechanism appears plausible in the light of modern theory but will require modification since Lillya, Kehoe, Philbin, Vickers and Wheeler [58] have very recently shown that the C_4-hydroxyl in the above case, is in fact equatorial.

Regarding the synthesis of flavan-3,4-diol racemates through use of the methods outlined, it has been found that:

a) 2,3-trans-3,4-trans-flavandiols are readily obtained by catalytic reduction of 2,3-trans-dihydroflavonols in neutral or acid medium, and also by reduction with either lithium aluminium hydride or sodium borohydride.

b) 2,3-trans-3,4-cis-flavandiols may be synthesized by reduction of 2,3-trans-dihydroflavonols with the "mixed reagent", lithium aluminium hydride-aluminium chloride. This isomer may also be obtained by the "oxime-amine" route.

c) The diacetates of 2,3-cis-3,4-trans-flavandiols result from the reduction of 3-bromoflavanones to bromoflavan-4-ols followed

by the reaction of the latter with potassium acetate and acetic anhydride.

d) 2,3-cis-3,4-cis-flavandiol racemates are obtained by catalytic reduction (with heating and pressure) of the flavonol with Raney nickel.

Flavan-3,4-Diols from Dihydroflavonols (Flavan-3-ol-4-ones).

The dihydroflavonols were discovered and synthesized long after the flavones and flavonols (flavone-3-ols). Oyamada [87] in 1934 showed that fustin (7,3',4'-trihydroxyflavan-3-ol-4-one) occurs with fisetin (7,3',4'-trihydroxyflavone-3-ol) in Rhus spp. and is 2,3-dihydrofisetin. The known, naturally-occurring dihydroflavonols isolated since, are all regarded as having the bulky C_2-aryl and the C_3-hydroxyl group equatorial and trans to one another and hence belong to the 2,3-trans-catechin series. Flavan-3,4-diols obtained by reduction of these dihydroflavonols will thus have the 2,3-trans-configuration. The stereochemistry of dihydroflavonols has been studied in detail by the Indian workers Mahesh and Seshadri [59], and Kulkarni and Joshi [60]. Dihydroflavonols are useful starting materials for the synthesis of flavan-3,4-diols as they are more readily reduced than flavonols, and in addition, their stereochemistry is now known.

a) Catalytic hydrogenation of dihydroflavonols.

Catalytic hydrogenation of dihydroflavonols was first applied with success to free phenolic forms by Freudenberg and Roux[61,62] (1954) using dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one) from Robinia pseudacacia. Hydrogenation over Adam's catalyst in methanol gave a leuco-robinetinidin (7,3',4',5'-tetrahydroxyflavan-3,4-diol) in high yield, the first crystalline flavandiol with free phenolic groups to be prepared by synthesis. Originally this compound was regarded as having a 2,3-trans-3,4-cis-configuration[63]. Application of the same conditions for the reduction of dihydroquercetin, (5,7,3',4'-tetrahydroxyflavan-3-ol-4-one) by Freudenberg and Weinges[64] was not successful, presumably due to hydrogen bonding between the C_4-keto group and the C_5-hydroxyl group. This problem was subsequently overcome by prior benzylation of the phenolic groups in the dihydroquercetin. Bognar and Rakosi[81] reduced dihydroflavonol by means of palladous charcoal in ethanolic or acetic acid solution and also by metal hydrides. The same flavan-3,4-diol was obtained from all these reductions and these workers concluded, on the basis of Barton's rules, that the diol had a 3,4-trans-configuration.

Reduction of (±)-fustin from Rhus glabra[62,65], (+)-fustin from acacia mearnsii[26] and (-)-fustin from Rhus cotinus[37] with platinum oxide in methanol yielded (±)-leuco-fisetinidin (7,3',4'-

trihydroxyflavan-3,4-diol, (+)-leuco-fisetinidin (mollisacacidin) and (-)-leuco-fisetinidin respectively. These compounds were tentatively assigned the 2,3-trans-3,4-cis configuration since all afforded isopropylidene derivatives in good yield.

Various methods of reduction have been applied by Shah and Kulkarni [66]. Reduction of the tetramethyl ether of dihydrorobinetin with platinum black and with lithium aluminium hydride, gave the methyl ether of the leuco-robinetinidin previously isolated by Freudenberg and Roux [61]. Attempts to isolate another isomer, thought to be present in the reaction mixture, were not successful. Hydrogenation of 7,8,3',4'-tetramethoxy-2,3-trans-dihydroflavonol (obtained via synthesis of the chalcone and chalcone dibromide) over platinum oxide in acetic acid gave Kulkarni and Joshi [67] a flavandiol of m.p. 132°, differing from the flavandiol (m.p. 179°), obtained by reduction of the same dihydroflavonol with lithium aluminium hydride. The lower melting isomer was regarded by these workers as being identical with (±)-melacacidin (7,8,3',4'-tetrahydroxyflavan-3,4-diol) tetramethyl ether. From a consideration of Barton's rules for unhindered ketones, Kulkarni and Joshi considered the C_4-hydroxyl in the diol m.p. 132° to be axial and equatorial in the other isomer. Furthermore, according to the Auwers-Skita [68] rule, the lower melting isomer should be the 3,4-cis-diol. Direct comparison of this diol

with (±)-melacacidin tetramethyl ether, m.p. 135° (which, from its method of reduction from 7,8,3',4'-tetramethoxyflavonol with Raney nickel [69] is a 2,3-cis-3,4-cis compound) by King and Clark-Lewis [70] indicated non-identity, thus proving the diol of the Indian workers to be a different isomer. Present knowledge suggests that the diol has in fact the 2,3-trans (being derived from a trans-dihydroflavanol)-3,4-trans configuration. The example illustrates clearly that an empirical application of Barton's rules or the Auwers-Skita rule may lead to erroneous conclusions.

b) Reduction of dihydroflavonols with metal hydrides.

Bauer, Birch and Hillis [71] attempted the reduction of dihydrokaempferol (5,7,4'-trihydroxyflavan-3-ol-4-one) with lithium aluminium hydride but obtained only an amorphous product, which on treatment with hydrochloric acid yielded pelargonidin. A flavan-3,4-diol structure, cf. Bate Smith [72], was suggested for the intermediate compound. Swain [73] converted dihydroquercetin (5,7,3',4'-tetrahydroxyflavan-3-ol-4-one) with sodium borohydride and alcohol into an amorphous leuco-cyanidin (5,7,3',4'-tetrahydroxyflavan-3,4-diol). Methylation of the leuco-compound yielded the crystalline tetramethyl ether which could also be obtained by reducing dihydroquercetin tetramethyl ether with sodium borohydride. No conclusions regarding the stereochemistry of the compound were drawn. Presumably a geometrical isomer of the tetramethyl ether obtained by Swain, was synthesized by Freudenberg and Weinges [64] through reduction

of the tetrabenzyl ether of (+)-dihydroquercetin with lithium aluminium hydride. The compound was assigned the 2,3-trans-3,4-cis-configuration, the latter designation being based on the ability of the diol to form an isopropylidene derivative. Ganguly and Seshadri [77] reduced dihydroquercetin tetramethyl ether with sodium borohydride and isolated a mixture of two isomeric leuco-cyanidin tetramethyl ethers. The stereochemistry of these diols has not been studied, but the higher melting isomer does not depress the m.p. of the tetramethyl ether of leuco-cyanidin obtained from Butea frondosa gum.

Reduction of (±)-6-methyl-4'-methoxydihydroflavonol by Kulkarni and Joshi [41,46] with lithium aluminium hydride gave a pair of diols differing in the configuration of the C_4-hydroxyl group. These workers regarded the higher melting diol (m.p.193°) as the trans-trans-racemate and the lower melting diol (m.p. 169°) as the trans-cis-racemate. The hydroxyl group was regarded as being axial in the latter compound and equatorial in the former. However, from studies of reduction products obtained by using the "mixed reagent", lithium aluminium hydride-aluminium chloride, Bokadia, Brown et al. [34] have proved that the assignments proposed by Kulkarni should be reversed.

A combination of sodium borohydride and boron trifluoride was used by Kashikar and Kulkarni [74] to effect conversion of the

6-methyl-4'-methoxyflavan-3,4-diol (m.p. 169°) mentioned above, to the diol of m.p. 193°. Bearing in mind the present assignment for the configuration of these two diols [34], the above reaction converts a 3,4-trans-diol to a 3,4-cis-diol.

Chandorkar and Kulkarni [75] reduced the trimethyl ether of fustin with lithium aluminium hydride and obtained two diols. The one of lower m.p. was shown by Clark-Lewis and Roux [36] to be identical with trimethyl ether of the flavandiol obtained by catalytic reduction of (±)-fustin [62]. Subsequent investigations by Drewes and Roux [76] have shown their higher melting isomer (m.p. 172°) to be a somewhat impure form of the 2,3-trans-3,4-cis-flavandiol m.p. 185°.

These examples illustrate that stereochemical deductions based purely on the method of synthesis are not always trustworthy and that a final assignment can only be made in conjunction with data derived from comparative oxidation rates [10,34] and n.m.r. spectra [9,10,42] of the diols.

c) Reduction of dihydroflavonols with the "mixed reagent", lithium aluminium hydride - aluminium chloride.

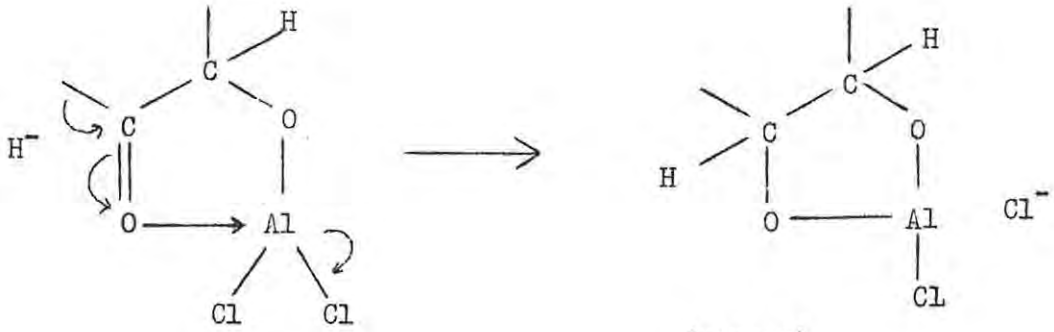
It has been pointed out by Dauben and co-workers [54] that where metal hydride reductions of ketones can give rise to diastereoisomeric alcohols, the ratio depends on the nature of the metal hydride used in the reduction. The first recorded use

of the mixed reagent was by Brown [78] for the synthesis of new cholestene diols. This method has been applied with success in the flavonoid field and Brown and co-workers [34] have developed a convenient preparative method for the synthesis of 2,3-trans-3,4-cis-flavandiols from 2,3-trans-dihydroflavonols. These workers examined the n.m.r. spectra of the diacetates of 2,3-trans-flavan-3,4-trans diol, m.p. 145° and 2,3-trans-flavan-3,4-cis-diol, m.p. 163°. The former compound was prepared by reduction of the dihydroflavonol with lithium aluminium hydride, sodium borohydride or palladium over charcoal whereas the trans-cis-diol was obtained by means of the "mixed reagent". The configuration at the C_2-position was expected to be the same for the two diols. Both diols showed a peak at τ 8.16 and this lay in the range of chemical shifts found for equatorial acetoxy groups for pyranose sugars. The difference between the diols thus depended on the conformation of the second acetoxy-group which was at a considerably lower field (τ 7.87) for the diol m.p. 163°. This isomer was accordingly regarded as having the second acetoxy-group axial, while the lower m.p. isomer had this group in the equatorial position. These assignments were in agreement with the oxidation rates of the two diols i.e. the diol of m.p. 163° was 3,4-cis and the diol of m.p. 145° was 3,4-trans.

Clark-Lewis, Jackman and Williams [9] have employed the mixed reagent for the synthesis of 6-methyl-3',4'-dimethoxy-2,3-

trans-flavan-3,4-cis-diol while trans-cis-derivatives based on leuco-fisetinidin and leuco-robinetinidin were prepared in a similar manner by Drewes and Roux [76] and Lillya, Drewes and Roux [79].

The mechanism of reduction with the mixed reagent is certain to be linked with the coordination properties of the aluminium. Clark-Lewis [9] has suggested that coordination of the aluminium with the keto alcohol coupled with hydride ion attack on the resulting complex, would result in the cis-diol complex shown in XXXVII.



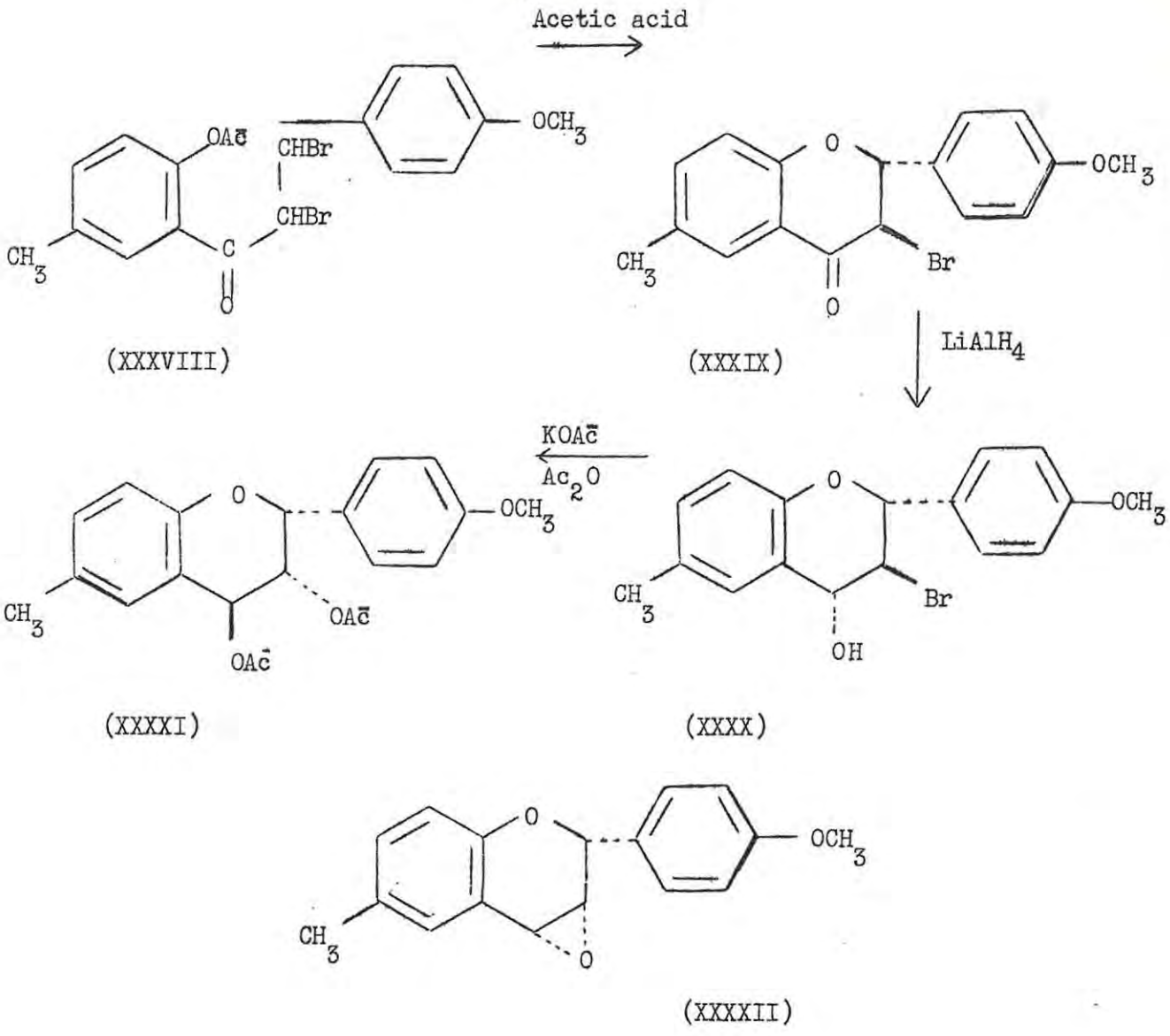
(XXXVII)

Another method for the synthesis of 2,3-trans-3,4-cis-diols is known. Bognar, Rakosi, Fletcher, Philbin and Wheeler [80] have developed a synthesis leading from the dihydroflavonol to the oximinoflavar which, on reduction, yields the amine and this in turn is convert . to the flavandiol. The method is, however, more laborious than the "mixed reagent" one.

Flavan-3,4-Diols from 3-Bromoflavanones.

This method leads to the 2,3-cis-3,4-trans-diols, the least accessible of the four racemic forms of the leuco-anthocyanidins. It was first employed by Kulkarni and Joshi [41,82] for the synthesis of the third racemate of 6-methyl-4'-methoxyflavan-3,4-diol, isolated as the diol, m.p. 150°. In this synthesis the chalcone dibromide XXXVIII was cyclised in acetic acid to give the 3-bromoflavanone XXXIX. The latter was reduced with lithium aluminium hydride to the bromoflavan-4-ol XXXX which was then converted to the diol diacetate XXXXI with potassium acetate and acetic anhydride. It was found that treatment of the 4-ol XXXX, with sodium hydroxide in dioxane furnished an epoxide XXXXII, which on treatment with acetic acid-sulphuric acid and acetylation afforded the same diol diacetate XXXXI.

The stereochemical course of this conversion has not been clarified despite subsequent investigation by Indian workers [83]. Recently Clark-Lewis, Spotswood and Williams [84] have deduced, on the basis of n.m.r. data, that the 3-bromoflavanone XXXIX m.p. 138° is the 2,3-trans-isomer ($J_{2,3}$ = 8.5 c.p.s.). Kulkarni and co-workers [83,85] previously regarded this as the 2,3-cis-isomer and their evidence was based chiefly on the velocity of dehydrobromination to the corresponding flavone. The compound which has now been identified by Clark-Lewis and co-workers [84] as the

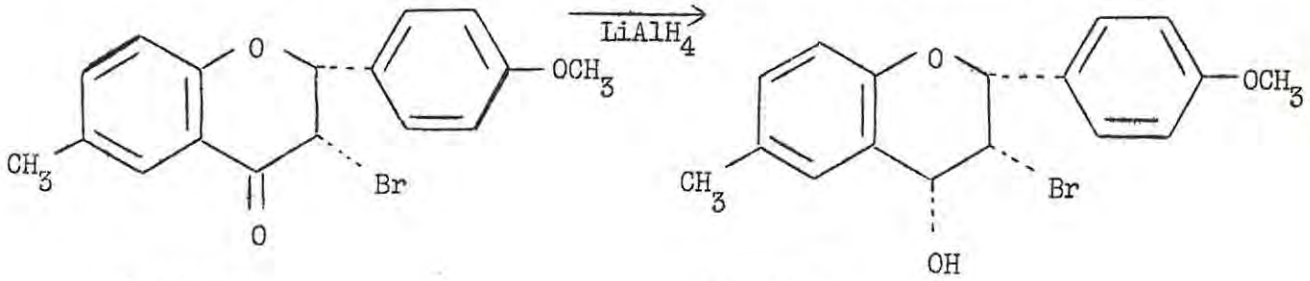


cis-isomer ($J_{2,3}$ = 1.8 c.p.s.) has m.p. 158 - 159°. Kulkarni had cited a m.p. 167° [85] and 152° [82] for this compound.

Reduction of the 2,3-trans- -bromoflavanone[84] XXXIX, with lithium aluminium hydride gave the corresponding

2,3-trans-3,4-trans-bromoflavan-4-ol XXXX m.p. 201 - 202°, ($J_{2,3}$ = 10.4 c.p.s.; $J_{3,4}$ = 9.3 c.p.s.) which was regarded as the 2,3-cis-3,4-trans-compound by Kulkarni [82,83]. Conversion of the 2,3-trans-3,4-trans-3-bromoflavan-4-ol XXXX to the 2,3-cis-3,4-trans-diacetate with potassium acetate and acetic anhydride apparently occurs by trans-opening of the cis-cis epoxide XXXXII formed from XXXX with inversion at C_3 [84].

Reduction of the 2,3-cis-bromoflavanone XXXXIII m.p. 158°, with lithium aluminium hydride gave the 2,3-cis-3,4-cis-bromoflavan-4-ol XXXXIV, ($J_{2,3}$ = 1.0; $J_{3,4}$ = 4.3 c.p.s.) which Kulkarni [41]



(XXXXIII) (XXXXIV)

had formerly regarded as the 2,3-trans-3,4-cis compound. 6-Methyl-3',4'-dimethoxy-2,3-cis-flavan-3,4-trans-diacetate has been prepared by methods similar to the above [9].

The inferences drawn by the Indian workers regarding configuration, from the velocity of dehydrobromination of the 3-bromoflavanones as judged by an increase in absorption at 320 mμ, has been criticized by Clark-Lewis and co-workers [84]. Conclusions

reached by Kulkarni [83] are regarded as invalid since alternative reactions leading from the trans-compound to the related chalcone and aurone, as well as dehydrobromination to the flavone, were not considered. Clark-Lewis et al. did, in fact, observe that the 2,3-cis-3-bromoflavanone became yellow on storage presumably through trans-elimination of hydrogen bromide whereas the 2,3-trans-3-bromoflavanone was completely stable.

Flavan-3,4-Diols from Flavonols.

The first successful reduction of a flavonol to a flavan-3,4-diol was by Mozingo and Adkins [86] who prepared a flavan-3,4-diol, m.p. 123 - 124° by reduction of flavonol over copper-chromic oxide catalyst. This compound is isomeric with flavan-3,4-diol m.p. 145 - 146° obtained by Bognar and Rakosi [81] from dihydroflavonol under a variety of conditions. The compound m.p. 145 - 146°, was regarded to be trans-trans, but in the light of present knowledge the diol of m.p. 123 - 124° is likely to have been the cis-cis-isomer.

King and Clark-Lewis [69,70] have shown that hydrogenation of flavonols with Raney nickel yields 2,3-cis-3,4-cis-flavandiols and to date this is the only method available for obtaining this stereoisomer. Catalytic reduction of ethylenic bonds proceeds by cis- addition and on this basis reduction of a flavonol should yield a product in which the C_2-aryl, C_3-hydroxyl and C_4-hydroxyl

substituents assume the cis-configuration.

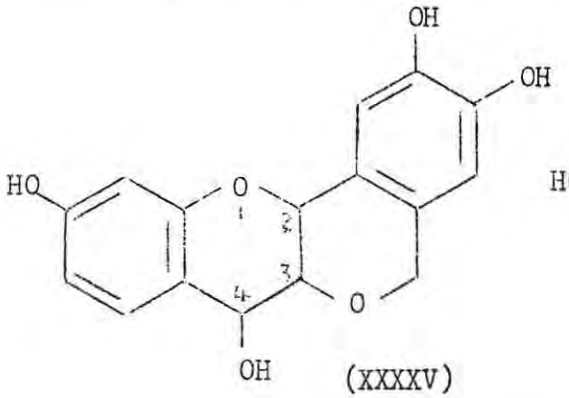
Hydrogenation over Raney nickel of 7,8,3',4'-tetramethoxyflavonol in ethanol gave the first crystalline synthetic methylated leuco-anthocyanidin, 7,8,3',4'-tetramethoxyflavan-3,4-diol [70]. The compound was regarded as being a cis-cis-diol by virtue of its ability to form a cyclic carbonate and an isopropylidene derivative, although now these two criteria are known to lead to erroneous conclusions [10]. The synthetic 2,3-cis-3,4-cis racemate has the same geometrical configuration as the naturally-occurring melacacidin. Recently Clark-Lewis and Katekar [29] have synthesized the methyl ether of cis-cis-leuco-fisetinidin (7,3',4'-O-trimethyl-2,3-cis-flavan-3,4-cis-diol) by the above method.

Comparison of the properties of flavandiols obtained by stereospecific synthesis with those of flavandiols isolated from Nature has proved valuable in establishing the stereochemistry of the naturally-occurring leuco-anthocyanidins.

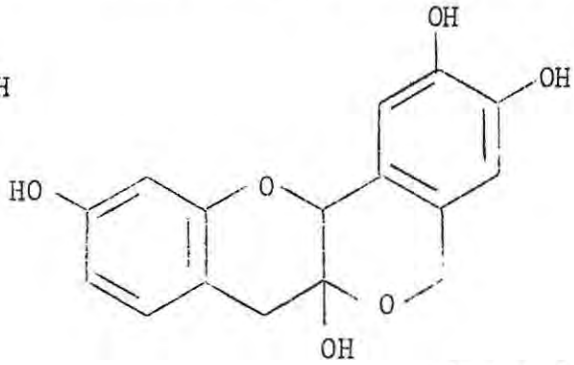
STEREOCHEMISTRY OF NATURALLY-OCCURRING LEUCO-ANTHOCYANIDINS.

Peltogynol and Peltogynol B.

Peltogynol XXXXV was one of the first leuco-anthocyanidins to be isolated and was obtained from the heartwood of Peltogyne porphyrocardia by Robinson and Robinson [88]. Originally the structure XXXXVI, based mainly on evidence from degradative studies and its conversion into an anthocyanidin on treatment with



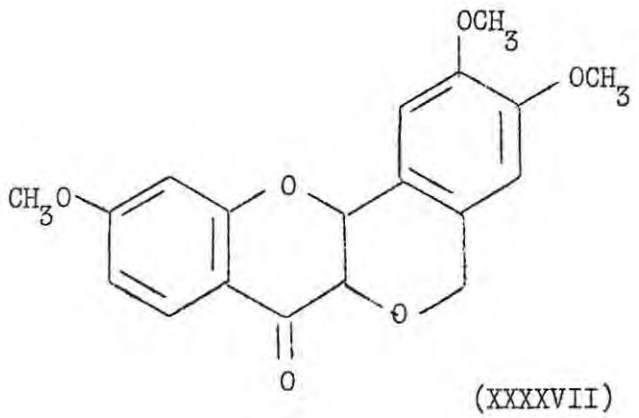
(XXXXV)



(XXXXVI)

acid, was suggested for peltogynol. The revised structure, XXXXV, established by Chan, Hassall and Forsyth [89,90], was deduced from the oxidation of peltogynol trimethyl ether with manganese dioxide to the optically active flavanone peltogynone trimethyl ether XXXXVII which yielded peltogynol trimethyl ether on reduction with sodium borohydride. This evidence clearly establishes the structure of peltogynol as a flavan-3,4-diol derivative.

Peltogynol is accompanied in Peltogyne porphyrocardia by a stereoisomer, Peltogynol B, which differs only in the

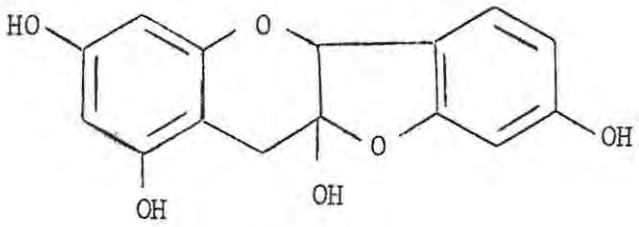


(XXXXVII)

configuration of the C_4-hydroxyl group [91]. When peltogynone trimethyl ether is reduced with sodium borohydride, only peltogynol trimethyl ether and no peltogynol B trimethyl ether is formed. If it is assumed that the ketonic group on C_4 behaves as an unhindered ketone, then reduction with borohydride should, by Barton's rules [51], yield the C_4-equatorial alcohol. Peltogynol B must thus have the C_4-hydroxyl axial. The B isomer also turns red (through anthocyanidin formation) when heated near the m.p. in air and this reaction involves the trans-elimination of the C_4-axial hydroxyl group. It will occur most readily when the C_3-hydrogen atom, the C_4-hydroxyl group and the carbon atoms at positions 3 and 4 are coplanar [51]. This is achieved when both the C_3-hydrogen atom and the C_4-hydroxyl group have axial conformations and this isomer may thus be regarded as a 3,4-cis-derivative. Peltogynol then has the 3,4-trans-diol configuration and both are considered as having the C_3-oxygen bond equatorial [90].

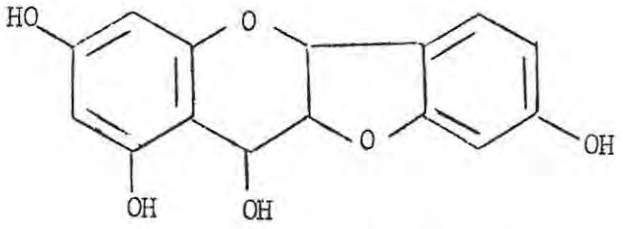
Cyanomaclurin.

Perkin and Cope [92] first isolated (1895) cyanomaclurin from jackwood - Artocarpus integrifolia. It was more fully investigated by Perkin [93] and by Appel and Robinson [94] who proposed the structure XXXXVIII. Freudenberg and Weinges [64,95]



(XXXXVIII)

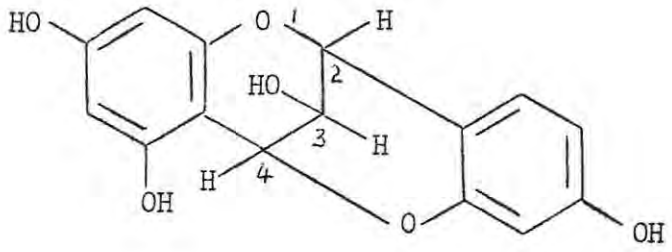
confirmed the presence of four hydroxyl groups in cyanomaclurin through formation of a tetramesyl derivative and provided additional evidence for the hemi-ketal formulation above by obtaining crystalline cyanomaclurin trimethyl ether monoacetate. However, on the available evidence, Clark-Lewis [96] regards XXXXIX as an alternative structure for the compound. Support for this formulat-



(XXXXIX)

ion has come from Seshadri and Chakravarty [97]. Recently Nair

and Venkataraman [98] proposed yet another structure for cyanomaclurin, L. This structure is in good agreement with the



(L)

n.m.r. data for cyanomaclurin and its trimethyl ether. The value of the spin-spin coupling constants from these spectra for the protons at C_2, C_3 and C_4 appear to indicate that protons at C_2 and C_4 are equatorial and cis to one another.

Leuco-anthocyanidins corresponding to the common anthocyanidins.

Several leuco-anthocyanidins corresponding to the common anthocyanidins have been isolated but in no instance has a study of the stereochemistry of these compounds been made. Leucopelargonidins (5,7,4'-trihydroxyflavan-3,4-diol) have been isolated by Ganguly and Seshadri [99] and Paris and Cubukcu [100]; leuco-cyanidins (5,7,3',4'-tetrahydroxyflavan-3,4-diol) by Seshadri and co-workers [101,102,103,104] and leuco-delphinidins (5,7,3',4',5'-pentahydroxyflavan-3,4-diol) by Hathway [105] and by Ganguly, Seshadri and Subramanian [106].

Melacacidin. [(-)-7,8,3',4'-tetrahydroxy-2,3-cis-flavan-3,4-cis-diol]

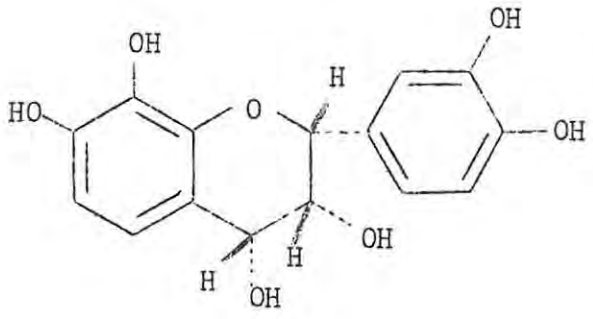
This was the first leuco-anthocyanidin isolated from natural sources and was obtained from the heartwood of Acacia melanoxylon by King and Bottomley [40]. Originally the compound could not be obtained crystalline but it yielded a crystalline tetramethoxy derivative. This leuco-anthocyanidin has subsequently been found in Acacia excelsa and Acacia harpophylla and obtained in crystalline form from these sources [107]. The structure of (-)-O- tetramethylmelacacidin has been confirmed by comparison with the synthetic (±)-7,8,3',4'-O-tetramethyl-2,3-cis-flavan-3,4-cis diol [70]. Both the racemic synthetic compound and the methylated (-)-melacacidin form cyclic carbonates. From this property, King and Clark-Lewis [70] surmised that the compound had a 3,4-cis-configuration. Since both compounds, in addition, yielded isopropylidene derivatives, this was regarded as further evidence for the 3,4-cis-configuration. At the time it was considered unlikely that cyclic acetals would result from 3,4-trans-diols. Although the original assignments were correct, the above criteria for 3,4-cis-diols are now known to be unreliable [10,34].

(-)-Melacacidin tetramethyl ether was hydrogenolysed over palladium to yield the (-)-2,3-cis-flavan-3-ol derivative [29,108]. This new flavan-3-ol was an analogue of (-)-epicatechin; its acetate was more laevorotatory than the alcohol and the tosylate was less

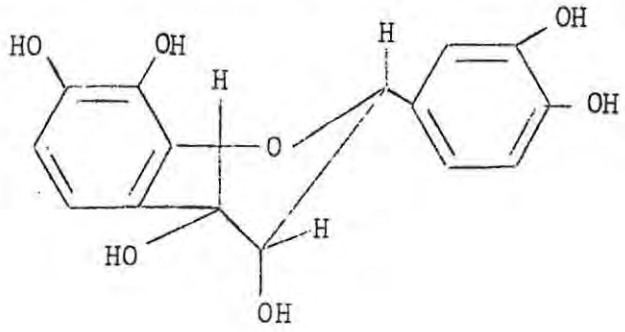
laevorotatory than the alcohol as is the case with (-)-epicatechin. Hence its 2,3-cis-configuration was established.

Two conformations of substituents 2(ax):3(eq):4(ax) and 2(eq):3(ax):4(eq) are possible for the natural flavan-3,4-diol [70]. Of these alternatives the 2(eq):3(ax):4(eq) disposition is regarded as the more favourable since the largest substituent i.e. the dihydroxy phenyl and the numerically larger number of groups other than hydrogen are equatorially situated.

Data obtained from n.m.r. spin-spin coupling constants for the protons at C_2, C_3 and C_4 have confirmed the cis-cis, 2(eq):3(ax):4(eq) assignments. Values for melacacidin tetramethyl ether were $J_{2,3}$ = ~ 1 c.p.s.; $J_{3,4}$ = 4.0 c.p.s. and for the diacetate $J_{2,3}$ = 1 c.p.s.; $J_{3,4}$ = 4.1 c.p.s.[29] The absolute configuration of the three asymmetric centres in melacacidin may now be specified, in the R and S notation of Cahn, Ingold and Prelog[21], as 2R : 3R: 4R and melacacidin may be represented as LI or LII.



(LI)

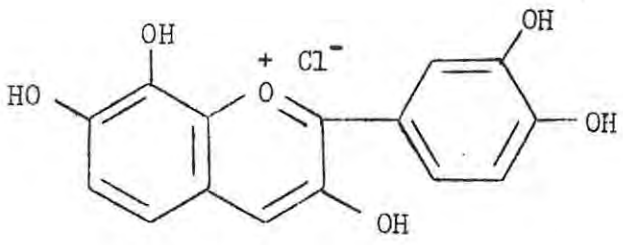


(LII)

Isomelacacidin (7,8,3'4'-tetrahydroxy-2,3-cis-flavan-3,4-trans-diol.)

In all the isolations of melacacidin it was accompanied by an isomer isomelacacidin [29,107]. The crude ethanolic extracts of A. melanoxylon, A excelsa and A. harpophylla contained three leuco-anthocyanidins - melacacidin, isomelacacidin and O-ethylisomelacacidin, the latter being an artefact formed from isomelacacidin and alcchol used during handling of the extract. Isomelacacidin in the extract could be converted to O-ethylisomelacacidin and the latter could then be separated from melacacidin by counter-current separation.

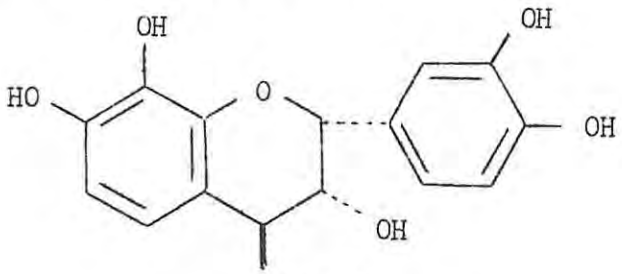
For deducing the structure of isomelacacidin, two observations were considered to be of importance [107]: a) melacacidin was readily converted to isomelacacidin under mild conditions (90% conversion with 0.1N hydrochloric acid for 10 min. at 100° and b) both leuco-anthocyanidins gave the same anthocyanidin LIII.



(LIII)

O-Ethylisomelacacidin yielded an amorphous tetramethyl ether which gave a crystalline toluene-p-sulphonate, thus indicating the presence

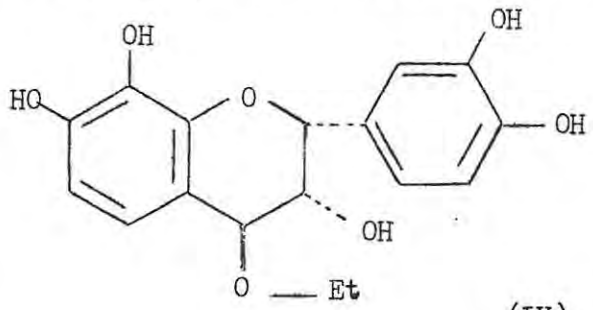
of four phenolic groups and an alcoholic hydroxyl group. In addition, isomelacacidin and O-ethylisomelacacidin gave the same sulphone LIV. Melacacidin gave the same sulphone but only under



(LIV)

more acid conditions which are known to result in epimerization [109]. This then established the stereochemical identity in melacacidin and isomelacacidin of those positions not affected by acid.

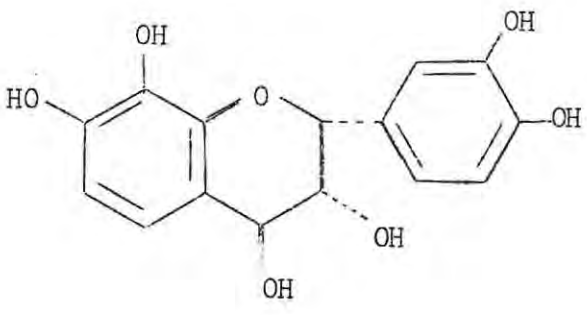
It remained to establish whether O-ethylisomelacacidin was LV or the analogous 3-ethoxy-4-hydroxy compound. The extreme lability of the ethyl group in O-ethylisomelacacidin excludes the



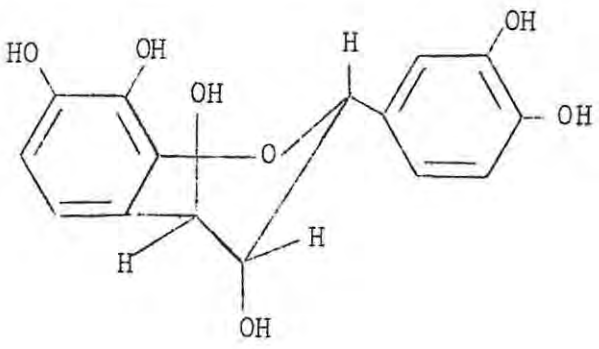
(LV)

latter possibility and favours the benzylic ether structure LV. Melacacidin and isomelacacidin were therefore regarded as epimers

differing only in the configuration of the C_4-hydroxyl group. Bearing in mind that the properties of isomelacacidin are in good accord with those of other benzyl alcohols activated by o- and p-hydroxyl groups, and that the reactivity of these alcohols is attributable to resonance stabilization of the benzyl carbonium ions [110], this indicates that the C_4-hydroxyl group in isomelacacidin is axial. In this conformation maximum resonance stabilization (and greater reactivity) of the C_4-carbonium ion is through coplanarity of the attached groups. If the C_4-hydroxyl were equatorial (as in melacacidin) the compound would be considerably less reactive. The fact that epimerization of melacacidin to isomelacacidin occurs as the free phenol and not as the methyl ether, is explained by the greater electron release from the hydroxyl group than from the methoxyl group. Isomelacacidin is thus represented as in LVI or LVII. Its absolute configuration may be designated as 2R:3R:4S.



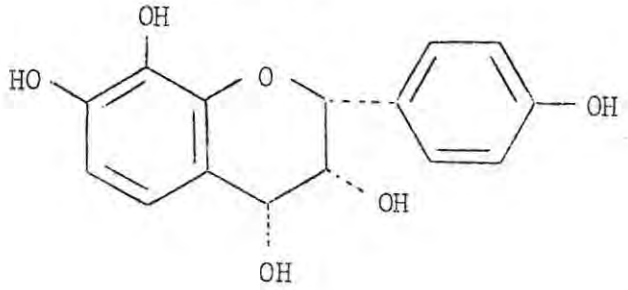
(LVI)



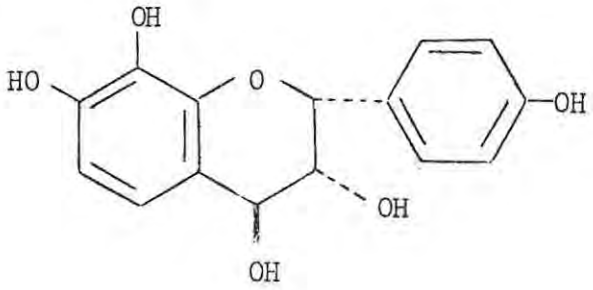
(LVII)

Teracacidin and isoteracacidin (7,8,4'-trihydroxyflavan-3,4-diol).

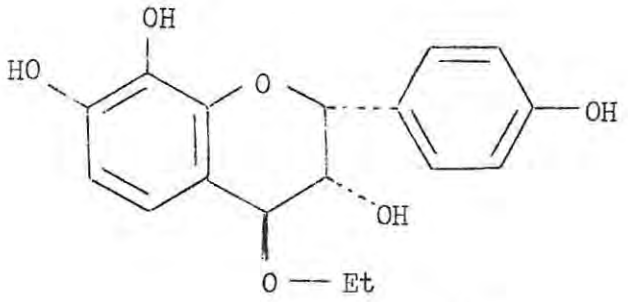
These compounds were isolated from Acacia intertexta by Clark-Lewis, Katekar and Mortimer [29,111]. The chemical and chromatographic behaviour of melacacidin, isomelacacidin and O-ethylisomelacacidin closely resembles that of the compounds in the teracacidin series and by analogy a similar relation is thought to exist between teracacidin LVIII, isoteracacidin LIX and O-ethylisoteracacidin LX.



(LVIII)



(LIX)



(LX)

The teracacidin structure was confirmed by synthesis of the 2,3-cis-3,4-cis-racemate of 7,8,4'-O-trimethylflavan-3,4-diol by catalytic hydrogenation of 7,8,4'-O-trimethylflavonol. Teracacidin

is thus stereochemically identical with melacacidin and can be specified as 2R:3R:4R. This assignment was confirmed by comparing the molecular rotations of some derivatives of these two compounds [29].

Unlike melacacidin and O-ethylisomelacacidin, the teracacidin compounds have not been obtained in crystalline form and formulation of isoteracacidin as the C_4-epimer and O-ethylisoteracacidin as its C_4-ethyl ether rests largely on analogy with the melacacidin compounds.

Mollisacacidin [(+)-Leuco-fisetinidin, 7,3',4'-trihydroxyflavan-3,4-diol] and Quebracho (-)-Leuco-fisetinidin.

The first crystalline leuco-anthocyanidin isolated from natural sources was mollisacacidin by Keppler [65] from the heartwood of Acacia mearnsii. Shortly afterwards Roux [112] and Freudenberg and Weinges [64] obtained the enantiomorph from quebracho wood. Gleditsin, isolated from Gleditsia japonica was proved to be identical with (+)-mollisacacidin [113]. (+)-Mollisacacidin and (-)-leucofisetinidin represent the first example of the occurrence in nature of enantiomorphous forms of a flavan derivative [36,114].

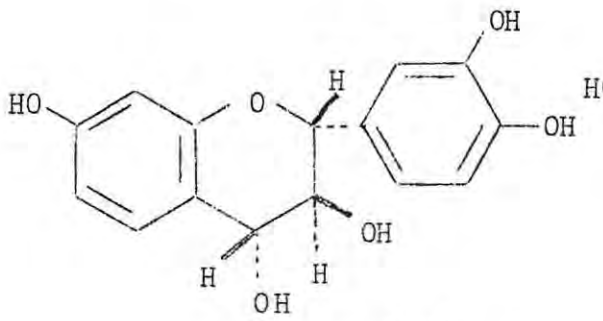
Keppler [65] found that mollisacacidin trimethyl ether could be oxidised rapidly at room temperature. A 1,2-glycol structure thus existed and he proposed a 3,4-cis-configuration on the grounds that an acidic borate complex was formed by the methylated leuco-anthocyanidin. Catalytic reduction of (±)-

fustin to (+)-7,3',4'-trihydroxyflavan-3,4-diol was regarded by Keppler as furnishing additional evidence for the 3,4-cis-configuration. Both deductions have now been shown to be erroneous.

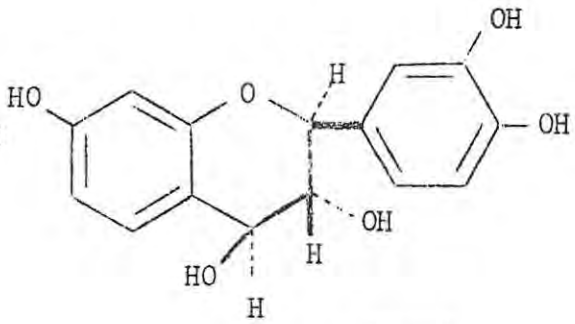
The 2,3-trans-configuration of (+)-mollisacacidin and (-)-leuco-fisetinidin has been established by their synthesis from 2,3-trans-dihydroflavonols [26,37] and conversion of the diols to flavan-3-ols of which the configuration was known [25,28]. Proof for the 2,3-trans nature of mollisacacidin has been extended [29] recently through hydrogenolysis of (+)-mollisacacidin trimethyl ether to (-)-fisetinidol trimethyl ether and conversion of the latter compound to (+)-2-ethoxy-7,3',4'-trimethoxyisoflavan. This type of molecular rearrangement is interpreted as a 1,2-arrangement of trans-groups.

Apart from Keppler, other workers, Clark-Lewis and Roux [36], Freudenberg and Weinges [37] and Roux and Paulus [63] had assigned a tentative 3,4-cis arrangement for the two flavandiols. These views were based mainly on the high yield (>60%) of isopropylidene derivative obtained from the methyl ether. Subsequent evidence by Drewes and Roux [10,76] and Clark-Lewis and Katekar [29] (although no experimental evidence is cited by these workers) has shown conclusively that (+)-mollisacacidin and (-)-leuco-fisetinidin possess 2,3-trans-3,4-trans configurations as in

LXI and LXII respectively. (+)-Mollisacacidin may be designated



(LXI)



(LXII)

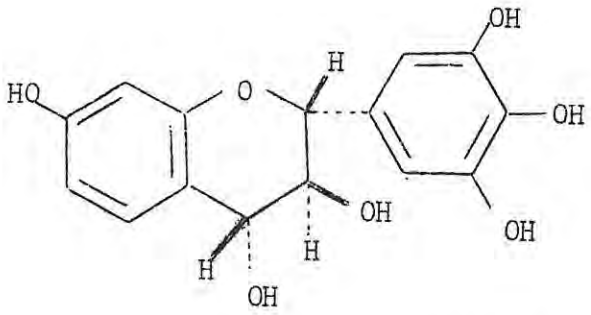
as 2R:3S:4R and (-)-leuco-fisetinidin as 2S:3R:4S. The substituents at C_2, C_3 and C_4 in these trans-trans compounds are 2(eq) : 3(eq) : 4(eq).

(+)-Leuco-robinetinidin [(+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol.]

Weinges [25] reported without detail the isolation of a (+)-leuco-robinetinidin from the heartwood of Robinia pseudacacia. He showed that the (+)-leuco-robinetinidin obtained from (+)-dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one), by the method of Freudenberg and Roux [61,62] could be reduced to (-)-robinetinidol (7,3',4',5'-tetrahydroxyflavan-3-ol). From molecular rotation differences of derivatives of the flavan-3-ol as compared with 2,3-trans-(+)-catechin [25], Weinges deduced that (+)-dihydrorobinetin, (-)-robinetinidol and (+)-leuco-robinetinidin were 2,3-trans and had the same absolute configuration at C_2 and C_3 as (+)-catechin. Recent n.m.r. measurements by Lillya, Drewes and Roux [79] of the coupling constant for the C_2- and C_3-protons, ($J_{2,3}$ = 9.0 c.p.s.) confirm this

assignment.

Roux and Paulus [63] extended the work on (+)-leuco-robinetinidin and proposed, with reservations, a 2,3-trans-3,4-cis-configuration. Data obtained by Lillya, Drewes and Roux [79], based on oxidation rates and n.m.r. measurements, however now prove that the diol has a 2,3-trans-3,4-trans-configuration as in the case of (+)-mollisacacidin. (+)-Leuco-robinetinidin may thus be represented as LXIII and its absolute configuration designated as 2R:3S:4R.



(LXIII)

Guibourtacacidin (7,4'-dihydroxyflavan-3,4-diol).

Chromatographic evidence for the presence of a 7,4'-dihydroxyflavan-3,4-diol in the heartwood of certain Guibourtia spp. was presented by Roux [115]. The chromatographic evidence suggested that the compound was a third member of the resorcinol series of flavan-3,4-diols. This investigation prompted Phatak and Kulkarni [116] to synthesize two flavan-3,4-diols "isomeric

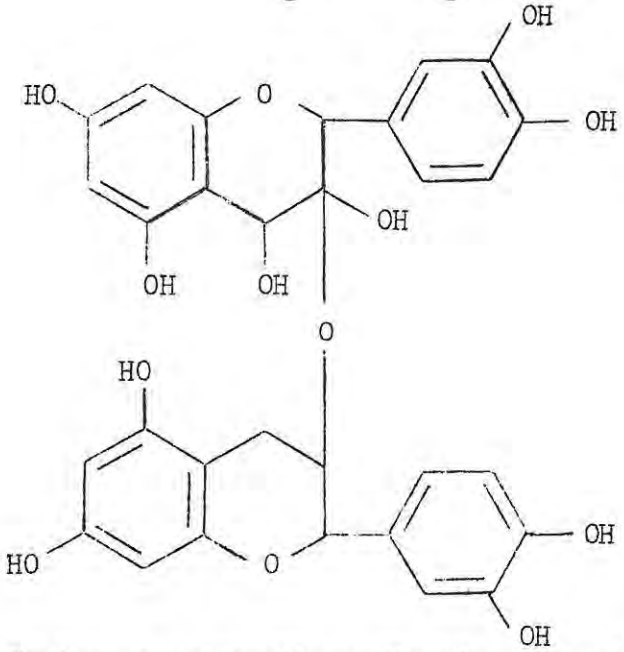
in position four and having the 7,4'-dihydroxyflavan-3,4-diol structure". To the one diol they assigned a 2,3-trans-3,4-cis-configuration and to the other a 2,3-trans-3,4-trans-configuration. In their opinion, the former isomer was likely to be stereochemically identical with guibourtacacidin.

Roux and De Bruyn [117] recently re-investigated the heartwood of Guibourtia coleosperma and isolated an amorphous 7,4'-dihydroxyflavan-3,4-diol in very low (0.004%) yield. This compound formed 3,7,4'-trihydroxyflavylium chloride in high (30%) yield. Infrared spectra of the chromatographically pure dimethyl ether of guibourtacacidin and the synthetic diol (supposedly trans-cis) of Phatak and Kulkarni [116], show a close similarity, but differences existed between the infrared spectra of the unmethylated synthetic trans-cis-diol [117] and guibourtacacidin. The results were therefore equivocal but limitations of material prevented extension of the work on their stereochemistry.

Cacao Leuco-cyanidin.

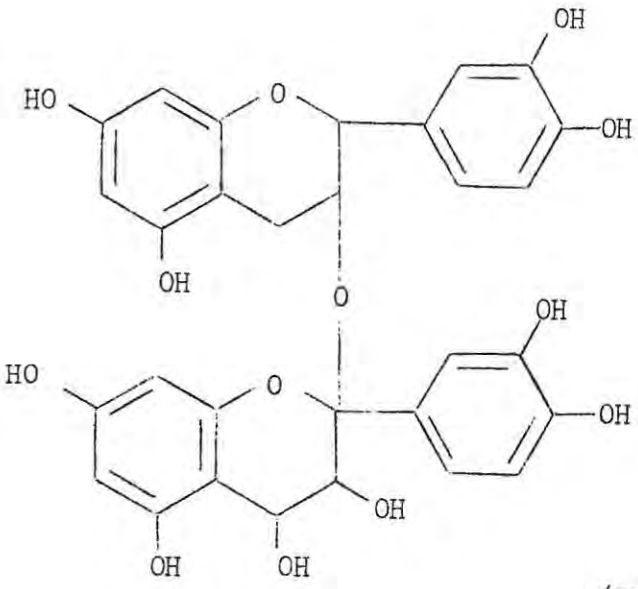
Fresh cacao beans contain a complex leuco-anthocyanidin first isolated by Forsyth [118,119]. The cacao leuco-cyanidin readily yields (-)-epicatechin when warmed with 0.1N hydrochloric acid and with stronger acids yields cyanidin. It forms an octa-acetate and a non-phenolic octamethyl ether which cannot be acetylated under mild conditions. Cyanidin 5,7,3',4'-tetramethyl ether is formed

when the octamethyl ether is treated with hot hydrochloric acid. Under milder conditions the leuco-cyanidin gave (-)-epicatechin 5,7,3',4'-tetramethyl ether and leuco-cyanidin methyl ether. Forsyth and Roberts [120] considered various possible structures for the new leuco-anthocyanidin but regarded as most likely the one shown in LXIV. Freudenberg and Weinges [121] have isolated from



(LXIV)

the fruit of Crataegus oxyacantha a dimeric compound for which they propose a possible structure LXV. The cacao leuco-cyanidin and the compound from Crataegus oxyacantha were found to have the same R_F values in various solvents and their infrared spectra were identical [121]. Freudenberg and Weinges [122] do not regard the open hemi-ketal formulation as shown in LXIV to be more



(LXV)

probable than their own closed ketal formulation LXV.

STEREOCHEMISTRY OF FLAVAN-4-OLS.

Flavans containing a hydroxyl group in the 4- position have been the subject of much recent study. Reduction of flavanones, catalytically and otherwise, has been studied closely and much attention directed to the configuration and conformation of the resulting 4-hydroxy group.

Freudenberg and Orthner [123] reduced flavanone with aluminium amalgam in aqueous ethanol and obtained a flavan-4-ol m.p. 118°. Later Karrer and co-workers [124] designated this as the 4α-ol and as β the second isomer, which together with flavan-4α-ol they obtained by using ammoniacal titanous chloride as reducing agent. The α- and β-designation does not imply any

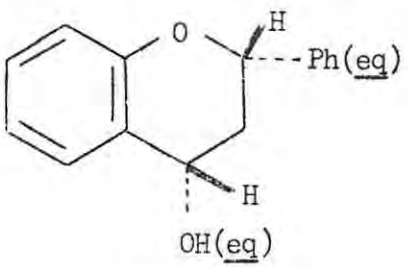
geometrical configuration as the stereochemistry of these flavan-4-ols was not known. Later workers adopted this nomenclature for other pairs of isomers.

Flavan-4α-ols. Besides Freudenberg and Orthner [123], Kashikar and Kulkarni [85] have employed aluminium amalgam for the synthesis of flavan-4α-ols. Bokadia, Brown and Cummings [125] introduced lead tetra-acetate as reagent for obtaining 4α-ols. By this method flavans are attacked first at the 4- position to yield 4α-acetoxy derivatives from which, on hydrolysis, flavan-4α-ols result. Bognar, Rakosi, Fletcher, Kehoe, Philbin and Wheeler [57,80] found that oximination of flavanones, followed by reduction to the amine and final deamination to the alcohol also gave flavan-4α-ols.

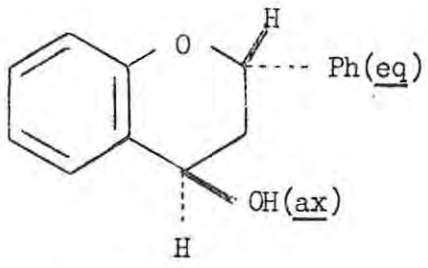
Flavan-4-β-ols. Catalytic reduction (copper-chromic oxide) of flavanone was employed by Mozingo and Adkins [86] to obtain flavan-4β-ol. Geissman and Clinton [126] used platinum oxide and also sodium amalgam for synthesis of the same compound. Numerous workers including Bokadia, Brown and Cummings [125], Kashikar and Kulkarni [85], Pew [127] and Row and co-workers [128] have employed metal hydrides such as lithium aluminium hydride and sodium borohydride to prepare flavan-4β-ols.

Application of n.m.r. methods to flavan-4-ols has served to establish the stereochemistry of these compounds. This

is well illustrated by the work on flavan-4α-ol and flavan-4β-ol by Wheeler and co-workers [57]. These workers had tentatively proposed the 2,4-cis-configuration [C_2-phenyl group (eq); C_4-hydroxyl group pseudo (eq)] for flavan-4α-ols and the 2,4-trans-configuration [C_2-phenyl group (eq); C_4-hydroxyl group pseudo (ax)] for flavan-4β-ols. The same conclusions regarding the conformation of flavan-4-ols had been reached independently by Kasahara and Mitsui [56]. Subsequent application of n.m.r. to the benzoates of the flavan-4-ols [58], showed that the previous assignments [57] required revision, and that in flavan-4β-ol LXVI, produced by catalytic reduction, the C_2-phenyl and the C_4-hydroxyl are both equatorial and cis to one another. The flavan-4α-ol LXVII, obtained from flavanone by oximination, reduction to the amine and deamination has the C_2-phenyl group equatorial, the C_4-hydroxyl group axial and these substituents are trans to one another. The



(LXVI)



(LXVII)

flavan-4β-ols may thus be designated as 2S:4S whereas the flavan-4α-ols are 2S:4R.

Flavan-4-ols with free hydroxyl groups.

The flavan-4-ols discussed above have in no case contained free phenolic hydroxyl groups. Apart from flavan-4-ol itself, they have included 4'-methoxyflavan-4-ol[125], 3-bromo-6-methyl-4'-methoxyflavan-4-ol [85], 5,7,4'-trimethoxyflavan-4-ol [126] and 6,7,3',4'-tetramethoxyflavan-4-ol [128]. The first synthesis of a flavan-4-ol with free hydroxyl groups was by Roux and Paulus [129] who prepared first 7,3',4'-trihydroxyflavan-4-ol and subsequently [38] 7,3',4',5'-tetrahydroxyflavan-4-ol by catalytic reduction of 7,3',4'-trihydroxyflavanone (butin) and 7,3',4',5'-tetrahydroxy-flavanone (robtin) respectively. These two flavan-4-ols have also been synthesized by Roux and Paulus [38] using a novel method which involves the reduction of the dihydroflavonol (flavan-3-ol -4-one) to the flavanone with zinc and hydrochloric acid followed by catalytic reduction of the flavanone. From the optically pure dihydroflavonols the first optically active flavan-4-ols were synthesized. The two flavan-4β-ols were originally assigned a 2,4-trans-configuration by analogy to the corresponding flavan-3,4-diols but n.m.r. examination of the C_2- and C_4-protons of the acetates of these two flavan-4-ols by Lillya, Drewes and Roux [79] has shown that these protons must be axial and in addition that the substituents have a 2,4-cis-arrangement. Previously Roux [163] had shown that the two flavan-4β-ols have the same configuration at C_2 and C_4 as the corresponding natural (+)-flavan-3,4-diols

(now known to be trans-trans) and on this basis the flavan-4-ols were 2,4-cis. These conclusions agree with the suggested configuration for these compounds by Clark-Lewis, Spotswood and Williams [130] based on n.m.r. measurements of 3-bromo-derivatives of flavan-4-ols.

EXPERIMENTAL AND RESULTS.

Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer using deuterochloroform as solvent and tetramethylsilane as internal standard. Band positions are expressed as p.p.m. downfield with the standard as origin. Coupling constants were measured with an accuracy of $\pm$ 0.2 c.p.s. Infrared spectra were supervised by Dr. P.R. Enslin, National Chemical Research Laboratory, C.S.I.R., Pretoria. The spectra (2.5 - 15μ) were determined from KBr discs using a Perkin Elmer Infracord spectrophotometer. The ultraviolet and visible range spectra were measured with a Beckman Model DU Quartz spectrophotometer. C, H, methoxyl and acetyl estimations were by K. Jones, Micro-Analytical Laboratory, C.S.I.R., Pretoria. All melting points are uncorrected and mixed m.p. were done on the crystalline residue remaining after dissolving equal weights of both compounds in a suitable solvent and allowing the latter to evaporate.

PART I.

ISOLATION OF NEW FLAVONOID COMPONENTS OF BLACK WATTLE BARK.

Extraction of Black Wattle Bark. Bark (3.5 Kg.), collected from healthy, mature (30 - 40 ft.) trees, was cut into thin slivers across the grain, with a stainless steel knife. The bark was immersed in ethyl acetate contained in 10 litre round bottom flasks within 2 hr. of being stripped from the tree. After 24 hr. at room temperature the ethyl acetate extract (Fig. 1) was concentrated to a pale brown powder in a rotary evaporator. More material was obtained by re-extracting the bark for an additional 48 hr. Wax was removed from the combined solids (700 g.) by extraction with light petroleum (b.p. 80-110°) in a large Soxhlet extractor.

Fractionation of the Extract. Preliminary experiments had indicated that the extract could be separated into higher and lower molecular weight fractions by using the countercurrent-partition principle. Accordingly, wax-free extract (100 g.) was dissolved in the aqueous phase (1 l.) of a 1:1 (v/v) mixture of water and ethyl acetate. This was placed in the first of ten 2 l. separating funnels each containing 1 l. of the aqueous phase. The extract was then fractionated by countercurrent-partition separation where each funnel was shaken for 3 min., left stationary while the phases separate (7 min.), and transfer of the upper phase to the next funnel was made. After completion of the 10-stage separation procedure the contents from both

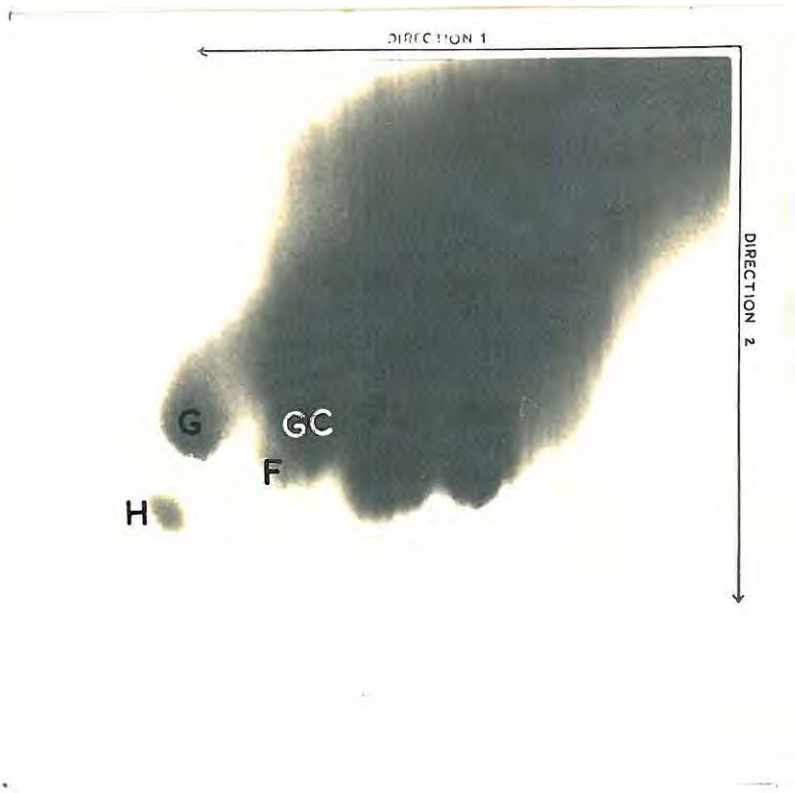


Fig. 1. Two-way chromatogram of whole fresh wattle bark extract sprayed with ammoniacal silver nitrate. G = (+)-catechin ; H = (-)-robinetinidol ; GC = (+)-gallocatechin. For all chromatograms water-saturated butan-2-ol was employed for direction 1 and 2% (v/v) acetic acid for direction 2.

upper and lower phases of each funnel were concentrated under vacuum. The enrichment procedure was repeated seven times and the contents of the corresponding tubes were united. The greatest proportion of monomeric flavonoid components was present in the upper phase of funnels 9 and 10. The solids from the tubes showed a gradation in colour from brown in fraction 1 to red-brown to yellow in fractions 9 and 10.

Table 1. Typical yields of fractions from 100 g. extract.

Tube no.	1	2	3	4	5	6	7	8	9	10
Wt.(g.) (Organic phase)	0.63	1.30	2.33	4.21	5.06	6.33	7.40	8.31	10.50	6.25

Separation of components. The total solids from tubes 9 and 10 (Fig. 2) were combined (126 g.) and dissolved in 400 ml. of the aqueous phase of a water-butan-2-ol-light petroleum (b.p. 40-150°) (5:4:1, by vol.) mixture. The very viscous solution was introduced into the first 8 tubes of a 160-tube, 50 ml. underphase automatic Craig machine (Glasapparatebau Göttingen, Helmut Rettberg). The upper and lower phases of the above mixture were used for countercurrent separation, and after 160 transfers the upper layer of every fifth tube was examined by two-dimensional paper chromatography on Whatman no. 1 peper. Solvents used were water-saturated butan-2-ol and then 2% (v/v) acetic acid. Residual chlorophyll and waxes, which, at the end of the run are concentrated in the last 30 tubes, tend to cause

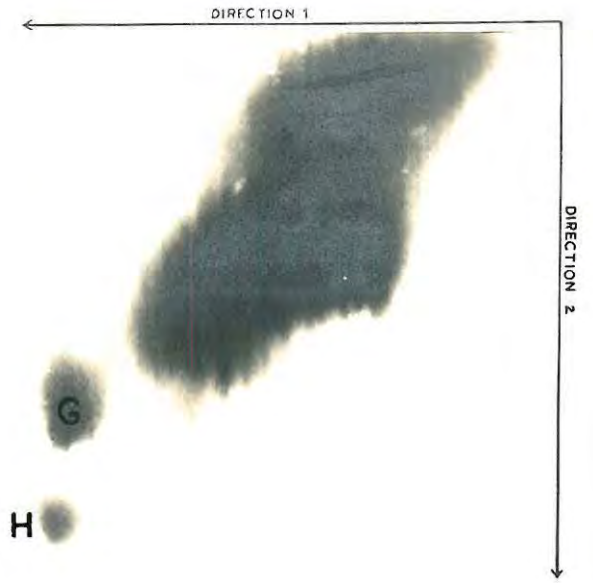


Fig. 2. Chromatogram of tubes 9 and 10 of the preliminary countercurrent separation.

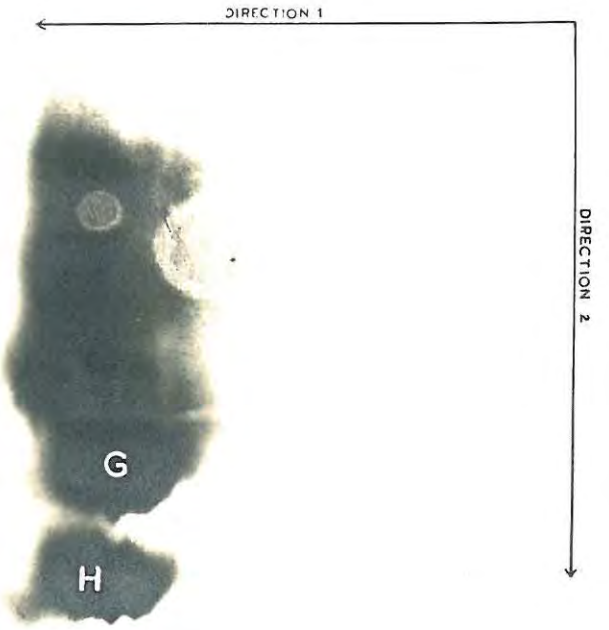


Fig. 3. Chromatogram of tubes 110-130 from Craig separation of tubes 9 and 10, (Fig. 2).

delay in the separation of the phases of these tubes due to emulsion formation.

Examination and Separation of Craig fractions.

The upper phases from tubes 110-130, 131-144 and 145-160 were grouped and concentrated to dryness in a rotary evaporator. The aqueous phases remaining in the tubes of each of these three fractions were extracted several times with ethyl acetate and the extract added to the appropriate fraction.

i) Tubes 110-130. The solids (7.23 g.) from these tubes (Fig. 3) contained relatively high concentrations of (-)-robinetinidol and (+)-catechin, and in addition (-)-fisetinidol, fustin and dihydrorobinetin. Separation of these components was achieved as follows: The total solids, in ethanol (150 ml.) were applied to thirty $22\frac{1}{4}$ x $18\frac{1}{2}$ in. Whatman no.3 paper sheets at 5 ml. per sheet (approx. 250 mg. per sheet) (cf. Roux and Evelyn [131]). The chromatograms were developed by the ascending technique in 2% (v/v) aqueous acetic acid for 14 hr.

The sheets were dried in a current of air and then examined under ultraviolet light. A side strip cut from one sheet was sprayed with ammoniacal silver nitrate [132] and this revealed two strongly reducing bands at R_F approximately 0.38 (catechin band) (Fig. 4) and R_F 0.48 (robinetinidol band) (Fig. 5). These two bands were cut out and eluted with 70% (v/v) ethanol. The combined eluates

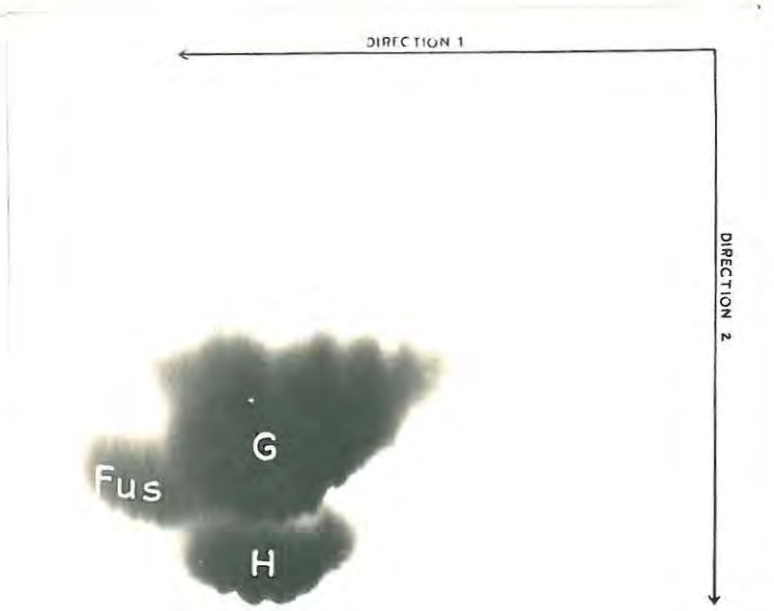


Fig. 4. Chromatogram of (+)-catechin band after adsorption separation of tubes 110-130, (Fig. 3). Fus = fustin.

Fig. 5. Chromatogram of (-)-robinetinidol band after adsorption separation of tubes 110-130, (Fig. 3). Fis = (-)-fisetinidol.

from the R_F 0.48 band on being concentrated to a small volume (12 ml.) yielded crystals of robinetinidol (350 mg.) m.p. and mixed m.p. with authentic (-)-robinetinidol [(-)-7,3',4',5'-tetrahydroxyflavan-3-ol], 204-206° (cf. Roux and Maihs [133]).

Fustin (7,3',4'-trihydroxyflavan-3-ol-4-one).

The eluates from the catechin band (R_F 0.38) were streaked onto 10 sheets of Whatman no.3 paper and developed with butan-1-ol-acetic acid-water (6:1:2, by vol.) as above. A strongly reducing band at R_F 0.70 was cut out and eluted with 70% (v/v) ethanol (Fig. 6). From the eluates a white sludge separated after concentrating to a small volume. Crystals of fustin (7 mg.) m.p. and mixed m.p. with (+)-fustin (soda glass) 212° (Roux and Paulus [26]) could only be obtained after running the compound once more on 2 sheets of paper in 2% (v/v) acetic acid. (It was found that the fustin, in these low concentrations, was readily converted to fisetin). The pure compound could not be separated from authentic (+)-fustin on two-dimensional chromatograms (R_F in 2% (v/v) acetic acid, 0.37). By comparison (-)-fustin has R_F 0.35 (Roux, Maihs and Paulus [134]). The infrared spectrum of the compound was identical with that of (+)-fustin over the range 2-15μ.

(-)-Fisetinidol [(-)-7,3',4'-trihydroxyflavan-3-ol]. The solution remaining after the removal of (-)-robinetinidol (above) through crystallization was streaked onto six sheets of Whatman no.3 paper

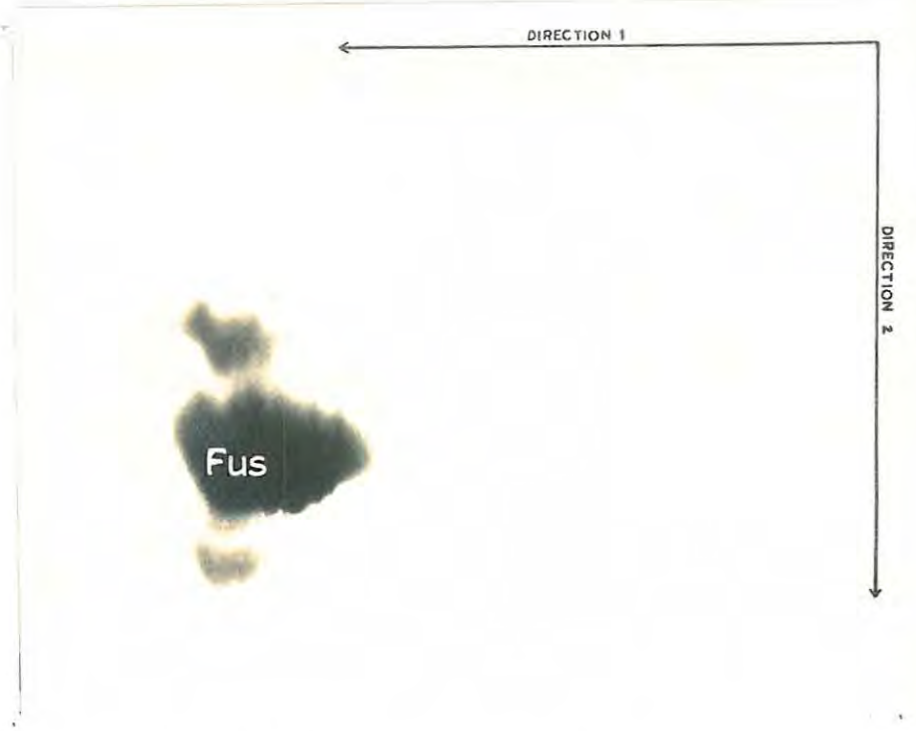


Fig. 6. Chromatogram of (+)-catechin band after adsorption and partition separation. Fus = fustin.

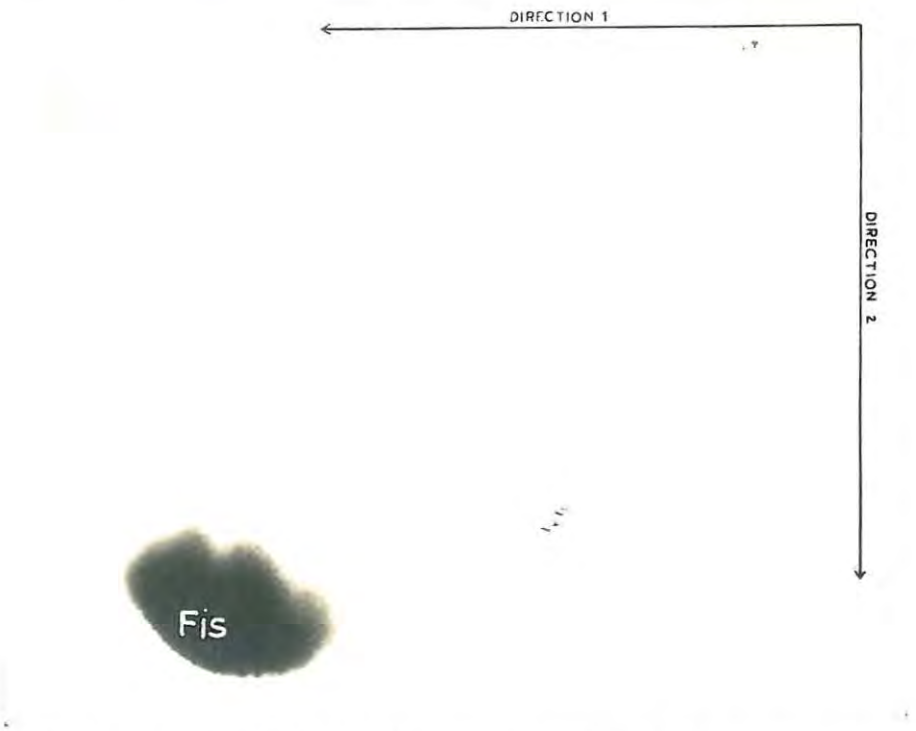


Fig. 7. Chromatogram of (-)-robinetinidol band after adsorption and partition separation. Fis = (-)-fisetinidol.

which had been prewashed with water and dried. The sheets were developed by the descending technique in butan-1-ol-acetic acid-water (6:1:2, by vol.). A strongly reducing band at R_F 0.67 was located with silver nitrate, cut out, eluted with alcohol as before and the combined eluates concentrated to a small volume (2 ml.). Two-dimensional paper chromatography showed the fraction to be impure still and chromatographic purity (Fig. 7) was achieved through repetition of the preparative chromatography on two cellulose sheets with 2% (v/v) acetic acid. From the final eluates (-)-fisetinidol (13 mg.) crystallized, m.p. 210-214°. The crystals came down in a gum initially, and were recrystallized with difficulty from water. Comparison of the compound with authentic (+)-fisetinidol and (-)-fisetinidol (Roux and Paulus [28]) was carried out on two-dimensional paper sheets. The compound was inseparable from (-)-fisetinidol. In 2% (v/v) acetic acid with reference substance (+)-fustin (R_F 0.37) the R_F values were: synthetic (+)-fisetinidol, 0.43; synthetic (-)-fisetinidol, 0.48; (-)-fisetinidol from black wattle bark, 0.48. The infrared spectrum over the range 2-15μ was identical with that of (+)- and (-)-fisetinidol.

Dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one).

After the above partition separation of the catechin band (R_F 0.38) for the isolation of fustin, the strongly reducing catechin band (R_F 0.60) in butan-1-ol-acetic acid-water (6:1:2, by vol.) on Whatman no.3 was cut out and eluted. (+)-Catechin (Roux and Maihs [133])

m.p. 172-174°, (47 mg.), crystallized from the combined eluates. The mother liquor was examined in more detail. Two-way chromatograms with the usual solvent systems, including water-saturated butan-1-ol for the first direction, had indicated that catechin and dihydrorobinetin migrated to identical positions. The presence of dihydrorobinetin in the mother liquor was suspected since the solution remaining after removal of all traces of (-)-robinetinidol (by running the mixture once more on one sheet of Whatman no.3 paper with 2% (v/v) acetic acid) gave an intense red on treatment with magnesium-hydrochloric acid (Pew [135]). It was also observed on a two-way chromatogram of the mother liquor that the catechin spot appeared more orange than the usual ochre colour observed with bis-diazotized benzidine. Attempts to separate the dihydrorobinetin and catechin with a solvent mixture of water - 90% formic acid (1:1 by vol.) were not successful. The entire mixture (16.3 mg.) was then streaked onto a single sheet of Whatman no.3 paper and developed with aq. 75% (w/v) phenol (Koeppen [136]). The dihydrorobinetin migrated as a discrete band of R_F 0.65 whereas the catechin had R_F 0.57. From this separation 2.5 mg. of dihydrorobinetin, m.p. 243-246°, was obtained. Two-way chromatograms, run in water-saturated butan-2-ol and then in 2% (v/v) acetic acid indicated that the compound was pure and also inseparable from optically pure (+)-dihydrorobinetin (Roux and Paulus [38]). The R_F values in the above solvents were 0.69 and 0.35 respectively. The pure dihydrorobinetin,

recrystallized from water, gave a deep red-blue with Mg-HCl. Its infrared spectrum was identical with that of (+)-dihydrorobinetin over the range 2.5 - 15μ.

Butin (7,3',4'-trihydroxyflavan-4-one). The total solids (1.8 g.) from tubes 131-145 of the Craig separation were dissolved in alcohol (40 ml.) and separated on paper sheets (5 ml. per sheet) with 2% (v/v) acetic acid as described above. A band with R_F 0.16 was cut out and eluted to give 106 mg. solids. Two-way chromatograms showed one predominant spot and several minor ones. This main spot was inseparable from that of (+)-butin (Roux and Paulus [137]) on two way chromatograms [R_F 0.20 in 2% (v/v) acetic acid, 0.83 in water-saturated butan-2-ol on Whatman no. 1 paper] and gave characteristic colour reactions on paper chromatograms (bis-diazotized benzidine-yellow, and ferric alum-light green). A red colour formed with Zn-HCl in solution. The isolation of butin was not attempted.

Butein (2',4',3,4-tetrahydroxychalcone). The solids (1.6 g.) from tubes 145-160 from the Craig separation, in alcohol, were applied to six Whatman no. 3 sheets at 5 ml. per sheet and separated with 2% (v/v) acetic acid as above. Only compounds remaining on the origin were cut out, eluted and then applied to six sheets which were developed with butan-1-ol-acetic acid-water (6:1:2, by vol.) A yellow band (orange-yellow when fumed with ammonia and which reduces

silver) was cut out at R_F 0.75 and eluted. It was attempted to purify the compound further by boiling it for a short while with hydrochloric acid, neutralizing the acid with alkaline Amberlite resin IRA 400 and separating it on a single sheet with water saturated butan-1-ol-acetic acid-water (6:1:2, by vol.) but this did not prove to be effective. Purification was finally achieved through chromatography in a water - 90% (v/v) formic acid [1:1 (v/v)] mixture. A yellow band, R_F 0.31, was eluted and its ultraviolet and visible range spectra were determined:

λ max. 260 and 383 mμ (in ethanol); 256 and 456 mμ (in M-sodium ethoxide); 275 and 436 mμ (in 0.02 M-$AlCl_3$ in ethanol). For synthetic butein Roux and Paulus [137] found: λ max. 262 and 382 mμ (in ethanol); 262 and 450 mμ (in M-sodium ethoxide); 272 and 437 mμ (in 0.02 M-$AlCl_3$ in ethanol. Chromatographic comparison with authentic butein in aq. 75% (w/v) phenol (R_F 0.73) and butan-1-ol-acetic acid-water (6:1:2, by vol.) (R_F 0.83) showed identity.

Robtein (2',4',3,4,5,-pentahydroxychalcone). The contents, 70 g. of separating funnel 8 of the preliminary enrichment procedure, were separated in the Craig machine as before. The combined solids (0.78 g.) from tubes 119-160 were separated on three no. 3 sheets by the preparative method in 2% (v/v) acetic acid. Compounds remaining on the origin (91 mg.) were examined for chalcones. Separation on two no. 3 sheets with water-saturated butan-1-ol-

acetic acid-water (6:1:2, by vol.) gave a yellow band of R_F 0.42 which became bright yellow on fuming with ammonia. The band was cut out, eluted and purified further on two sheets with 80% (v/v) formic acid-water (3:2. v/v) when a yellow band R_F 0.59 was obtained. This fraction had the same R_F (0.66) as synthetic robtein (Roux and Paulus [38]) in butan-1-ol-acetic acid-water (6:1:2, by vol.) and gave the same characteristic brick-red when fumed with ammonia. The band was eluted with ethanol and its ultraviolet and visible spectra were determined:

λ max. 258 and 370 mμ (in ethanol); 275 and 425 mμ (in 0.02 M-$AlCl_3$ in ethanol). Synthetic robtein had: λ max. 260 and 385 mμ (in ethanol); 275 and 445 mμ (in 0.02 M-$AlCl_3$ in ethanol).

Compound F. This compound (Fig. 1) is present in low concentration in wattle bark extract. Separating funnel 8 of the preliminary enrichment procedure appeared to have the highest concentration of this compound and this fraction was accordingly separated in the Craig machine as above. Tubes 58-68 (9.5 g.) contained the highest proportion of F, and this fraction was streaked onto 45 sheets of Whatman no. 3 paper and chromatographed with 2% (v/v) acetic acid by upward migration. The band, R_F 0.37 (yellow with bis-diazotized benzidine), was cut out and eluted with 70% (v/v) ethanol. The combined eluates were concentrated to a small volume at 70° in a rotary evaporator and then to dryness in a vacuum desiccator (280 mg.). Two-way chromatography with water-saturated butan-2-ol and

2% (v/v) acetic acid showed that F was still contaminated by (+)-gallocatechin (Roux and Maihs [133]) and a compound F' running just ahead of F in the second direction.

250 mg. of impure F were again separated on 5 sheets of no. 3 paper with 2% (v/v) acetic acid. The reducing band (silver nitrate) at R_F 0.37 and the F' band just ahead of it were cut out and eluted with 70% (v/v) ethanol. This gave 131 mg. of F and 13.5 mg. of F'. Other solvent systems such as 8% (v/v) acetic acid, 2% (v/v) acetic acid: ethanol (3:1, by vol.) and aq. 75% (w/v) phenol[136] were used in an attempt to separate F from gallocatechin and F' but 2% (v/v) acetic acid appeared to effect the best separation.

Colour reactions of F. With toluene-p-sulphonic acid [138] (orange-yellow), vanillin-toluene-p-sulphonic acid [139] (bright red), bis-diazotized benzidine [140] (yellow), ammoniacal silver nitrate [132] (grey-black) and ferric alum (green) were obtained.

The first two reagents suggest the presence of a phloroglucinol A nucleus whereas the diazotized benzidine reaction indicates a resorcinol A nucleus and catechol B nucleus (cf. Roux and Maihs [141]). Orthodihydroxy groups in the B nucleus are shown by the silver nitrate reagent.

Anthocyanidin formation of F. F (1.5 mg.), isopropyl alcohol (4 ml.) and 3N-hydrochloric acid (1 ml.) were refluxed for 1 hr. according to

the conditions of Pigman, Anderson, Fischer, Buchanan and Browning[142]. A reddish colour developed. Some of the red solution was spotted onto a sheet of no. 1 Whatman paper and developed with 90% (w/v) formic acid-3N-HCl (1:1, v/v) [143]. An anthocyanidin with the same R_F (0.43) as fisetinidin (3,7,3',4'-tetrahydroxyflavylium chloride), which was run as reference compound, was obtained. This solvent mixture has been shown by Roux [143] to differentiate very effectively between a wide range of anthocyanidins.

Micro-fusion of F with potassium hydroxide. The fusion was carried out on about 2 mg. of compound as described by Roux [144]. Fusions were also done on catechin, leuco-fisetinidin, gallocatechin and F' for comparison. Degradation products were identified by chromatographing the reaction mixture (separated into an acidic and a phenolic fraction by the bicarbonate technique) on no. 1 paper with butan-1-ol-water-acetic acid (6:1:2, by vol.) together with known reference compounds. By spraying chromatograms with diazotized benzidine (for the phenolic fraction) and ferric alum and silver nitrate (for the acidic fraction) all products could be identified by virtue of their characteristic colours with these spray reagents and by their R_F values.

Table 2. Degradation products obtained from the alkali fusion of F and other reference compounds. (+++) denotes a high concentration.

Compound	Resorcinol	Phloro-glucinol	β resorcylic acid	Proto-catechuic acid	Gallic acid
F	+++	+++	+++	+++	Trace
Catechin	+	+++		+++	Trace
Leuco-fisetinidin	+++		+++	+++	Trace
Gallocatechin	+	+++	+		+++
F'	+++	+++	+++	+++	

Spectrophotometric examination. The ultraviolet absorption curve of F had a single maximum at 280 mμ while the infrared curve was that of a typical flavonoid compound. No carbonyl group is present.

Analysis of F. For the purest fraction found: C, 61.7; H, 5.4%. ($C_{30}H_{26}O_{12}$ req: C, 62.2; H, 4.5%).

Methylation of F. F (94 mg.) in methanol was methylated at 0° for 48 hr. with diazomethane (10 g.). The amorphous product (106 mg.) obtained from the solution was redissolved in alcohol and filtered into water. A white precipitate (50 mg.) was filtered off.

(Found: C, 65.9; H, 6.6; OCH_3, 31.6. $C_{37}H_{40}O_{12}$ req: C, 65.7; H, 5.9; OCH_3, 32.1%).

Reaction of F with acid. F (2 mg.) was gently warmed with ethanolic 5N-hydrochloric acid until an orange-red colour appeared. Two-way chromatography of the reaction mixture in water-saturated butan-2-ol and 2% (v/v) acetic acid showed the presence of two compounds in

higher concentration and several others of low concentration. The one major component, R_F 0.48 in the first direction and R_F 0.37 in the second direction, was inseparable from authentic F and gave the same colour reactions. The other main component, R_F 0.66 in the alcohol direction and R_F 0.35 in the 2% (v/v) acetic acid direction gave the following colour reactions: diazotized benzidine (ochre), ferric alum (green) and ammoniacal silver nitrate (grey-black). This evidence suggests a compound with a phloroglucinol A nucleus and a catechol B nucleus. Catechin (5,7,3',4'-tetrahydroxyflavan-3-ol) meets these requirements and this compound was found to be inseparable from the degradation product on two-way chromatograms using water-saturated butan-2-ol for the first direction and 2% (v/v) acetic acid for the second.

(+)-Leuco-robinetinidin [(+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol] and (+)-leuco-fisetinidin [(+)-7,3',4'-trihydroxyflavan-3,4-diol].

Previous chromatographic evidence for the presence of traces of monomeric leuco-robinetinidin and leuco-fisetinidin in fresh black wattle bark (Roux and Evelyn [131]) was confirmed as follows: Mature black wattle bark (500 g.) was cut into slivers with a stainless steel knife and covered with ethyl acetate (2 l.) at 0°, 10-30 min. after being stripped from the tree. After extraction for 48 hr. at -15°, the solution was streaked on 15 Whatman no. 3 sheets (5 ml. per sheet) and the chromatograms were developed with 2% (v/v) acetic acid without delay. Bands (red, with toluene-p-sulphonic acid) were cut at R_F 0.60

and 0.50 and the eluates examined by one- and two-way paper chromatography on Whatman no. 1 paper. (+)- and (-)-7,3',4'-Trihydroxyflavan-3,4-diol and (±)- and (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol were used as reference compounds. The eluates of both bands contained compounds corresponding to (+)-leuco-fisetinidin and (+)-leuco-robinetinidin in R_F [0.52 and 0.46 respectively in 2% (v/v) acetic acid] and in colour reactions. By comparison their enantiomers, (-)-leuco-fisetinidin and (-)-leuco-robinetinidin, which may be completely separated by this particular solvent system, cf. Roux, Maihs and Paulus [134], had lower R_F values (0.47 and 0.40 respectively) in the same solvent.

It was found that these leuco-anthocyanidins were not detectable in black wattle extracts which had either been heated or which had been handled for prolonged periods at room temperature. This is presumably owing to condensation of the monomeric components and explains the absence of these monomers in commercial bark extracts.

Flavonol Glycosides.

Extraction and examination of young bark. Bark, (0.9 kg.) freshly stripped from immature black wattle trees (average diameter 0.25 in.) was cut across the grain to give small chips. These were extracted in the cold with ethyl acetate (15 l.) over 10 days. Chlorophyll and waxes (6.5%) were present and these were removed with light petroleum (b.p. 80-110°) from the solid extractives (76.5 g.). Apart from its

almost white colour, this extract also differed from extract obtained from mature trees in the following respects:

i) Two-way paper chromatograms of this extract indicated the presence, in much higher concentration of a component which appeared as a dark spot under ultraviolet light, gave a lime green colour with ferric alum, appeared as a yellow area which fluoresced yellow-green under ultraviolet light after treatment with toluene-p-sulphonic acid, reduced ammoniacal silver nitrate and became intensely yellow on fuming it with ammonia.

ii) A small quantity of the extract (1.5 mg.) when treated in alcoholic solution with Mg-HCl developed a cherry red colour. Mature bark extract on the other hand tends to give a reddish brown colour under the same conditions.

The characteristic colour under ultraviolet light and the behaviour on fuming with ammonia cf. Roberts, Cartwright and Wood [145] suggested the presence of a flavonol glycoside, possibly quercitrin or myricitrin. Further evidence for this contention was the fact that both myricetin and quercetin gave red colours with the Mg-HCl reagent.

Enrichment of the extract. The extract (72 g.) was enriched in flavonoid components by dissolving it in a mixture of ethanol (220 ml.) ethyl acetate (720 ml.), adding chloroform (720 ml.) and filtering of the resulting precipitate (15 g.). Two-way chromatograms of the

filtrate indicated that a considerable proportion of the high molecular weight phenolic fraction in the extract had been removed from the filtrate through this procedure. The filtrate was concentrated to dryness in a rotary evaporator (57 g.) and the last traces of wax and chlorophyll were removed at this stage with benzene.

Separation of the extract. Small scale separations by paper chromatography of the extract indicated that the new compound(s) was readily converted to polymeric material which did not migrate from the area of application on the paper sheets in any solvent if it was:

a) heated above 70° even for a short period,

b) allowed to come into contact with mineral acid.

For further separations these precautions were observed.

Enriched extract (15 g.) was dissolved in ethanol (250 ml.) and applied to 50 sheets (i.e. about 300 mg. per sheet) of Whatman no. 3 paper. Components were separated with 2% (v/v) acetic acid by upward migration. A broad band, R_F 0.27, dark brown under ultraviolet light, was cut out and eluted with 70% (v/v) ethanol. The combined eluates were concentrated under vacuum at 45° to a volume of 200 ml. This solution was applied to 40 prewashed sheets and developed in water-saturated butan-1-ol by downward migration. In this instance this solvent was found to be more effective than butan-1-ol-acetic acid-water (6:1:2, by vol.). Two bands, R_F 0.51 and 0.66,

both yellow in visible and dark brown under ultraviolet light were cut out and eluted.

The combined eluates from each fraction were concentrated to a small volume under vacuum at 45° and then taken to dryness in a vacuum desiccator. The residue was dissolved in alcohol-water, treated with charcoal and filtered. Crystals slowly separated over a period of days. Several crops of crystals could usually be filtered off, the first being the least pure. From the above isolation 240 mg. (1.6% on enriched extract and 0.10% based on weight of bark) of crude product of lower R_F (myricitrin) and 63 mg. (0.4% on enriched extract and 0.03% based on weight of bark) of crude product of higher R_F (quercitrin) was obtained.

Myricitrin (3,5,7,3',4',5'-hexahydroxyflavone-3-rhamnoside). The compound which was recrystallized from water in long yellow needles with great difficulty had m.p. 201-206° with decomposition after sintering at 194-197°. It exhibited the following colour reactions: yellow in visible and brown under ultraviolet light, orange yellow with bis-diazotized benzidine, brown with ammoniacal silver nitrate, olive green with ferric alum, bright yellow with ammonia, yellow-green fluorescent in ultraviolet light after treatment with toluene-p-sulphonic acid and dark purple with Mg-HCl in solution. These colour reactions would also be given by myricetin (5,7,3',4',5'-pentahydroxyflavone-3-ol) but the fact that the compound only

fluoresced under ultraviolet light after treatment with toluene-p-sulphonic acid and also that it migrated in water (whereas myricetin would not), suggested that it was myricetin glycoside.

Furthermore, the compound was inseparable from authentic myricitrin obtained from Myrica rubra (Shimizu, Ohta, Yoshikawa and Kasahara [146]) in the following solvents: 2% (v/v) acetic acid (R_F 0.25), water-saturated butan-1-ol (R_F 0.56) and butan-1-ol-acetic acid-water (6:1:2, by vol.) (R_F 0.66).

Authentic myricitrin melts at 197° and a mixed m.p. with the compound from wattle bark showed strong sintering at 194-197° with decomposition at 202°. Hörhammer [147] cites the m.p. as 197-198° and Hattori [148] quotes 199-200° with sintering from 197°. (Found for a sample dried for 1 hr. under vacuum at 160°: C, 54.5, H, 5.2. Calculated for $C_{21}H_{20}O_{12}$: C, 54.3; H, 4.3%).

Alkali micro-fusion. The fusion under anhydrous conditions [144] was done on 1.5 mg. of the compound and also on an equal quantity of myricetin. Degradation products were identified by examining the reaction mixture, together with reference compounds, by chromatography and through the use of selective spray reagents as described previously. In both cases the major fusion products were phloroglucinol and gallic acid. The phloroglucinol exhibits a vivid purple on being sprayed with diazotized benzidine whereas the gallic acid

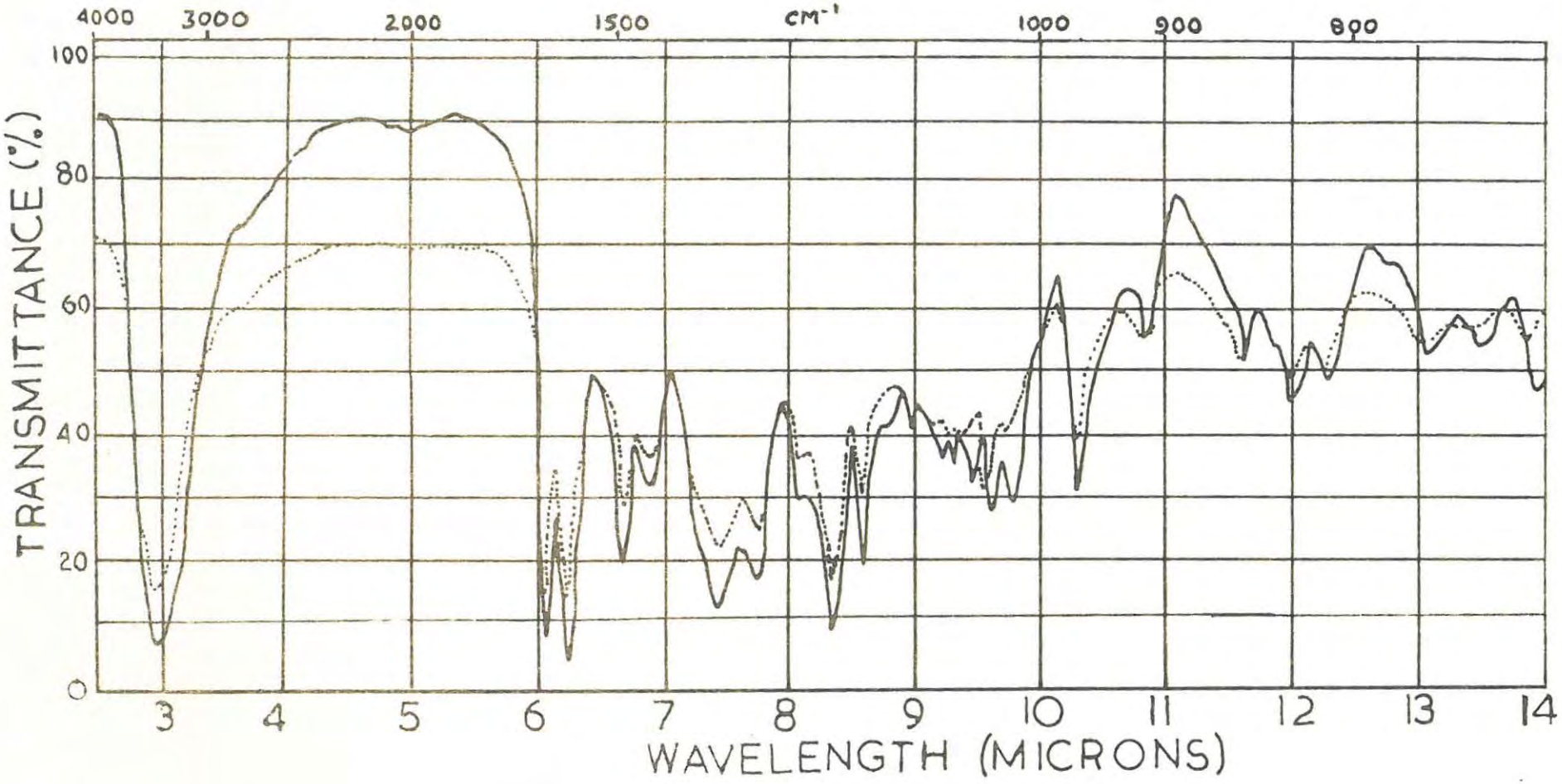


FIG. 8. INFRARED ABSORPTION SPECTRUM OF MYRICITRIN (——) AND MYRICITRIN FROM BLACK WATTLE BARK(····)

turns a characteristic brown-black with ammoniacal silver nitrate.

Spectrophotometric examination. Ultraviolet absorption curves of the compound gave: λ max. in ethanol, 256 and 355 mμ (log ε, 3.94 and 3.83 respectively); in 0.2M-$AlCl_3$ in ethanol, 268 and 422 mμ (log ε, 3.98 and 3.85). Found for authentic myricitrin : λ max in ethanol, 258 and 355 mμ (log ε, 4.01 and 3.94); in 0.2M-$AlCl_3$ in ethanol, 265 and 423 mμ (log ε, 4.08 and 3.96).

The infrared absorption curves of the two compounds were identical over the range 2.5-15μ, (Fig. 8).

Hydrolysis of the compound. Hydrolysis of the glycoside would be expected to give the aglycone (myricetin) and the sugar (rhamnose) in this case. The pure dry compound (71.5 mg.) was hydrolysed with 2N-HCl (20 ml.) for 2 hr. at 100°. The yellow myricetin precipitate was filtered off after keeping the solution at 0° overnight. The yield (46.5 mg.) represents 94.8% of the calculated value of myricetin in myricetin rhamnoside. The aglycone (21 mg.), after crystallization from aqueous ethanol was acetylated and the acetate was recrystallized three times from aqueous ethanol to give white needles (7 mg.) m.p. and mixed m.p. with authentic hexa-acetylmyricetin similarly prepared from myricetin (L. Light and Co. Ltd.) showing no depression, 213°. Ultraviolet-absorption curves of the aglycone gave: λ max. in ethanol, 255 (shoulder) and 375 mμ; in 0.02M-$AlCl_3$ in ethanol 275 and 438 mμ.

Found for authentic myricetin: λ max. in ethanol, 255 and 375 mμ; in 0.02 M-$AlCl_3$ in ethanol 272 and 440 mμ.

Identification of the sugar. The filtrate from the hydrolysis was neutralised with alkaline Amberlite IRA-400 resin. Chromatography on Whatman no. 1 paper of the neutral solution (with rhamnose and glucose as reference compounds) with benzene-butan-1-ol-pyridine-water (1:5:3:3, by vol.) (due to W. Matthias; cf. Hermann [149]) showed the sugar to be identical with rhamnose, R_F 0.45. Glucose had R_F 0.22. Aniline hydrogen oxalate, as described by Horrocks and Manning [150] was used as spray reagent. The sugars appeared as yellow spots after heat treatment of the paper at 105° for 10 min. A positive osazone test was also obtained for the sugar.

Quercitrin (3,5,7,3',4'-pentahydroxyflavone-3-rhamnoside).

Considerable difficulty was experienced in obtaining this compound crystalline. It was recrystallized from water in yellow needles sintering at 178° and melting at 180-185°.

On chromatograms the compound was yellow in visible and dark brown under ultraviolet light. It gave the following colours with spray reagents: orange-red with bis-diazotized benzidine, deep black with ammoniacal silver nitrate, lime green with ferric alum, intense yellow with ammonia and deep red with Mg-HCl in solution. The only differences with myricitrin are a deep black (instead of brown) with the silver nitrate reagent and the deep red (as opposed to purple) with the Mg-HCl reaction. This suggested that in this case the B ring of the glycoside contained the catechol nucleus

The compound and quercitrin (L. Light and Co. Ltd.) were inseparable on chromatograms in the following solvent systems: 2% (v/v) acetic acid (R_F 0.31), water-saturated butan-1-ol (R_F 0.66) and butan-1-ol-acetic acid-water (6:1:2, by vol.) (R_F 0.76).

Authentic quercitrin melts at 182-185°, cf. Hattori [148], and a mixed m.p. with quercitrin showed no depression, m.p. 180-185°. (Found for a sample dried at 140° under vacuum for 1 hr: C, 56.3; H, 5.3. Calculated for $C_{21}H_{20}O_{11}$: C, 56.3; H, 4.5%).

Micro-degradation. Alkali fusion on the unknown compound and on

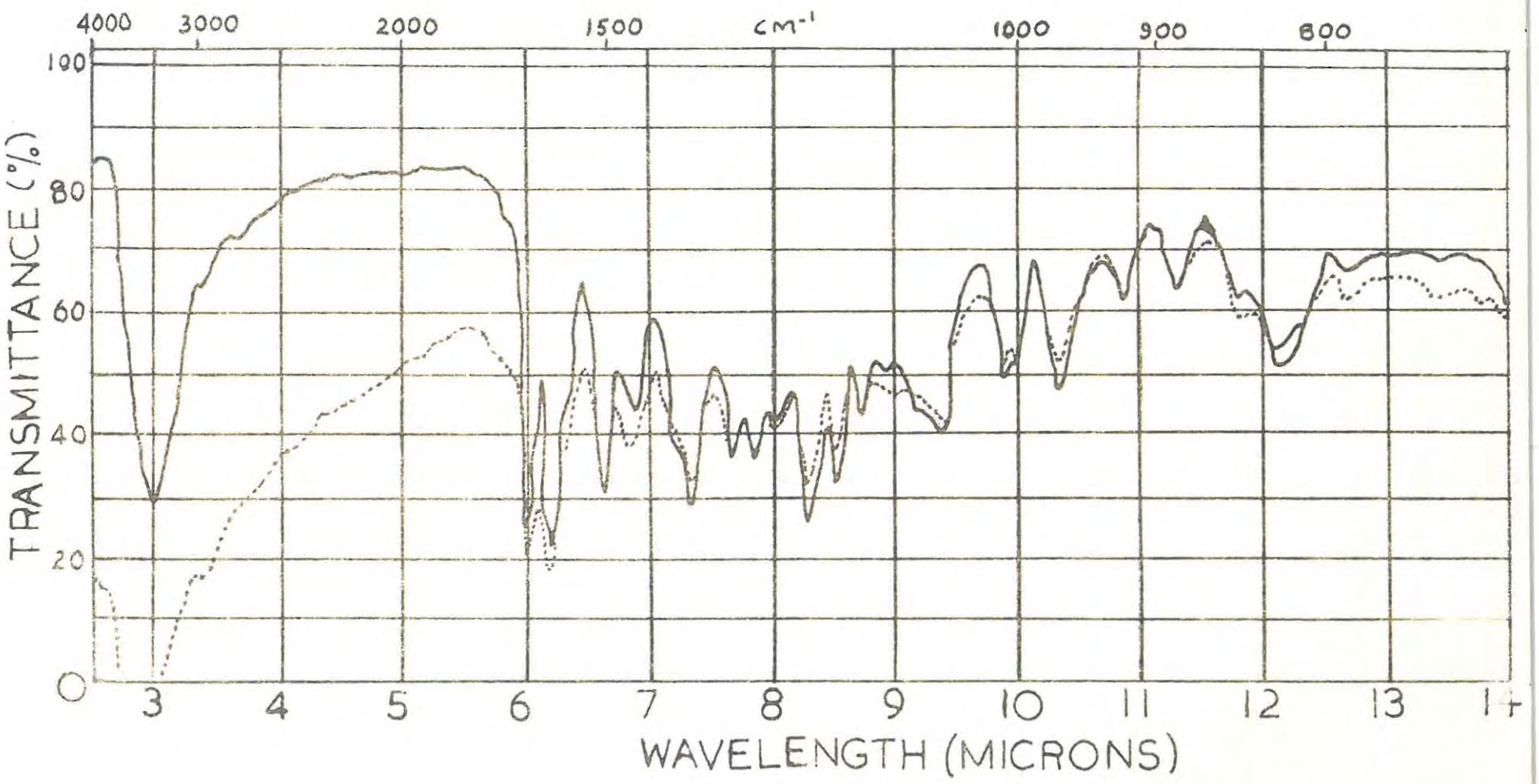


FIG.9. INFRARED ABSORPTION SPECTRUM OF QUERCITRIN (___) AND QUERCITRIN FROM BLACK WATTLE BARK (. . .)

quercetin yielded phloroglucinol and protocatechuic acid as the main products. These were identified by chromatographing the reaction mixture and reference compounds on no. 1 Whatman paper with butan-1-ol-acetic acid-water (6:1:2, by vol.). Phloroglucinol was readily identified as before, while protocatechuic acid gives a characteristic metallic green-black with the silver nitrate reagent and a typical green colouration with ferric alum.

Spectrophotometric examination. Ultraviolet-absorption curves of the compound gave: λ max. in ethanol 256 and 352 mμ(log. ε, 4.24 and 4.10 respectively); in 0.02 M-$AlCl_3$ in ethanol 273 and 420 mμ (log. ε, 4.32 and 4.12); in M-sodium ethoxide, 272, 330 and 403 mμ (log. ε, 4.36, 4.03, and 4.15). Found for authentic quercitrin: λ max. in ethanol, 256 and 352 mμ(log. ε, 4.29 and 4.17); in 0.02 M-$AlCl_3$ in ethanol, 274 and 418 mμ(log. ε, 4.37 and 4.18); in M-sodium ethoxide 273, 332 and 403 mμ(log. ε, 4.43, 4.09 and 4.32). The infrared-absorption curve of the compound and of quercitrin were identical over the range 2.5-15 μ, (Fig. 9).

Hydrolysis of the compound. The glycoside (67.04 mg.) was hydrolysed with hydrochloric acid as described for myricitrin. 43.5 mg. of aglycone were obtained and this represents 96.3% of the calculated value of quercetin in quercetin monorhamnoside. The aglycone formed, had the same R_F, 0.80, as authentic quercetin on Whatman no. 1 paper in butan-1-ol-acetic acid-water (6:1:2, by vol.). The ultraviolet

spectrum gave: λ max. in ethanol 258 and 373 mμ; in 0.02 M-$AlCl_3$ in ethanol, 270 and 435 mμ. Found for quercetin: λ max. in ethanol 257 and 373 mμ; in 0.02M-$AlCl_3$ in ethanol, 270 and 435 mμ. The sugar was shown to be rhamnose by paper chromatography, as described for myricitrin.

PART II.

STEREOSPECIFIC SYNTHESES OF WATTLE LEUCO-ANTHOCYANIDINS AND THEIR ISOMERS.

For some of these syntheses two naturally-occurring dihydroflavonols, (±)-fustin(7,3',4'-trihydroxyflavan-3-ol-4-one) and (±)-dihydrorobinetin(7,3',4',5'-tetrahydroxyflavan-3-ol-4-one) were used.

Isolation of fustin. The method for obtaining racemic fustin was similar to that described by Roux and Freudenberg [62]. Drillings from the heartwood of Rhus glabra (11.5 kg.) were extracted in the cold, first with acetone (30 l.) and then methanol (16 l.) for a total of 14 days. Concentration of the extracts in a rotary evaporator yielded 616 g. of bright yellow powder. This powder, in addition to fustin, contains a high percentage of fisetin. These two components were separated by column chromatography as follows:

Solka floc (Brown Paper Mills) cellulose powder (90 g.) in hot water (1½ l.) was introduced into a glass column (45 x 3.5 cm.) as a slurry, a slight vacuum being maintained during this filling procedure. The cellulose was washed with more boiling water (500 ml.) before the extract, 10 g., dissolved in a minimum of hot water was introduced carefully. Elution of the column with hot water was continued. Fisetin remained as a yellow mass at the top of the column but fustin migrated rapidly. Its front was marked by a blue

zone. Complete elution of fustin was indicated by a marked change from brown to bright yellow of the eluates. The combined fustin fractions were concentrated to dryness under vacuum, dissolved in alcohol, filtered to remove insoluble material and treated with charcoal. White crystals of (+)-fustin, (2.8 g. m.p. 225°) were obtained from water.

Isolation of dihydrorobinetin. The method employed was similar to that of Roux and Paulus [63]. Drillings from the heartwood of Robinia pseudacacia (10 kg.) were extracted in the cold with methanol (30 l.) for 5 days and again with fresh methanol after a further 5 days. The methanolic solution was concentrated to dryness in a rotary evaporator and gave 285 g. brown powder. Wax was removed by extracting the solid material with petroleum ether (40-70°), 267 g.

Wax-free solids (120 g.) were dissolved in the lower phase of a water-butan-2-ol-petroleum ether (5:3:2, by vol.) mixture (600 ml.) and introduced into the first 12 tubes of a Craig machine. The upper and lower phases of the above mixture were used for counter-current separation. Examination of every fifth tube by two-way chromatography indicated that the dihydrorobinetin was present as a very major component in tubes 52-102. Ethyl acetate extracts of the aqueous phases of these tubes were combined with their organic phases, and concentrated in a rotary evaporator to a small volume. Dilution with water yielded first robinetin (2 g. m.p. 324°) which could be

separated off and then dihydrorobinetin 28 g. m.p. 225-226°, $[\alpha]_D^{20}$ + 5.5°c, 1.0 in acetone-water (1:1 v/v). Methylation with diazomethane gave the tetramethyl ether m.p. 165°.

Synthesis of 2,3-trans-3,4-trans-Leuco-Fisetinidin.

(±)-7,3',4'-Trihydroxy-2,3-trans-flavan-3,4-trans-diol by catalytic reduction of (±)-fustin in acetic acid.

Platinum oxide (250 mg.) in glacial acetic acid (25 ml.) was allowed to absorb hydrogen. (±)-Fustin (500 mg.) in acetic acid (60 ml.) was then introduced into the hydrogenation vessel. In order to dissolve fustin the acetic acid solution was heated to boiling point. Hydrogenation proceeded rapidly and after two hours 95.2 ml. had been absorbed. The reaction was stopped at this stage since this volume of hydrogen represents more than twice the calculated amount (41 ml.) of hydrogen. In addition, the uptake of hydrogen was still fairly rapid - 3.6 ml. in 15 min.

The catalyst was filtered off from the greenish solution which was concentrated to dryness under vacuum. Since the residue could not be induced to crystallize, it was examined by two-way chromatography in water-saturated butan-1-ol and 2% (v/v) acetic acid. This showed that, in addition to a leuco-compound, unreacted fustin, fisetin, some high molecular weight material and other products in low concentration were present. The total residue was dissolved in alcohol (25 ml.) and streaked onto 5 sheets of Whatman

no. 3 paper and developed with 2% (v/v) acetic acid. A band (which gave a pink colour with toluene-p-sulphonic acid) R_F 0.50 was cut and eluted. The combined eluates were concentrated to a small volume (2 ml.) under vacuum, whereupon white needles (83 mg.) separated on standing. These had m.p. 145-147° and melt with reddening. Keppler [65] gives the m.p. of (±)-leucofisetinidin obtained from (±)-fustin by reduction in methanol as 125-130° and Roux and Freudenberg [62] cite 126-130°.

The above reduction was repeated with the following modifications:

i) The fustin, which was not completely soluble in the acetic acid in the cold, was introduced into the apparatus as a suspension in the solvent.

ii) The reaction was terminated when between $1\frac{1}{4}$ and $1\frac{1}{2}$ times the theoretical amount of hydrogen had been absorbed.

Side-reactions were not eliminated by these means but much improved yields of the leuco-anthocyanidin resulted. From fustin (500 mg.), leuco-fisetinidin (215 mg.) was obtained after the absorption of 68.5 ml. ($1\frac{1}{2}$ times theoretical) of hydrogen. The leuco-anthocyanidin was again obtained by separation of the reduction mixture on preparative sheets with 2% (v/v) acetic acid. In addition, unchanged fustin (90 mg. m.p. 225°) was recovered.

Two-way chromatography indicated that the compound was pure,

R_F 0.50 and 0.55 in 2% (v/v) acetic acid [separation into the (+)- and (-)-isomer] and R_F 0.60 in water-saturated butan-1-ol. The compound was inseparable from (±)-leucofisetinidin obtained by catalytic reduction in methanol of (±)-fustin [62].

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-trans-diol. The diol (233 mg.) in methanol was methylated with excess diazomethane for 48 hr. at 0°. The trimethoxy derivative (153 mg.) was recrystallized from ethanol and had m.p. 149-150°. Mixed m.p. with the methyl ether of racemic leuco-fisetinidin (from catalytic reduction in methanol [62]) showed no depression.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-trans-diacetate. The trimethoxy derivative (above) (70 mg.) was acetylated with a mixture of acetic anhydride (0.3 ml.) and pyridine (0.2 ml.) for 20 hr. at room temperature. From water 77.2 mg. product was isolated m.p. 119-120° (alcohol). Mixed m.p. with the same derivative of (±)-leuco-fisetinidin (catalytic reduction in methanol [62]) gave no depression. The n.m.r. spectra of these two compounds were also identical and showed the 2,3-trans-3,4-trans configuration of these diols (Fig. 18).

Isopropylidene derivative. The method employed was similar to that of King and Clark-Lewis [70]. The trimethyl ether of the leuco-fisetinidin (72.2 mg.) was dissolved in acetone (4.9 ml.) containing HCl

(1 drop in 100 ml.) and left at room temperature, in the dark for 72 hr. Triethylamine (2 drops) was added and the solution concentrated to dryness under vacuum. The residue crystallized from ethanol to give 36 mg. (50%) needles m.p. 132-134°.

(±)-7,3',4'-Trihydroxy-2,3-trans-flavan-3,4-trans-diol by reduction of (±)-fustin with potassium borohydride.

The method was similar to that employed by Swain [151]. (±)-Fustin (250 mg.) from Rhus glabra was dissolved in an ethanol-water mixture (4:2, by vol.). Potassium borohydride (125 mg.) was dissolved (only partial solution achieved) in ethanol (2 ml.). To the stirred solution of fustin, borohydride was added in the following manner over a $1\frac{1}{4}$ hr. period:

i) For the first 15 min. only borohydride solution was added in small quantities.

ii) Over the next hr. solid crystals of borohydride and borohydride solution were added. The reaction mixture did not warm up to any extent.

At the end of the period the solution became slightly turbid. It was acidified carefully with glacial acetic acid thus clarifying it again. The solution was immediately streaked onto three Whatman no. 3 sheets and developed with 2% (v/v) acetic acid. Strips cut from the chromatographic sheet indicated a single very strongly reducing band at R_F 0.56. Spraying with toluene-p-sulphonic

acid indicated a pink band at R_F 0.56 and a weaker blue band at R_F 0.30 which faded on prolonged heating. Both bands were cut out and eluted. The combined eluates from the higher R_F band were concentrated to a small volume under vacuum (10 ml.) when fine white needles (194 mg., 78% yield) of m.p. 157-162° (reddening) were obtained.

(Found: C, 62.2; H, 5.3. Calculated for $C_{15}H_{14}O_6$: C, 62.1; H, 4.8%.)

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-trans-diol.
The diol (150 mg.) was methylated with diazomethane to give the trimethyl ether (93 mg.) m.p. 151-152° (ethanol). A mixed m.p. with the trimethyl ether derived from the catalytic reduction in methanol of (±)-fustin [62] gave no depression.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-trans-diacetate.
The trimethyl ether above (60 mg.) was acetylated with acetic anhydride-pyridine. This gave the diacetate (63 mg.) which was recrystallized from ethanol to give needles m.p. 121-122°. A mixed m.p. with the same derivative derived from the catalytic reduction in methanol of (±)-fustin [62] gave no depression. In addition the n.m.r. spectra of these two compounds were identical, showing the 2,3-trans-3,4-trans configuration of the diol obtained by reduction with potassium borohydride.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-trans-diol from (±)-fustin by catalytic reduction in methanol.

(±)-Fustin (500 mg.) in methanol (50 ml.) was hydrogenated over platinum oxide catalyst (250 mg.) using the method of Roux and Freudenberg [62]. After 4 hr. 49.6 ml. hydrogen had been absorbed and hydrogenation was complete. (±)-Leuco-fisetinidin (450 mg.)was isolated from the reduction and methylated with diazomethane. The methyl derivative was recrystallized from an alcohol-water mixture and had m.p. 150°. Acetylation gave the diacetate m.p. 121-122°. The melting points of these compounds were in agreement with those of Roux and Freudenberg [62].

Synthesis of Methylated 2,3-trans-3,4-cis-Leuco-Fisetinidin.

(±)-7,3',4'-O-trimethyl-2,3-trans flavan-3,4-cis-diol by reduction of (±)-O-trimethylfustin with metal hydride.

The reduction was similar to that described by Bokadia, Brown, Kolker, Love, Newbould and Somerfield [34]. O-Trimethylfustin (500 mg.) m.p. 140-141° in dry tetrahydrofuran (distilled over sodium) (25 ml.) was added to a mixture of aluminium chloride (1.05 g.) and lithium aluminium hydride (0.3 g.) in tetrahydrofuran (15 ml.). On adding the fustin, vigorous frothing of the solution occurred. The solution was refluxed for 1½ hr. and then allowed to cool. The lithium complex was decomposed with wet ether and 0.5N hydrochloric acid (2 ml.). Water (5 ml.) was added, the aqueous layer was repeatedly extracted with ether and the combined extracts were dried over sodium sulphate.

The dry ethereal solution was concentrated to a small volume (2.5 ml.) when white needles (300 mg.) separated, m.p. 173-174°. Two recrystallizations from acetone raised the m.p. to 185°. The crystals tended to redden on handling as long as impurities were still present. (Found: C, 65.1; H, 6.3; OCH_3, 28.8. $C_{18}H_{20}O_6$ requires: C, 65.1; H, 6.1; OCH_3, 28.0%). The solubility properties of the compound are somewhat unusual. It dissolves only with great difficulty in glacial acetic acid (50 mg. in 50 ml.) and it is sparingly soluble in the common organic solvents in the cold.

The compound was shown to be entirely free of the isomeric trans-trans-diol by comparison of the n.m.r. spectra of their diacetates (Tables 14 and 16.,Figs. 18 and 19). A mixed m.p. with the isomeric trans-trans racemate (m.p. 150-151°) sintered from 145° and melted completely at 180-185°. Their infrared-absorption curves differed markedly in the region 10-15μ. (Figs. 25 and 26).

Similar compounds (trans-cis-diols), but of lower m.p., to which configurations were not assigned were synthesized by Chandorkar and Kulkarni [75] (m.p. 172°, dibenzoate m.p. 148°) by reduction of the same compound with lithium aluminium hydride and also by Fujise, Hishida, Onuma, Adachi, Fujise and Munekata [152,153] by similar methods (m.p. 172-173°, isopropylidene derivative m.p. 135°).

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-cis-diacetate. Acetylation of the diol (100 mg.) with acetic anhydride and pyridine yielded a non-crystalline diacetate which sinters at 54-57° and melts at 60-61°. (Found: C, 63.5; H, 6.1; OCH_3, 22.9; $CO.CH_3$, 20.3. $C_{22}H_{24}O_8$ requires: C, 63.5; H, 5.8; OCH_3, 22.4; $CO.CH_3$, 20.7%). Details of the n.m.r. spectrum of this compound are shown in Tables 14 and 16 and Fig. 19.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-cis-dibenzoate. The trans-cis-diol (60 mg.) was dissolved in a minimum of pyridine (1 ml.) and the solution was cooled to 0°. Benzoyl chloride (0.15 ml.) was added slowly while the flask was shaken. The solution assumed a dark red colour and crystalline pyridine hydrochloride separated out. It was kept in the dark for 24 hr. at room temperature and then poured onto crushed ice. A white solid, which hardened rapidly, was formed. The solid was filtered off (101 mg.) and dissolved in alcohol. Clusters of fine needles (85 mg.) separated on cooling, m.p. 84-86°. (Found for material dried at room temperature: C, 69.4; H, 6.0. $C_{32}H_{28}O_8 . C_2H_5OH$ requires: C, 69.6; H, 5.8%). The n.m.r. spectrum of the crystals confirmed the presence of ethanol. Some of the dibenzoate was dried for 2 hr. at 78°. The crystals now sintered at 84-86° but finally melted at 148°. (Found: C, 71.0; H, 5.1. $C_{32}H_{28}O_8$ requires: C, 71.1; H, 5.2%).

Isopropylidene derivative of (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol.

The method was similar to that of King and Clark-Lewis [70]. The diol (73.5 mg.), in view of its relative insolubility, was dissolved in a fairly large volume of acetone (15 ml.) containing hydrochloric acid (1 drop in 100 ml). The stoppered solution was kept in the dark for 72 hr. at room temp., neutralized with one drop of triethylamine and then concentrated to dryness under vacuum. The residue crystallized from ethanol in clusters of needles, and concentration of the mother liquor to a small volume yielded further crystals over 7 days. A total of 61 mg. (82% yield) m.p. 132-134° was obtained. (Found: C, 67.5; H, 6.6. $C_{21}H_{24}O_6$ requires: C, 67.7; H, 6.5).

Isopropylidene derivatives of 7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-trans-diol.

These derivatives of the racemic trans-trans-diol (m.p. 150-151°) and from (+)-O-trimethyl-mollisacacidin from Acacia mearnsii (m.p. 126-130°) (cf. Keppler [65], Clark-Lewis and Roux [36]) were prepared under the identical conditions described earlier. The former isopropylidene derivative had m.p. 134° and was obtained in 55% yield while the latter had m.p. 120-122° and was prepared in 64% yield. Mixed m.p. of the isopropylidene derivatives of the trans-cis and trans-trans racemates showed no depression, m.p. 132-134°. Their infrared absorption spectra were superimposable over the range 2.5 to 15μ but differed slightly from the corresponding derivative of the

optically pure (+)- compound m.p. 120-122°.

Hydrolysis of trans-trans-isopropylidene derivatives.

The isopropylidene derivative (m.p. 134°) of (+)-O-trimethyl-2,3-trans-flavan-3,4-trans-diol (m.p. 150-151°) (54 mg.) in absolute ethanol (2 ml.) and N hydrochloric acid (0.5 ml.) was heated at the steam bath temperature (97°) for 4 min. The acid was neutralized with triethylamine, the solution concentrated to dryness under vacuum and the white residue dissolved in acetone. Needles of triethylamine hydrochloride (m.p. 251°) were filtered off, and a white sludge, recovered from water (25 ml.), was dissolved in ethanol. Crystals (6 mg.) separated after 3 days at room temperature. These had m.p. 184° after recrystallization from ethanol and mixed m.p. with the trans-cis-diol (m.p. 185°) showed no depression (m.p. 185°).

Similarly the isopropylidene derivative (m.p. 120-122°) of (+)-O-trimethylmollisacacidin gave a diol (m.p. 182-184°) which showed no depression on admixture with the corresponding racemic trans-cis-diol m.p. 182-184°. The infrared spectra of both hydrolysis products were superimposable on that of the racemic trans-cis-diol.

Cyclic carbonates of trans-cis-and trans-trans-7,3',4'-O-trimethylflavan-3,4-diols.

The method used was similar to that described by Kulkarni and Joshi.[41] Trans-cis diol (100 mg.) was dissolved in a mixture of benzene (6 ml.)

and dioxane (6 ml.). Freshly distilled ethyl chloroformate (b.p. 94°, 1 ml.) was added to the solution. Triethylamine (1 ml.) was finally added dropwise. This last reaction was exothermic and caused the solution to boil. At the same time a white crystalline precipitate (m.p. 255°) formed. The solution was left overnight, the precipitate was filtered off and the filtrate concentrated under vacuum to a small volume. From the sweet-smelling residue crystals separated after two days at room temperature. White plates m.p. 177° (61% yield) were obtained from ethanol. (Found: C, 63.4; H, 5.3. $C_{19}H_{18}O_7$ requires: C, 63.7; H, 5.1).

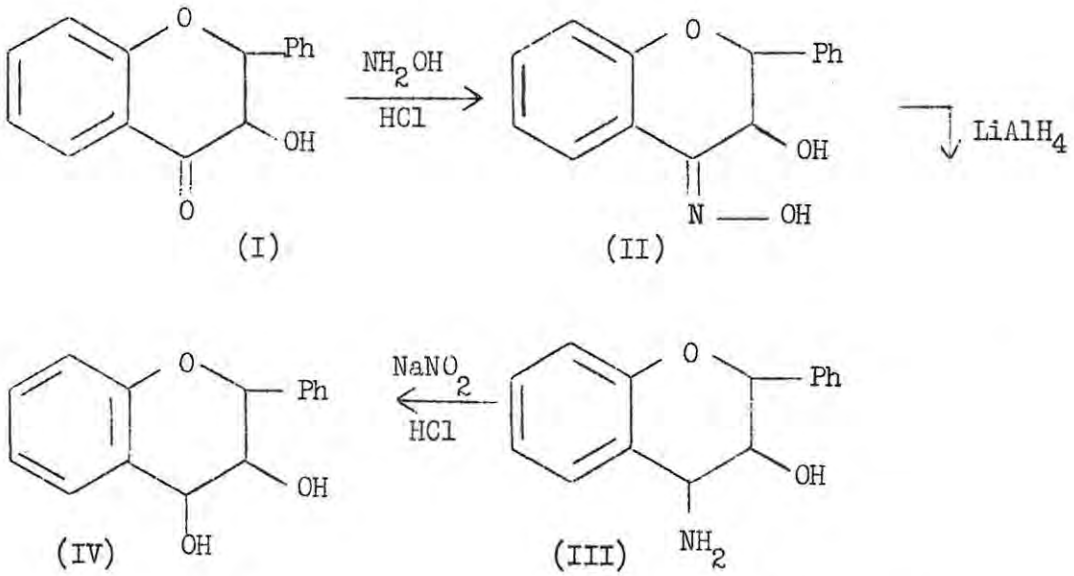
The cyclic carbonate prepared from (+)-trans-trans diol (m.p. 126-130°) was obtained in 3% yield and had m.p. 183-184°. The carbonate derived from racemic trans-trans-diol (m.p. 150-151°) was isolated with difficulty. Initially a red oil, which failed to crystallize, was obtained from the reaction. However, solution of the oil in a small quantity of ethanol, followed by a larger volume of petrol ether (b.p. 40-60°) which, after slight warming, was decanted again, left a residue which crystallized within 24 hr. The crystals, m.p. 184-186° (from ethanol) were obtained in 11% yield. (Found: C, 64.1; H, 5.5. $C_{19}H_{18}O_7$ requires: C, 63.7; H, 5.1).

The infrared curves for the carbonates derived from the racemic trans-cis- and trans-trans-diols were identical over the range 2.5-8μ, but differed from 8-15μ.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-cis-diol by the oxime-amine method.

This route for the preparation of trans-cis-diols was first suggested by Bognar, Rakosi, Fletcher, Kehoe, Philbin and Wheeler [80,154].

3-Hydroxyflavanone I is converted to the oxime II, reduced to the amine III and the latter converted to the flavandiol IV.



3,7,3',4'-Tetrahydroxy-4-oximinoflavan. To (+)-fustin (500 mg.) from Rhus glabra and hydroxylamine hydrochloride (750 mg.) were added pyridine (5 ml.) and water (2.5 ml.) and the solution was kept at the steam bath temperature (97°) for 1 hr. The solution was diluted with ethanol to 75 ml., applied to 15 sheets of Whatman no. 3 paper, and developed with 2% (v/v) acetic acid. A strongly reducing band R_F 0.51 was cut out and eluted. The oxime (398 mg.) from the paper sheets could not be obtained in crystalline form. It had m.p. 110° after

sintering at 80-85°. Two-way paper chromatography indicated that the substance was pure and not contaminated with fustin. In water-saturated butan-1-ol, fustin and its oxime had the same R_F 0.69 but in 2% (v/v) acetic acid they were well separated, R_F 0.40 and 0.52 respectively. The oxime was methylated in the usual way with diazomethane to yield an amorphous product (390 mg.) sintering at 57-62°.

3-Hydroxy-7,3',4'-trimethoxy-4-aminoflavan.

Attempts to reduce the methylated fustin oxime to the amine with palladium catalyst proved unsuccessful. Lithium aluminium hydride was used instead.

To lithium aluminium hydride (150 mg.) in dry tetrahydrofuran (25 ml.) was added the methylated fustin oxime (390 mg.) also in tetrahydrofuran (10 ml.) and the brownish solution refluxed for 1½ hr. The lithium complex was decomposed with wet ether and dilute hydrochloric acid and the aqueous layer extracted several times with ether. The combined extracts were dried (sodium sulphate) and concentrated to dryness under vacuum. An orange-yellow powder (300 mg.), was obtained which was examined by two-way chromatography. Apart from the amine which could be detected with bis-diazotized benzidine, various other contaminants were present in the reduction product. The entire yield was applied to 4 sheets of Whatman no. 3 paper and separated with 10% (v/v) acetic acid. The amine, R_F 0.83, readily located with benzidine, was cut out and eluted. The eluates were concentrated to dryness

and the residue dissolved in a minimum of acetone. After 4 days clusters of needles (21 mg., m.p. 170-174°) formed. Recrystallization from alcohol raised the m.p. to 177-179°.

(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-cis-diol.

The amine (17 mg.) in acetic acid (0.5 ml.) was treated with 2% (v/v) hydrochloric acid (2 ml.). Aqueous sodium nitrite [2% (v/v), 1 ml.] was added dropwise to the stirred solution at 0°. After 1 hr. at this temperature, the solution was heated to 100° in 30 min. and then cooled. After keeping at 0° overnight, a solid separated. The solution was left for 10 days at 0° and the crude crystals filtered off (5 mg., m.p. 136°). These were recrystallized twice from ethanol and then had m.p. 182-183°. A mixed m.p. with authentic (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol (m.p. 185°) prepared as above (lithium aluminium hydride-aluminium chloride reduction) showed no depression, m.p. 184-185°. The 2,3-trans-3,4-cis configuration of this diol was confirmed by chromatographic comparison with the authentic 2,3-trans-3,4-cis compound on borate impregnated paper. (Table 18).

By this method it was also possible to show that the crude crystals of 2,3-trans-3,4-cis-diol (m.p. 136°) contained a small quantity of another isomer of higher R_F and which was presumably the 2,3-trans-3,4-trans-isomer. Examination of the pure trans-cis-diol by paper ionophoresis showed that it had the same positive

migration (Table 19) as the authentic 2,3-trans-3,4-cis-diol.

Synthesis of Methylated 2,3-cis-3, 4-cis-Leuco-Fisetinidin.

(±)-7,3',4'-O-Trimethyl-2,3-cis-flavan-3,4-cis-diol from reduction of 7,3',4'-O-trimethylflavonol with Raney nickel.

(±)-Fustin from Rhus glabra was methylated with diazomethane to give (±)-7,3',4'-O-trimethylflavan-3-ol-4-one (O-trimethylfustin) m.p. 143° (cf. Roux and Paulus [26]). The O-trimethylfustin (930 mg.) in ethanolic N sulphuric acid (50 ml.) was refluxed for 5½ hr. while oxygen was bubbled through the solution. When left overnight, buff crystals (482 mg.) of 7,3',4'-O-trimethylflavonol separated. These were recrystallized once from acetone-alcohol, m.p. 187°. (Found: C, 65.5; H, 5.0. Calc. for $C_{18}H_{16}O_6$: C, 65.9; H, 4.9%). Van Kostanecki and Nitkowski [155] cite m.p. 186° for the same compound.

Raney nickel, prepared from Raney's alloy [156] (Hopkin and Williams nickel/aluminium 42/58 powder) and aged for one month at room temperature, was used for the reduction of the flavonol. To 7,3',4'-O-trimethylflavonol (215 mg.) dissolved in ethanol: methanol (3:1 v/v) (50 ml.) was added Raney nickel (0.4 g.) and the mixture heated with shaking, under hydrogen for 7 hr. at 95-100° and 55 Kg/sq. cm. The catalyst was removed, and the green-yellow solution concentrated to dryness under vacuum at a temperature not exceeding 55°. The residue was dissolved in warm ethanol and the yellow precipitate (m.p. 186°) which formed on cooling, was filtered off. The solution was concentrated (1 ml.) and gave needles (52 mg.) at 0°, m.p. 70-90°.

After recrystallization from ethanol these sintered at 75-90°, but resolidified and melted at 148°. (Found for sample dried at 80°: C, 65.2; H, 6.4, OCH_3, 29.4. Calculated for $C_{18}H_{20}O_6$: C, 65.1, H, 6.1, OCH_3, 28.0%). The infrared absorption spectrum of this cis-cis-diol (Fig. 27) is compared with the spectra of the trans-trans- and trans-cis-diols in Figs. 25 and 26.

(±)-7,3',4'-O-Trimethyl-2,3-cis-flavan-3,4-cis-diacetate.

Acetylation of the diol (32 mg.) with acetic anhydride (0.15 ml.) and pyridine (0.15 ml.) gave the diacetate (30 mg.) which crystallized as needles from ethanol, m.p. 129°. (Found: C, 63.9; H, 6.2; OCH_3, 23.1, $CO.CH_3$, 20.9. Calculated for $C_{22}H_{24}O_8$: C, 63.5; H, 5.8, OCH_3, 22.4; $CO.CH_3$, 20.7%). Details of the n.m.r. spectrum of this compound are shown in Tables 15 and 17 and Fig. 20.

Fujise, Hishida, Onuma, Adachi, Fujise and Munekata [152] recently (1962) quoted a m.p. 151-152 for a 7,3',4'-trimethoxyflavan-3,4-diol prepared as above, while Clark-Lewis and Katekar [29] give m.p. 148-149° for the diol, and m.p. 128-129° for its diacetate, the diol requiring chromatography on alumina following hydrogenation of the flavonol.

Synthesis of Methylated 2,3-trans-3,4-trans-Leuco-Robinetinidin.

(±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-trans-diol by catalytic reduction of (±)-dihydrorobinetin.

This compound (m.p. 230-231°) was obtained by catalytic

hydrogenation (platinum oxide) in methanol of (±)-dihydrorobinetin followed by methylation with diazomethane. The flavandiol was first prepared by Freudenberg and Roux [61]. Acetylation of the methyl ether with acetic anhydride and pyridine gave the diacetate m.p. 113-114°. Details of the n.m.r. spectrum of this compound are shown in Tables 14 and 16 and Fig. 21.

Synthesis of Methylated 2,3-trans-3,4-cis-Leuco-Robinetinidin.

(±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-cis-diol by reduction of (±)-O-tetramethyldihydrorobinetin with metal hydride.

The method of Bokadia et al.[34] was used with some modifications, particularly with regards to reaction temperature. Anhydrous aluminium chloride (1.05 g.) in dry tetrahydrofuran (10 ml.) to which lithium aluminium hydride (0.15 g.) had been added, was cooled to -5° in an ice-salt bath. The (±)-O-tetramethyldihydrorobinetin (0.5 g.) in tetrahydrofuran (20 ml.) was introduced slowly and the solution stirred for 1 hr. The lithium complex was decomposed with wet ether and dilute hydrochloric acid and the product worked up as before, when white needles (178 mg.), m.p. 210-215°, were obtained. The mother liquor of these crystals was taken to dryness under vacuum, redissolved in ethyl alcohol: acetone (1:1 v/v), and this afforded crystals (26 mg.) m.p. 180-200° after 3 hr. at room temperature. A further 148 mg. crystals m.p. 145-160° were obtained after 12 hr. at 0°. Finally 46 mg., m.p. 145° with sintering at 96° separated after 36 hr. at 0°.

Repeated recrystallization (five times) of the first crop

of crystals from acetone: alcohol (1:1 v/v) gave crystals (76 mg.) m.p. 229-230°. Mixed m.p. with authentic trans-trans isomer obtained by catalytic reduction, showed no depression, m.p. 229-231°. The diacetate, m.p. 114° was prepared. (Found: C, 61.6, H, 6.2. Calculated for $C_{23}H_{26}O_9$: C, 61.9; H, 5.9%). It gave mixed m.p. 113-114° with the diacetate derived from (±)-dihydrorobinetin by catalytic hydrogenation (Freudenberg and Roux [61]).

Four recrystallizations of the third crop of crystals from alcohol gave the isomeric trans-cis diol (59 mg.), m.p. 167°. (Found: C, 63.4; H, 6.4; OCH_3, 33.8. $C_{19}H_{22}O_7$ requires: C, 63.0; H, 6.1; OCH_3, 34.3%). Mixed m.p. of the trans-trans and trans-cis isomers gave m.p. 205-215°. Comparison of the n.m.r. spectra of the diacetates of the isomers showed that both were pure (Figs. 21 and 22).

Prior to the above successful conversion of the O-tetramethyldihydrorobinetin to the trans-cis-diol, similar reductions, but at an elevated temperature, had been done. In one such reduction the solution was kept at room temperature for 45 min. whereafter it was refluxed for another 45 min. This yielded mainly the trans-trans-diol m.p. 229-231° and very little trans-cis-diol. In another reduction at 0° for 1 hr. both the trans-trans- and the trans-cis-diols were obtained but these could not be separated by fractional crystallization.

(±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-cis-diacetate. Acetylation of the trans-cis-diol m.p. 167° (43 mg.) gave clusters of white needles (40 mg.) from ethanol, m.p. 124°. (Crystals formed readily from the pure trans-cis-diol, but those from very slightly impure diol remained non-crystalline after acetylation). (Found: C, 62.0; H, 6.2; OCH_3, 28.0; $CO.CH_3$, 19.4. $C_{23}H_{26}O_9$ requires: C, 61.9; H, 5.8; OCH_3, 27.8; $CO.CH_3$, 19.3%). A mixed melt of the 3,4-cis- and 3,4-trans diacetates had m.p. 114-117°.

The details of the n.m.r. spectrum of this compound are shown in Tables 14 and 16 and Fig. 22.

Isopropylidene derivatives of (±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-cis-and-3,4-trans-diols.

Conditions identical to those described in the leuco-fisetinidin series were used for formation of isopropylidene derivatives but reaction proceeded for 84 hr. Reaction with the trans-cis-diol (30 mg.) gave needles, (24.5 mg., 82% yield), m.p. 138°. (Found: C, 66.0; H, 6.9. $C_{22}H_{26}O_7$ requires: C, 65.6; H, 6.5%). The racemic trans-trans-diol (30 mg.) also gave needles (25.7 mg., 86% yield), m.p. 137-138°. Repetition of this synthesis from the trans-trans-diol on a larger scale (80.4 mg.) likewise gave an 86% yield. (Found: C, 65.0; H, 6.5. Calculated for $C_{22}H_{26}O_7$: C, 65.6; H, 6.5%). Mixed m.p. of the racemic isopropylidene derivatives from the trans-cis and trans-trans-diol, showed no depression and their infrared absorption curves were superimposable over the range 2.5-15μ.

The isopropylidene derivative of (+)-7,3',4',5'-O-tetramethylflavan-3,4-diol from Robinia pseudacacia heartwood (Weinges [25]), present in tubes 17-51 in the Craig separation, was obtained in 71% yield under the above conditions, compared with 65% cited previously by Roux and Paulus [38]. It crystallized in rosettes m.p. 138° from alcohol and mixed m.p. with the isopropylidene derivatives of the isomeric racemates gave no depression m.p. 136-138°.

Hydrolysis of trans-trans-isopropylidene derivatives.

Milder conditions were employed than for the corresponding (±)-7,3',4'-O-trimethyl derivative. The isopropylidene derivative of the racemic

trans-trans-diol m.p. 137-138° (20 mg.) was hydrolysed with 1 ml. ethanolic hydrochloric acid (1 drop in 10 ml.) for 2½ min. on the steam bath (97°). The acid was neutralized with a drop of triethylamine and the solution concentrated to dryness in a vacuum desiccator. The dry residue was dissolved in ethanol giving rosettes (13.5 mg.), m.p. 148-150°. After recrystallizing from ethanol, the compound melted partly at 154-155°, with complete melting at 166-167°. Further recrystallizing did not alter the m.p. Mixed m.p. of the hydrolysis product with the racemic trans-cis-diol, m.p. 167°, gave m.p. 153-155°.

Synthesis of Methylated-2,3-cis-3,4-cis-Leuco-Robinetinidin and 2,3-cis-Flavan-3-ol Analogue.

(±)-7,3'4'5'-O-Tetramethyl-2,3-cis-flavan-3,4-cis-diol by reduction of 7,3'4'5'-O-tetramethylflavonol with Raney nickel.

Considerable difficulty was encountered in establishing the appropriate reaction conditions for the above diol. (±)-Dihydrorobinetin from Robinia pseudacacia [157] was methylated with diazomethane to give (±)-7,3',4',5-O-tetramethylflavan-3-ol-4-one, m.p. 165°. The methylated dihydroflavonol (500 mg.) in ethanolic N sulphuric acid (20 ml.) was refluxed for 6 hr. while oxygen was bubbled through the solution (cf. Freudenberg and Hartmann [157] and Shah and Kulkarni [66]). The colour of the solution darkened and after 2 hr. crystals formed. More came down on leaving the solution overnight (344 mg.). Buff needles of 7,3',4',5'-O-tetramethylflavonol (300 mg.) m.p. 194-195° were obtained after recrystallizing once from ethanol. (Found: C, 63.7;

H, 5.1. Calculated for $C_{19}H_{18}O_7$: C, 63.7, H, 5.0%). Dean and Nierenstein [158] and Shah and Kulkarni [66] record m.p. 193° and 194-195° respectively for the same compound.

7,3',4',5'-O-Tetramethylflavonol (192 mg.) was dissolved in ethanol: methanol (3:1 v/v) (50 ml.) and hydrogenated with freshly prepared Raney nickel (0.4 g.) for $4\frac{3}{4}$ hr. at $38\frac{1}{2}$ Kg./sq. cm. and 90-95°. After removal of the catalyst the solution was concentrated to a small volume (2 ml.). White needles (92.4 mg.), m.p. 160°, separated at room temperature (48% yield). Recrystallization from ethanol raised the m.p. to 165°. (Found: C, 63.5; H, 6.33; OCH_3, 33.5. $C_{19}H_{22}O_7$ requires: C, 63.0; H, 6.1; OCH_3, 34.2%).

The infrared absorption spectrum of this diol (Fig. 30) is compared with the spectra of the isomeric trans-trans- and trans-cis-diols in Figs. 28 and 29.

(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3,4-cis-diacetate. Acetylation of the diol (33 mg.) with acetic anhydride and pyridine gave the diacetate (37 mg.) which was recrystallized from ethanol, m.p. 169°. (Found: C, 61.9; H, 5.8; OCH_3, 27.6; $CO.CH_3$, 19.6. $C_{23}H_{26}O_9$ requires: C, 61.9; H, 5.8; OCH_3, 27.8; $CO.CH_3$ 19.3%). Details of the n.m.r. spectrum of this compound are shown in Tables 15 and 17 and Fig. 23.

(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3-ol. More drastic reaction conditions than those used for the hydrogenation giving the above flavandiol also gave the corresponding racemic 2,3-cis-flavan-3-ol. This compound was readily obtained in pure form as follows:- 7,3',4',5'-O-Tetramethylflavonol (150 mg.) in ethanol: methanol (3:1 v/v) (40 ml.) was hydrogenated with fresh Raney nickel (1 g.) for 3½ hr. at 110-118° and 99 Kg./sq. cm. After removal of the catalyst from the colourless solution, the latter was reduced to a small volume (2 ml.) under vacuum when needles (36 mg.) separated, m.p. 132-135°. On recrystallizing twice from ethanol this gave needles (21 mg.), m.p. 158°. (Found: C, 65.7; H, 6.5; OCH_3, 35.8. Calculated for $C_{19}H_{22}O_6$: C, 65.9; H, 6.4; OCH_3, 35.8%).

(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3-acetate. The above flavan-3-ol (40 mg.) was acetylated as before to give the acetate (43 mg.), m.p. 142-143° after recrystallizing from ethanol. (Found: C, 65.1; H, 6.3; OCH_3, 32.2; $CO.CH_3$, 12.1. Calculated for $C_{21}H_{24}O_7$: C, 65.0; H, 6.2; OCH_3, 32.0; $CO.CH_3$, 11.1%). Details of the n.m.r. spectrum of this compound are shown in Tables 15 and 17 and Fig. 24.

Kashikar, Kulkarni, Borkar and Kulkarni [159] recently synthesized this compound by hydrogenation of 3-benzyl-7,3',4',5'-O-tetramethylflavonol. However, the m.p. of both their flavan-3-ol (150-152°) and acetate (136-138°) are 5 to 6° below those found above.

Oxidation Rates of Methylated Flavan-3,4-diols.

Oxidations with lead tetra-acetate.

Lead tetra-acetate oxidations were done using Cordner and Pausacker's [35] and Brown and co-workers' [34] modification of the earlier methods developed by Criegee, Rank and Kraft [33] and Criegee, Buchner and Walther [32].

All materials used in the oxidations were of analytical reagent quality. Water was twice distilled from potassium permanganate in an all-glass apparatus; acetic acid was purified by the method of Eichelberger and La Mer [160], and lead tetra-acetate was crystallized from acetic acid.

General Method.

The water bath was maintained at 20 ± 0.05°. The reactions were done in standard 50 ml. flasks. Between 50 and 80 mg. of the pure, dry methylated diol was weighed into a flask and acetic acid (37.5 ml.) containing 10.4 moles % of water was added. A solution of lead tetra-acetate in acetic acid, as above, was placed in a second flask. When both solutions had attained the water bath temperature, 12.5 ml. of the tetra-acetate solution was added to the diol and the flask shaken. An aliquot (5 ml.) was withdrawn as soon as possible and pipetted into 5 ml. of stopping solution consisting of potassium iodide (100 g.) and sodium acetate (500 g.) in water (1 l.). Before titrating, the solution was diluted with water (50 ml.). The iodine

liberated was titrated against 0.005 N sodium thiosulphate (starch). The second order velocity constant, k, was calculated from the usual relation:

$$k = \frac{2.303}{t(b-a)} \log \frac{a}{b}\left(\frac{b-x}{a-x}\right)$$

by a graphical method where $\log \frac{a}{b}\left(\frac{b-x}{a-x}\right)$ was plotted against t, a = first diol concentration, and b = first tetra-acetate concentration.

Rate constant for (+)-7,3',4'-O-trimethyl-2,3-trans flavan-3,4-trans-diol. (O-Trimethyl-leuco-fisetinidin).

The optically pure diol $[\alpha]_D^{12}$ + 31.4° (0.8% in 1:1 $COMe_2$-H_2O)[36] from the heartwood of Acacia mearnsii was methylated with diazomethane, m.p. 126-130°, $[\alpha]_D^{16}$ - 10.3 (1.12% in $C_2H_2Cl_4$).[36] Before use it was dried at 78° under vacuum over phosphorus pentoxide.

Results and Calculation.

Concentration of diol = 0.004695M.

Concentration of thiosulphate = 0.0050990N.

Concentration of tetra-acetate (by titration against thiosulphate) = 0.17795M (1.25 ml. tetra-acetate ≡ 8.72 ml. thiosulphate).

Table 3. Volume of thiosulphate consumed at various time intervals.

t(min.)	Vol. thiosulphate (ml.)
0	8.62
12	6.65
22	6.56
38	5.86
60	5.22
83	4.92
103	4.59
120	4.55
143	4.31

Thus:

a). Initial concentration of diol = 0.004695

$$= \frac{.004695}{.017795} \times \frac{8.72}{1} \times \frac{3}{1} = 6.90 \text{ ml. thiosulphate.}$$

b). Initial concentration of tetra-acetate = 8.72 ml. thiosulphate.

c). First tetra-acetate estimation = 8.62 ml. thiosulphate (b).

d). Equivalent diol concentration = 6.90-(8.72-8.62) = 6.80 ml. thiosulphate (a).

The value of $\log \frac{a}{b}\left(\frac{b-x}{a-x}\right)$ may now be evaluated, thus when t=12, the log function is $\log \frac{6.80}{8.62}\left(\frac{6.65}{4.83}\right)$.

Table 4. Variation of log function with time.

t(min.)	$\log \frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$
12	3.58
22	3.82
38	5.84
60	8.31
83	9.76
103	11.62
120	11.88
143	13.51

When these log. values were plotted against time, a straight line relationship was obtained, Fig. 10. The slope of the line was calculated to be 0.0009368.

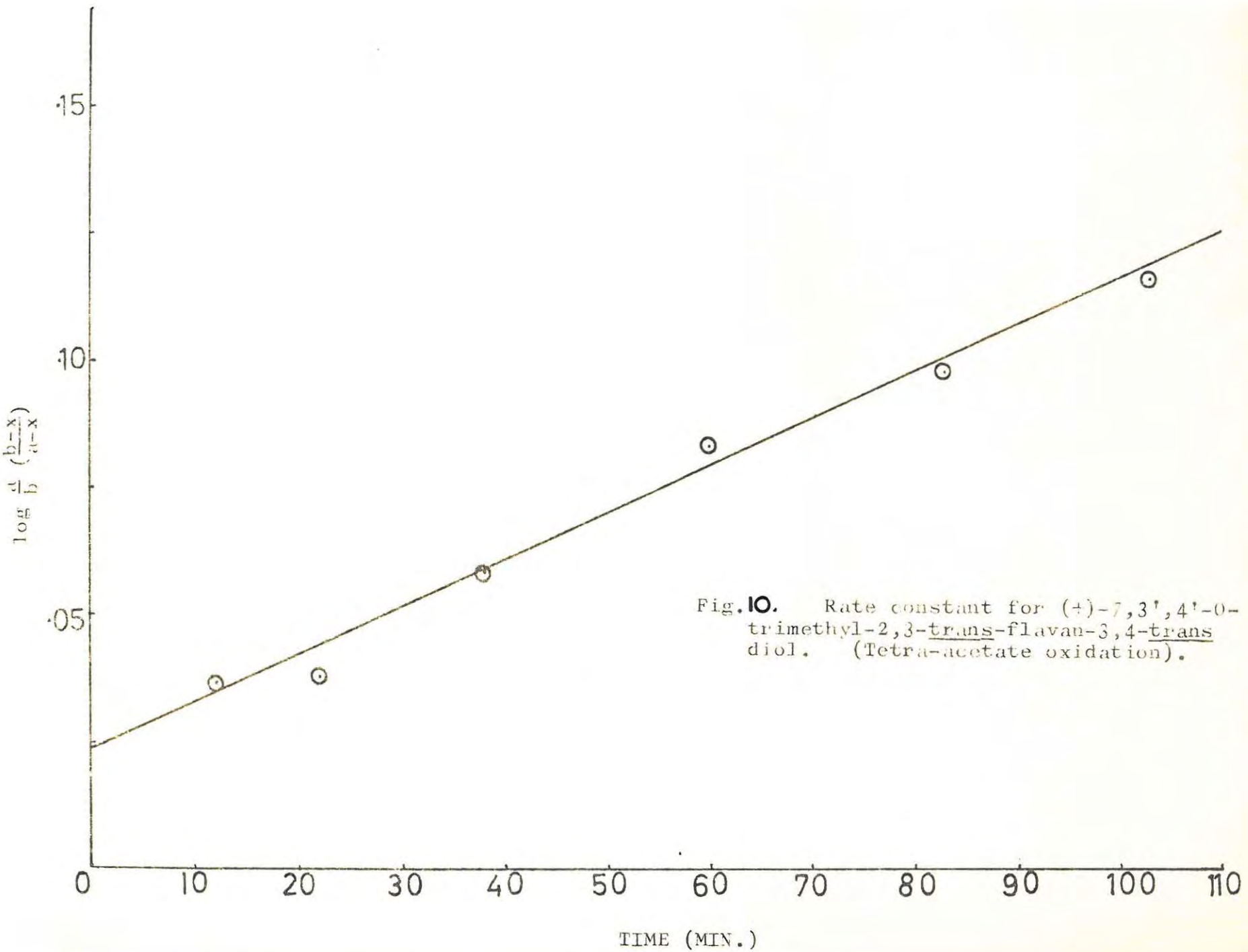


Fig. 10. Rate constant for (+)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-trans diol. (Tetra-acetate oxidation).

Thus:

$$k = \frac{2.303}{(b-a)} \times \text{Slope}$$

$$= \frac{2.303}{.0037105} \times .0009368$$

$$= 0.5815 \text{ mole.}^{-1} \text{ l. min.}^{-1}$$

Three further oxidations, as above, gave the following values of k: 0.6563, 0.6540, and 0.6020 mole.$^{-1}$ l. min.$^{-1}$ Mean: 0.623 mole.$^{-1}$ l. min.$^{-1}$

Rate constant for (±)-7,3',4'-O-trimethyl-2,3-trans flavan-3,4-cis-diol.

For this diol the reaction rate was very rapid, and agreement between results was, therefore, not as good as in the case of the trans-trans-diol.

Results and Calculation.

Concentration of diol = 0.004773M.

Concentration of thiosulphate = 0.0050990N.

Concentration of tetra-acetate = 0.017668M. (1.25 ml. tetra-acetate ≡ 8.66 ml. thiosulphate).

Table 5. Volume of thiosulphate consumed and variation of log. function with time.

t (min.)	Vol. thiosulphate (ml.)	log. $\frac{a}{b}\left(\frac{b-x}{a-x}\right)_{\times 10^{-2}}$
0	6.90	-
5	5.21	4.61
9	4.34	8.82
14	3.61	14.52
23	3.19	19.53
29	3.11	20.76
45	2.93	23.83
67	2.80	26.48
90	2.87	25.02

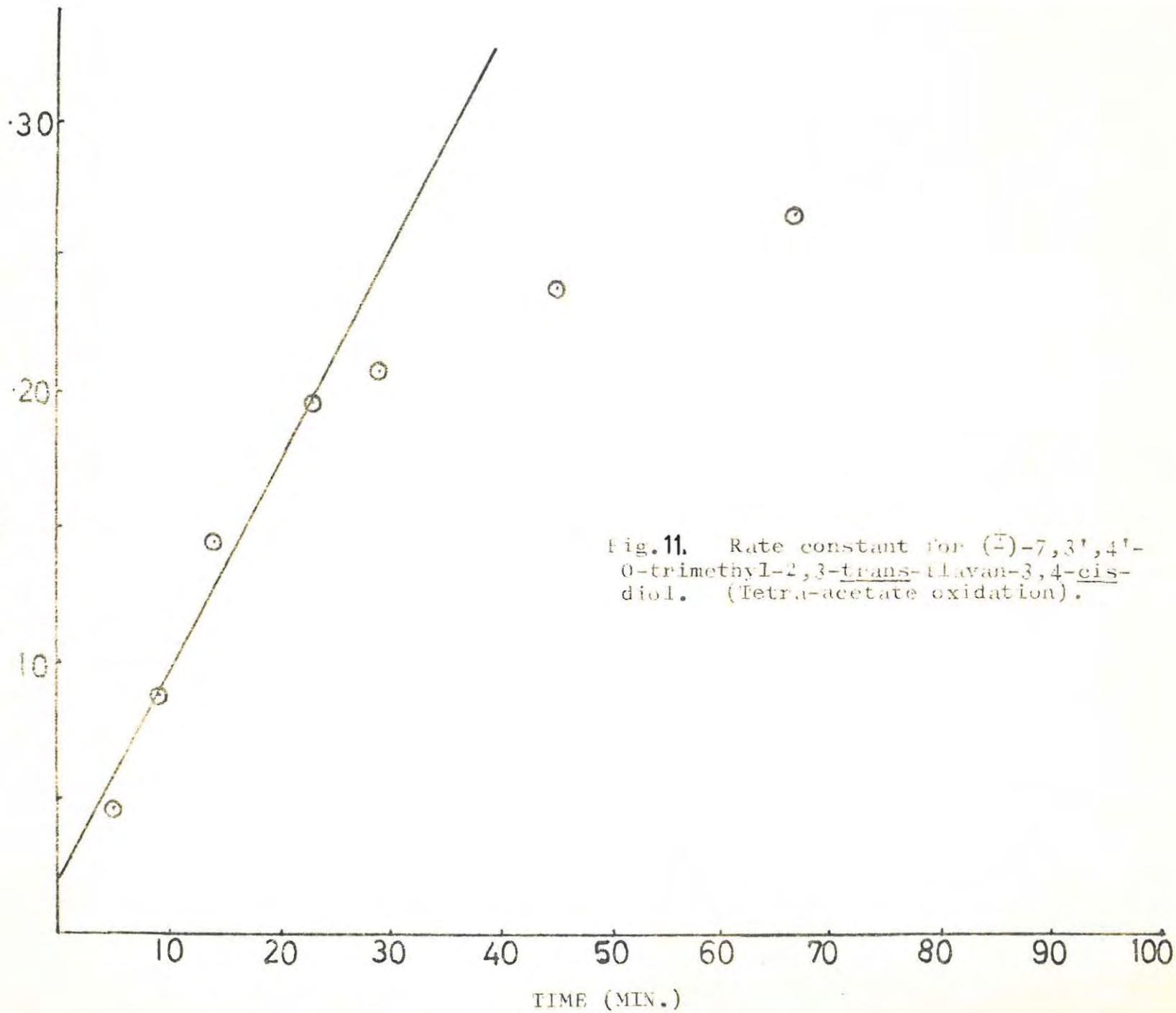


Fig. 11. Rate constant for (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol. (Tetra-acetate oxidation).

The plot of log. $\frac{a}{b}\left(\frac{b-x}{a-x}\right)$ against t for this compound is shown in Fig. 11.

$$k = \frac{2.303}{(b-a)} \times \text{Slope}$$

$$= \frac{2.303}{.003348} \times .007692$$

$$= 5.289 \text{ moles}^{-1} \text{ l. min.}^{-1}$$

Another oxidation under identical conditions gave 4.94 moles^{-1} l. min.$^{-1}$

Oxidations with periodic acid.

Periodic acid, as reagent for glycols, was introduced by Malaprade [30]. In this work the experimental procedure as outlined by Jackson [31] was largely followed. All reagents were of analytical quality.

General method.

All oxidations were done at 20 $\pm$ 0.05°. The pure, dry methylated flavandiol (40-50 mg.) was dissolved in ethanol (50 ml.) in a standard 100 ml. volumetric flask. Distilled water (40 ml.) was added and the solution allowed to reach equilibrium in the water bath. Periodic acid solution (about 0.05M) (10 ml.), also at the water bath temperature, was then pipetted into the solution containing the flavandiol and the solution made up to the 100 ml. mark. The mixture was shaken and an aliquot (5 ml.) was withdrawn as soon as possible. This was run into an iodine flask containing standard arsenite solution (0.005N, 12 ml.) together with saturated sodium bicarbonate (5 ml.) and a few crystals of potassium iodide. The flask was stoppered and

left to stand for 15 min. before titrating with iodine (0.005N) and starch indicator.

From the value of the volume of iodine consumed by the various aliquots, the quantity of residual periodate in each of these aliquots may be calculated. Immediately on mixing a 5 ml. aliquot contains 0.5 ml. periodic acid. The number of ml. of standard arsenite solution equivalent to 0.5 ml. of periodate solution was determined by titration. It is thus possible to calculate the quantity of arsenite equivalent to the periodate in each aliquot as the reaction proceeds. k May be obtained graphically as before from the relation $k = \frac{2.303}{t(b-a)} \log \frac{a}{b}\left(\frac{b-x}{a-x}\right)$

Rate constant for (+)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-trans-diol. [(+)-O-Trimethyl mollisacacidin]

(+)-Mollisacacidin, from the heartwood of Acacia mearnsii, was methylated with diazomethane. The methyl ether (m.p. 129°) was dried at 78° under vacuum over phosphorus pentoxide for 3 hr.

Results and calculation.

Concentration of diol = 0.001472M.

Concentration of sodium arsenite = 0.0050602N

Concentration of periodic acid = 0.04615M.

(0.5 ml. of periodate solution = 9.12 ml. sodium arsenite)

Table 6. Volume of iodine consumed and residual iodate at various time intervals.

t(min.)	Vol.iodine (ml.)	Residual periodate (ml.)
3	3.14	0.4735
5	3.35	0.4611
10	3.87	0.4306
15	4.14	0.4148
23	4.42	0.3984
47	4.88	0.3713
60	4.92	0.3690
69	4.96	0.3667
87	5.02	0.3631
105	4.90	0.3702
120	5.03	0.3625

Thus:

a) Initial concentration of diol $= \left(\frac{.001472}{.04615} \times \frac{9.12}{1} \times \frac{9}{1}\right)$

$\equiv$ 2.62 ml. sodium arsenite solution.

b) Initial concentration of periodate $\equiv$ 9.12 ml. sodium arsenite.

c) First iodate estimation $\equiv \frac{.4735}{0.5} \times \frac{9.12}{1}$

$\equiv$ 8.64 ml. sodium arsenite (b).

d) Equivalent diol concentration $\equiv$ 2.62-(9.12-8.64)

$\equiv$ 2.14 ml. sodium arsenite (a).

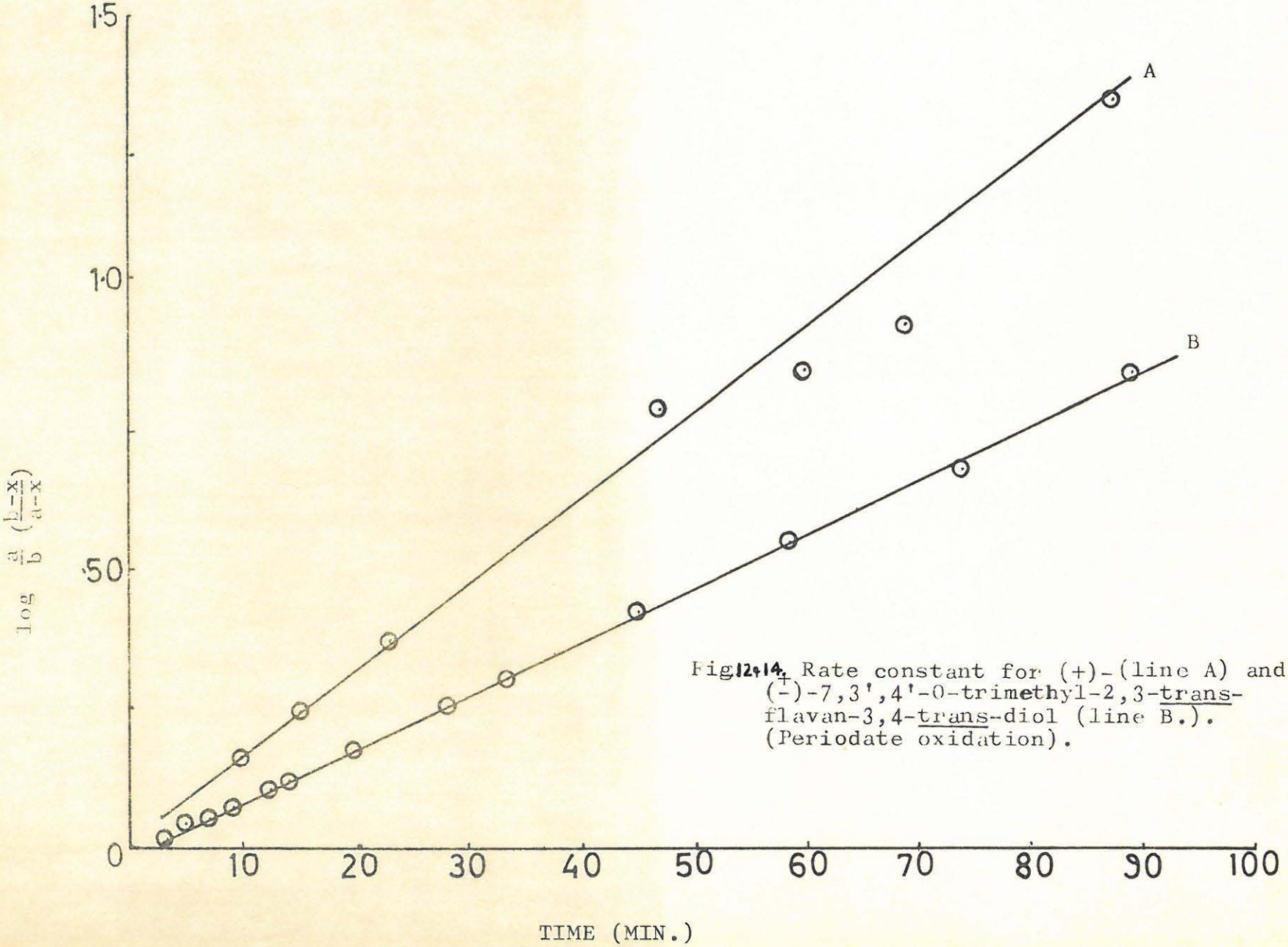


Fig 12.14. Rate constant for (+)- (line A) and (-)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-trans-diol (line B.). (Periodate oxidation).

Table 7. Variation of log. function with time and calculation of the rate of consumption of periodate.

t(min.)	$\log \frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$	No. of moles of periodate consumed.
3	-	0.1850
5	3.78	0.2709
10	15.84	0.4833
15	24.35	0.5934
23	36.89	0.7080
47	79.31	0.8963
60	86.01	0.9130
69	94.05	0.9283
87	135.4	0.9534
120	172.6	0.9576

A plot of $\log \frac{a}{b}\left(\frac{b-x}{a-x}\right)$ against t gave a straight line relationship as shown in Fig. 12. The slope of the straight line was 0.015636 so that:

$$k = \frac{2.303}{(b-a)} \times \text{Slope}$$

$$= \frac{2.303}{.03290} \times \frac{.015636}{1}$$

$$= 1.094 \text{ moles}^{-1} \text{ l. min.}^{-1}$$

Rate constant for (±)-7,3',4'-O-trimethyl-2,3-trans flavan-3,4-cis-diol.

The trans-cis-diol (m.p. 185°) was oxidised with periodic acid in the same manner as the foregoing (+)-O-trimethyl mollisacacidin.

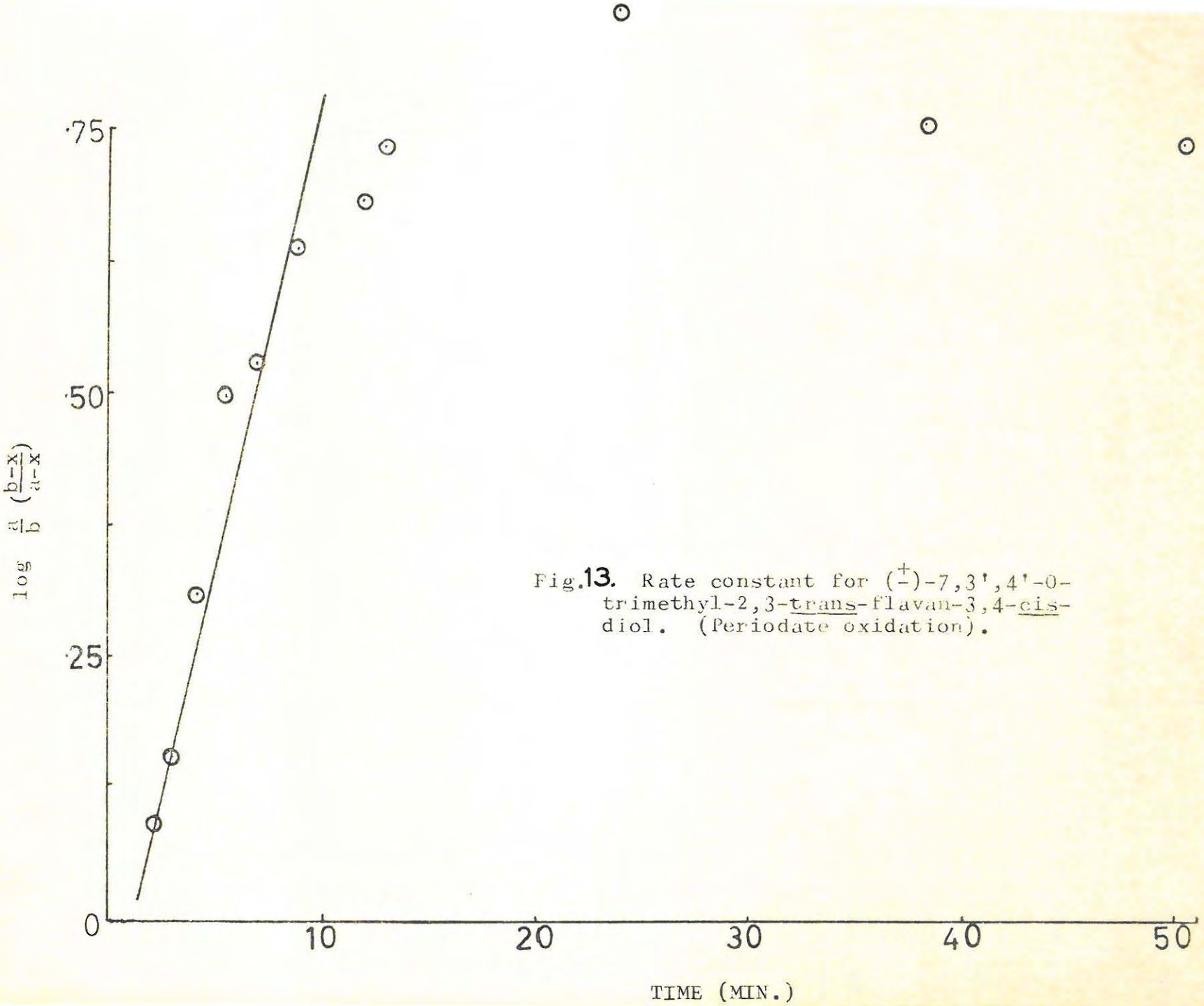


Fig.13. Rate constant for (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol. (Periodate oxidation).

Table 8. Variation of log. function with time and calculation of the rate of consumption of periodate.

t(min.)	Residual periodate (ml.)	No. of moles of periodate consumed.	$\log.\frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$
1.5	0.4282	.4660	-
2.25	0.4097	.5856	9.31
3.0	0.3996	.6511	15.62
4.25	0.3818	.7666	31.02
5.5	0.3681	.8554	49.50
7.0	0.3670	.8626	52.59
9.0	0.3616	.8976	63.74
12.0	0.3604	.9053	68.28
13.0	0.3586	.9170	73.41
24.0	0.3549	.9410	86.13
35.0	0.3537	.9488	94.36
38.5	0.3579	-	75.27
50.5	0.3585	-	73.41
62.5	0.3585	-	73.41

Concentration of diol = 0.001561M.

Concentration of periodic acid = .04559M.

From Fig. 13, $k = 6.238$ moles^{-1} l. min.$^{-1}$.

Rate constant for (±)-7,3',4'-O-trimethyl-2,3-trans flavan-3,4-trans-diol.

This diol m.p. 151° was oxidised as above, and gave the following results:

Concentration of diol = 0.001480M.

Concentration of periodic acid = 0.04636M.

Table 9. Variation of log. function with time and calculation of the rate of consumption of periodate.

t(min.)	Residual periodate (ml.)	No. of moles of periodate consumed.	$\log \frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$
1.5	0.4863	0.0935	-
3.0	0.4810	0.1297	1.36
5.0	0.4711	0.1973	4.03
7.0	0.4688	0.2130	4.64
9.0	0.4612	0.2649	7.01
11.5	0.4524	0.3249	10.11
14.0	0.4483	0.3530	11.52
20.0	0.4349	0.4444	17.15
28.0	0.4191	0.5520	25.29
33.5	0.4115	0.6040	30.12
45.0	0.3963	0.7079	42.60
58.5	0.3858	0.7800	54.54
74.0	0.3776	0.8360	67.75
89.0	0.3700	0.8880	86.17
105.0	0.3712	-	82.96
120.0	0.3618	0.9434	250.0
135.0	0.3630	-	172.4

From Fig. 14, $k = 0.686$ moles^{-1} l. min.$^{-1}$

Rate constant for flavandiols of the leuco-robinetinidin series.

(+)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-trans-diol.

This diol, derived from Robinia pseudacacia, m.p. 164-166° gave the following values:

Concentration of periodate = 0.04648M.

Concentration of diol = 0.001351M.

Concentration of sodium arsenite = 0.0051431N.

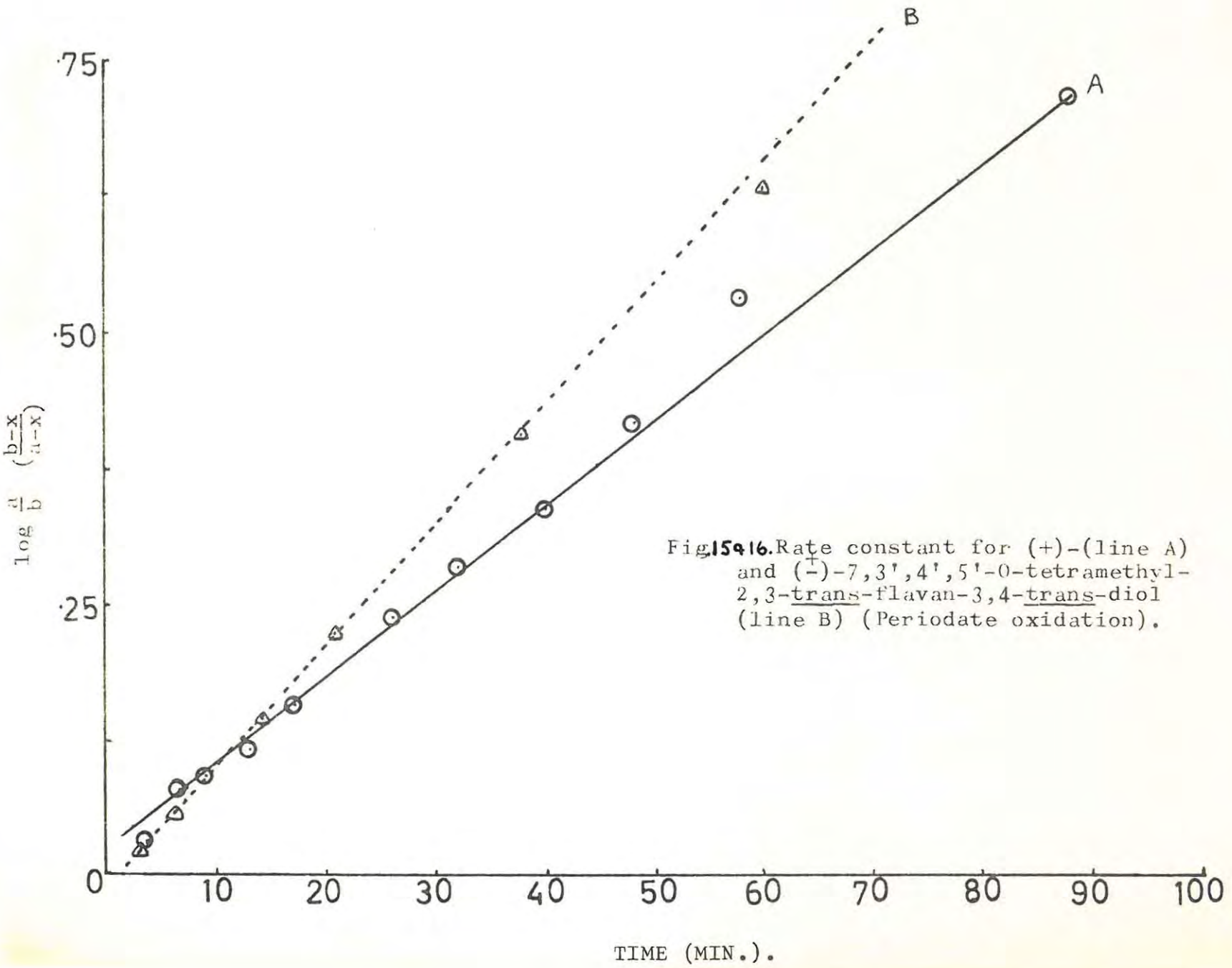


Fig 15 16. Rate constant for (+)-(line A) and (±)-7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-trans-diol (line B) (Periodate oxidation).

Table 10. Variation of log. function with time and calculation of rate of consumption of periodate.

t(min.)	Residual periodate (ml.)	No. of moles of periodate consumed.	$\log \frac{a}{b}$	$(\frac{b-x}{a-x}) \times 10^{-2}$
1.5	0.4799	0.1535		-
3.5	0.4709	0.2219		2.98
6.5	0.4643	0.2731		7.85
9.0	0.4548	0.3456		8.99
13.0	0.4492	0.3880		11.39
17.0	0.4403	0.4562		15.62
26.0	0.4269	0.5586		23.68
32.0	0.4202	0.6097		28.37
40.0	0.4141	0.6567		33.32
48.0	0.4057	0.7207		41.47
58.0	0.3968	0.7889		52.79
73.0	0.3867	0.8656		71.56
88.0	0.3867	0.8656		71.56
108.0	0.3829	0.8954		82.25
120.0	0.3834	0.8912		80.53
138.0	0.3762	0.9465		115.9
168.0	0.3812	0.9083		87.85
388.0	0.3550	1.10		-

From Fig. 15, $k = 0.525$ moles^{-1} l. min.$^{-1}$

(±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-trans-diol.

This diol m.p. 229-231°, gave the following results:

Concentration of periodate = 0.04648M

Concentration of diol = 0.001254M.

Concentration of sodium arsenite = 0.005143N.

Table 11. Variation of log. function with time and calculation of rate of consumption of periodate.

t(min.)	Residual periodate (ml.)	No. of moles of periodate consumed.	$\log \frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$
1.5	0.4872	0.1056	-
3.0	0.4799	0.1654	2.36
6.0	0.4710	0.2389	5.50
9.0	0.4626	0.3074	8.89
14.0	0.4504	0.4088	14.55
21.0	0.4364	0.5238	22.56
29.0	0.4303	0.5742	26.81
38.0	0.4152	0.6983	40.75
49.0	0.4119	0.7259	44.62
60.0	0.3996	0.8270	63.59
74.0	0.3907	0.9004	87.21
89.0	0.3868	0.9327	104.37
109.0	0.3829	0.9648	134.06
136.0	0.3762	1.0190	-

From Fig. 16, $k = 0.728$ moles^{-1} l. min.$^{-1}$

(±)-7,3',4',5'-O-Tetramethyl-2,3-trans flavan-3,4-cis-diol.

Due to the rapid reaction of this diol, m.p. 167°, numerous readings were taken over the first ten minutes. The following results were obtained:

Concentration of periodate = 0.04648M.

Concentration of diol = 0.001279M.

Concentration of sodium arsenite = 0.005143N.

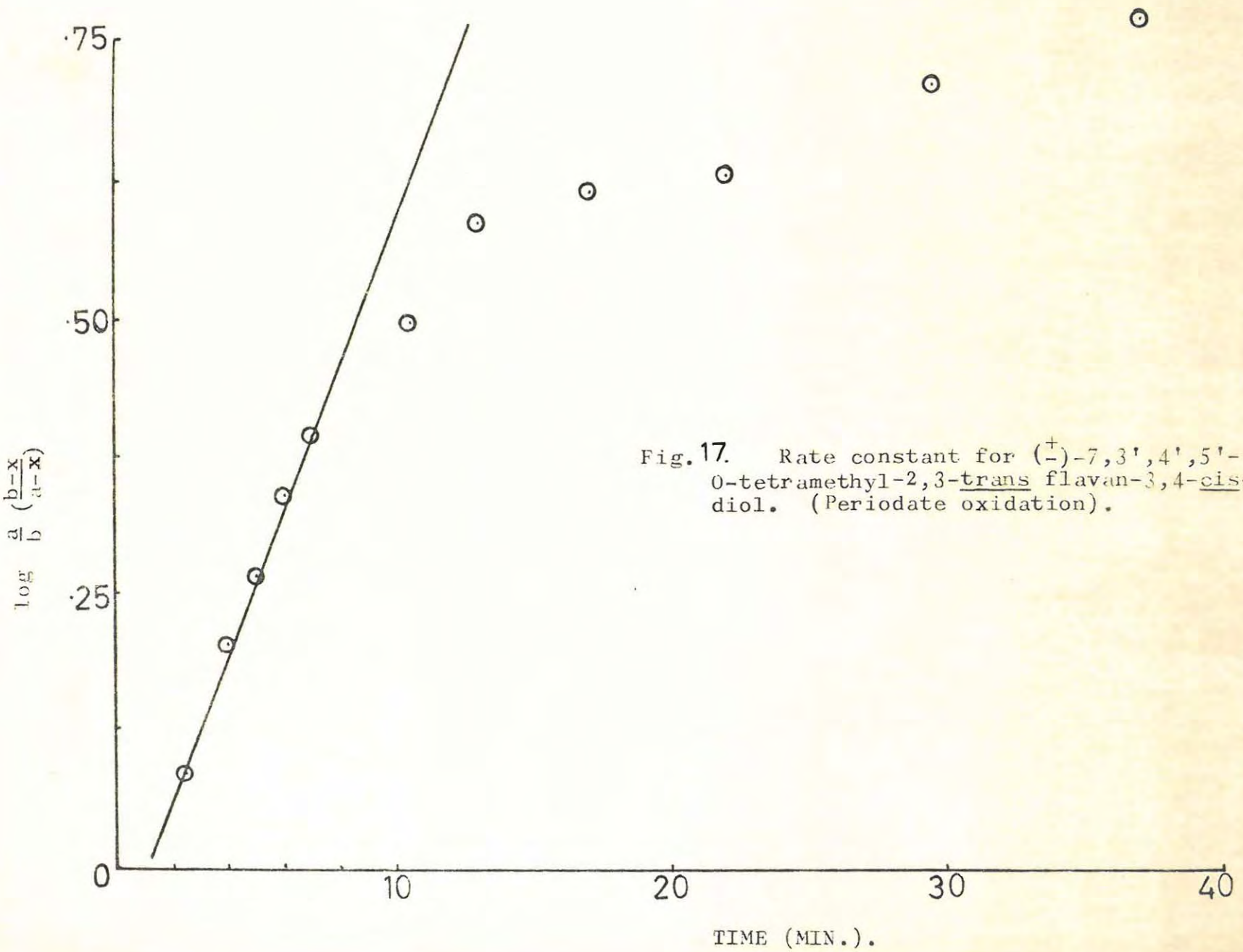


Fig. 17. Rate constant for (±)-7,3',4',5'-O-tetramethyl-2,3-trans flavan-3,4-cis-diol. (Periodate oxidation).

Table 12. Variation of log. function with time and calculation of rate of consumption of periodate.

t(min.)	Residual periodate (ml.)	No. of moles of periodate consumed.	$\log \frac{a}{b}\left(\frac{b-x}{a-x}\right) \times 10^{-2}$
1.5	0.4492	0.4096	-
2.5	0.4342	0.5310	8.46
4.0	0.4191	0.6527	20.36
5.0	0.4130	0.7022	26.41
6.0	0.4069	0.7518	33.69
7.0	0.4030	0.7832	39.18
10.5	0.3968	0.8328	49.83
13.0	0.3929	0.8643	58.51
17.0	0.3918	0.8733	61.38
22.0	0.3912	0.8778	62.91
29.5	0.3884	0.9003	71.50
37.0	0.3868	0.9138	77.68
46.0	0.3812	0.9588	109.48
61.0	0.3806	0.9633	145.5
170.0	0.3711	1.039	-

From Fig. 17, $k = 4.487$ moles^{-1} l. min.$^{-1}$

Summary of oxidation rates. The oxidation rates of the methylated 2,3-trans-flavandiols studied are summarized below.

Table 13. Velocity constants of periodate and lead tetra-acetate oxidations of methylated 2,3-trans-3,4-trans- and 2,3-trans-3,4-cis-diols.

	m.p.	k (moles^{-1} l. min.$^{-1}$)	
		Periodate	Lead tetra-acetate
O-Trimethyl-2,3-trans-flavan-3,4-diols (leuco-fisetinidins)			
(±)-3,4-trans *	150-151°	0.68	-
(+)-3,4-trans +	126-130°	1.09	0.62
(±)-3,4-cis *	185°	6.24	5.28
O-Tetramethyl-2,3-trans-flavan-3,4-diols (leuco-robinetinidins)			
(±)-3,4-trans *	228-230°	0.73	-
(+)-3,4-trans +	164-166°	0.53	-
(±)-3,4-cis *	167°	4.49	-

* Synthetic compounds
\+ Derivatives of natural compounds.

NUCLEAR MAGNETIC RESONANCE SPECTRA OF DERIVATIVES OF METHYLATED FLAVAN-3-4-DIOLS AND A FLAVAN-3-OL ANALOGUE.

In general the diacetyl derivatives of the methylated flavan-3,4-diols were most suitable for n.m.r. study since in these derivatives the least overlap of signals of the 2,-3- and 4-protons occurs. The τ value (in parts per million) and coupling constants J, (cycles per second) calculated from the n.m.r. spectra of the derivatives of the methylated flavan-3,4-diols are shown in Tables 14, 15, 16 and 17. Designation of the 3- and 4-acetyl signals was by comparison with the corresponding derivatives of flavan-3-ol-and flavan-4-ol analogues. Similarly the designation of 7-, and grouped 3',4'- or 3',4',5'-methoxyl signals was by comparison with the corresponding synthetic 7,4'-O-dimethylflavan-3,4-diol derivatives and with published data by Clark-Lewis, Jackman and Williams [9] for 4'-O-methylflavan-3,4-diol derivatives.

The 5-proton was identified by its strong shift downfield in derivatives of the dihydroflavonol and flavanone analogues, the signal being split by spin-spin coupling with the 6-proton. Hence it was readily recognized in the 3,4-diacetates and cyclic derivatives of the methylated flavan-3,4-diols.

Table 14. τ Values (p.p.m.) for derivatives of 2,3-trans-flavan-3,4-diols.

	Acetyl	Me	O Me					
7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-diacetate								
	3-,	4-	7-,	3'+4'	2H	3H	4H	5H
(±)-3,4-trans	8.15	7.97	6.23	6.12	4.95	4.42	3.73	~2.92
(+)-3,4-trans	8.15	7.97	6.24	6.12	4.94	4.42	3.74	~2.91
(±)-3,4-cis	8.15	7.87	6.22	6.10	4.80	4.47	3.82	2.80
7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-dibenzoate								
			O Me					
			7-,	3'+4'	2H	3H	4H	
(±)-3,4-cis			6.21	6.18	4.49	4.17	3.44	
Cyclic derivatives of (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol								
	CH_3		O Me					
			7-,	3'+4'	2H	3H	4H	5H
Carbonate			6.20	6.10	5.25	5.10	4.33	2.63
Isopropylidene	8.53	8.45	6.22	6.10	5.73	5.45	4.88	2.61
7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-diacetate								
	Acetyl	Me	O Me					
	3-,	4-,	7-,	3',4',5'	2H	3H	4H	5H
(±)-3,4-trans	8.12	7.97	6.22	6.13	4.95	4.42	3.77	2.92
(+)-3,4-trans	8.12	7.97	6.21	6.13	4.96	4.43	3.77	2.93
(±)-3,4-cis	8.13	7.85	6.20	6.12	4.80	4.48	3.82	2.80

Table 15. τ Values (p.p.m.) for derivatives of 2,3-cis-3,4-cis-flavan-3,4-diols and a related 2,3-cis-flavan-3-ol.

Acetyl Me		O Me				
3-,	4-,	7-,	3',4',(5')	2H	3H	4H
(±)-7,3',4'-O-Trimethyl-2,3-cis-flavan-3,4-cis-diacetate						
8.06	7.88	6.16	6.10	4.68	4.32	3.64
(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3,4-cis-diacetate						
8.03	7.85	6.14	6.09	4.65	4.24	3.62
(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3-acetate						
8.06	-	6.20	6.13	4.93	4.35	6.90*

* methylene group at C_4

Table 16. Coupling constants (c.p.s.) for 2-, 3- and 4-protons of derivatives of 2,3-trans-flavan-3,4-diols.

	$J_{2,3}$	$J_{3,4}$
(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-diacetate		
(±)-3,4-trans	8.9	7.1
(+)-3,4-trans	8.9	7.1
(±)-3,4-cis	10.3	3.3
(±)-7,3',4'-O-Trimethyl-2,3-trans-flavan-3,4-dibenzoate		
(±)-3,4-cis	10.1	2.9
Cyclic derivatives of (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol		
Carbonate (from trans-diol)	9.4	6.0
Carbonate (3,4-cis)	9.0	6.0
Isopropylidene (3,4-cis)	9.9	5.0
(±)-7,3',4',5'-O-Tetramethyl-2,3-trans-flavan-3,4-diacetate		
(±)-3,4-trans	8.9	7.1
(+)-3,4-trans	9.0	6.9
(±)-3,4-cis	10.0	3.4

Table 17. Coupling constants (c.p.s.) for 2-, 3- and 4-protons of derivatives of 2,3-cis-3,4-cis-flavan-3,4-diols and a related 2,3-cis-flavan-3-ol.

(±)-7,3',4'-O-Trimethyl-2,3-cis-flavan-3,4-cis-diacetate	
$J_{2,3}$	$J_{3,4}$
1.0	3.8 (4.2)
(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3,4-cis-diacetate	
1.0	4.1 (4.0)
(±)-7,3',4',5'-O-Tetramethyl-2,3-cis-flavan-3-acetate	
1.0	-

FIG. 18. N.M.R. SPECTRUM OF (±)-7,3',4'-O-TRIMETHYL-2,3-TRANS-FLAVAN-3,4-TRANS-DIACETATE

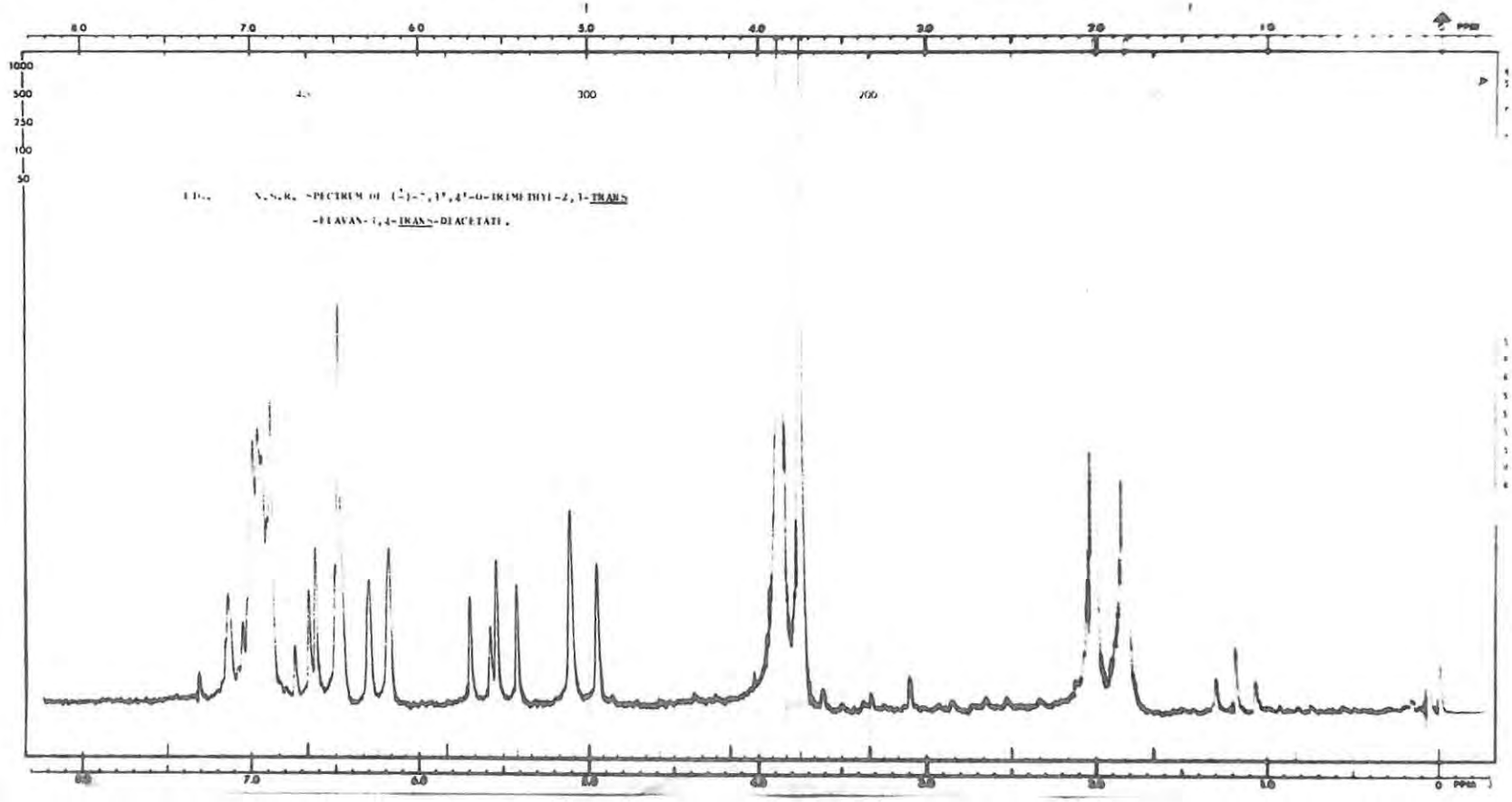


FIG. 19. N.M.R. SPECTRUM OF (±)-7,3',4'-O-TRIMETHYL-2,3-TRANS-FLAVAN-3,4-CIS-DIACETATE.

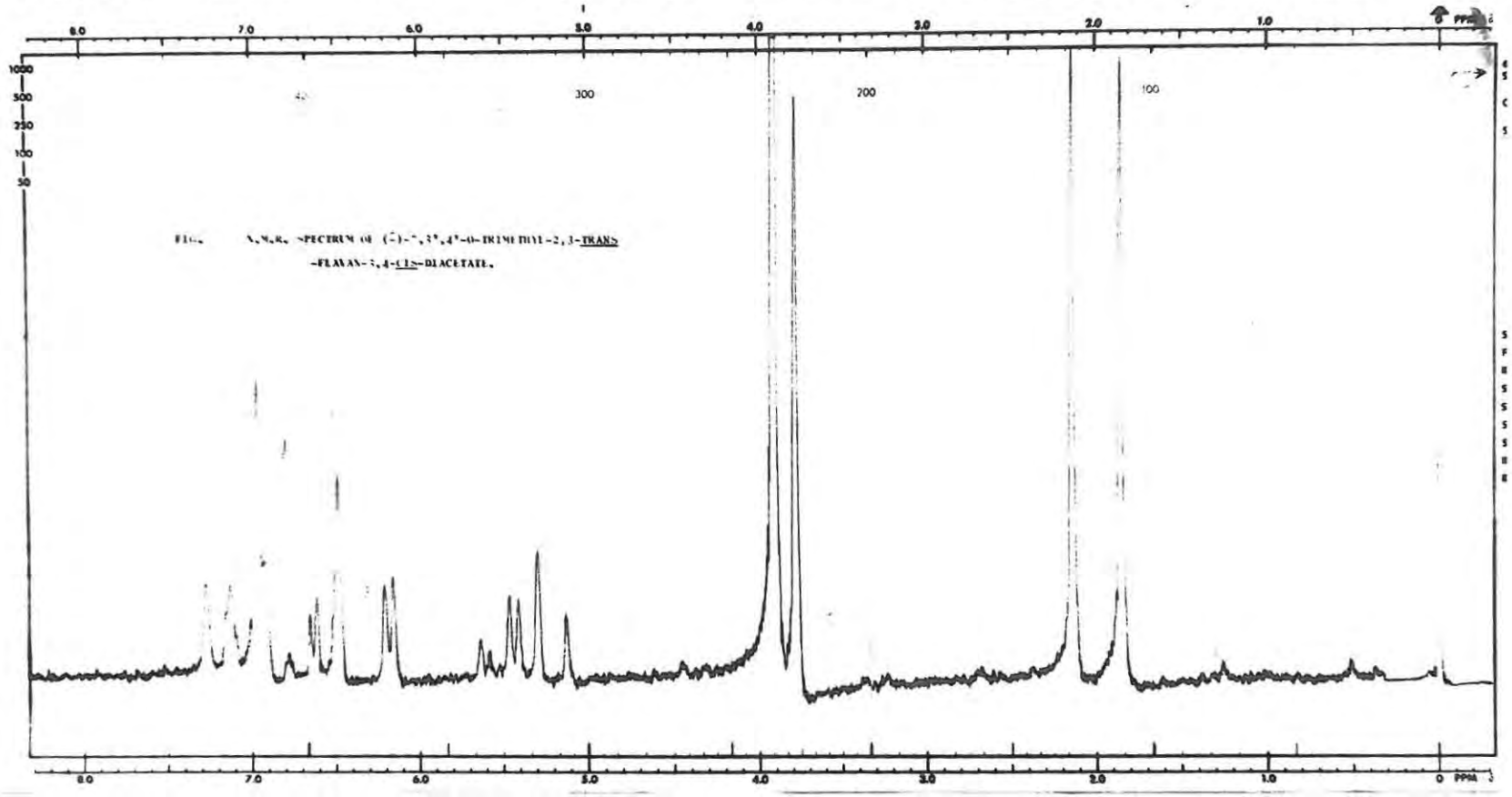


FIG. 20. N.M.R. SPECTRUM OF (±)-7,3',4'-O-TRIMETHYL-2,3-CIS-FLAVAN-3,4-CIS-DIACETATE

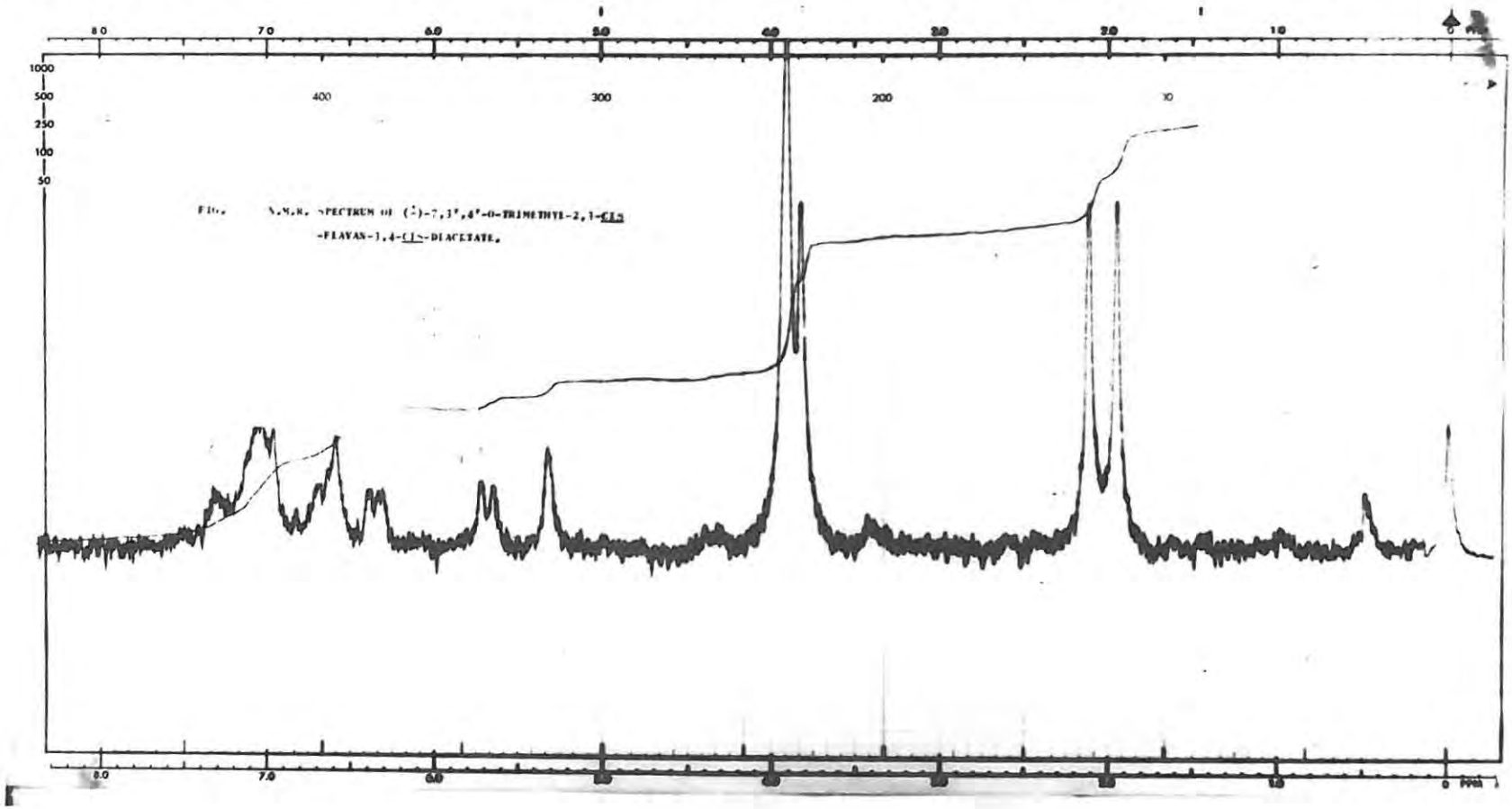


FIG.21. N.M.R. SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL-2,3-TRANS-FLAVAN-3,4-TRANS-DIACETATE

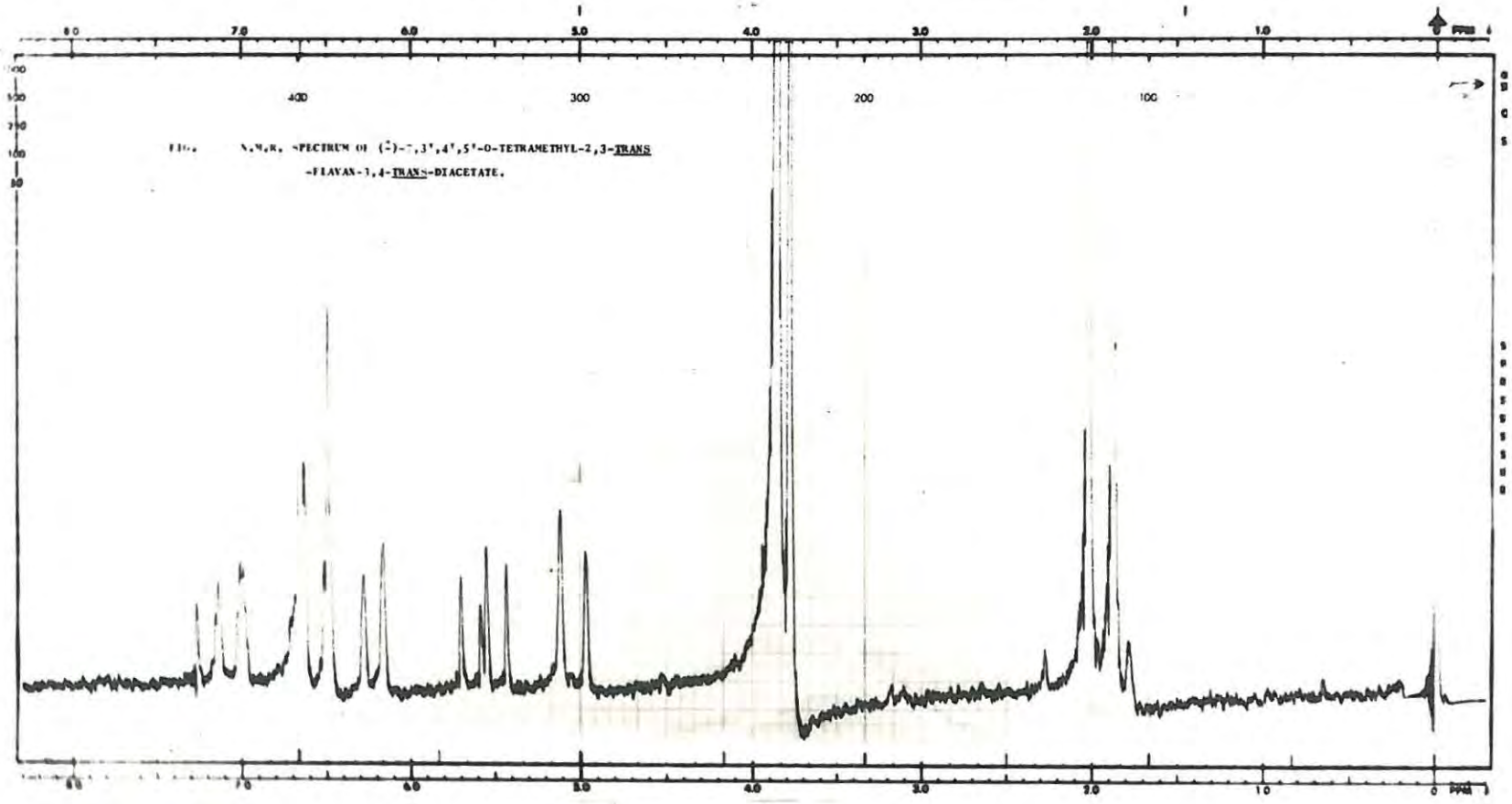


FIG. 22. N.M.R. SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL-2,3-TRANS-FLAVAN-3,4-CIS-DIACETATE

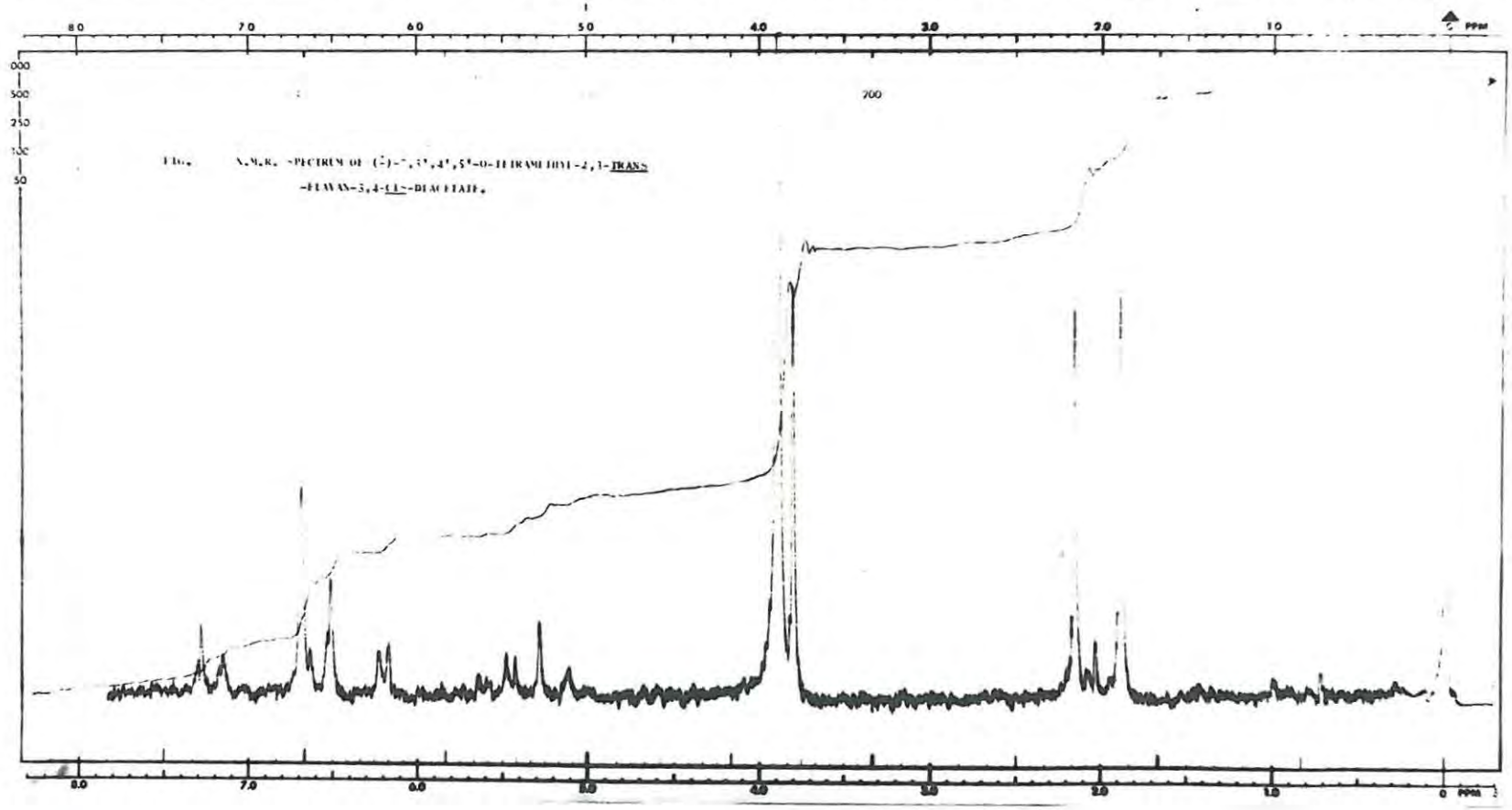


FIG. 23. N.M.R. SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL-2,3-CIS-FLAVAN-3,4-CIS-DIACETATE

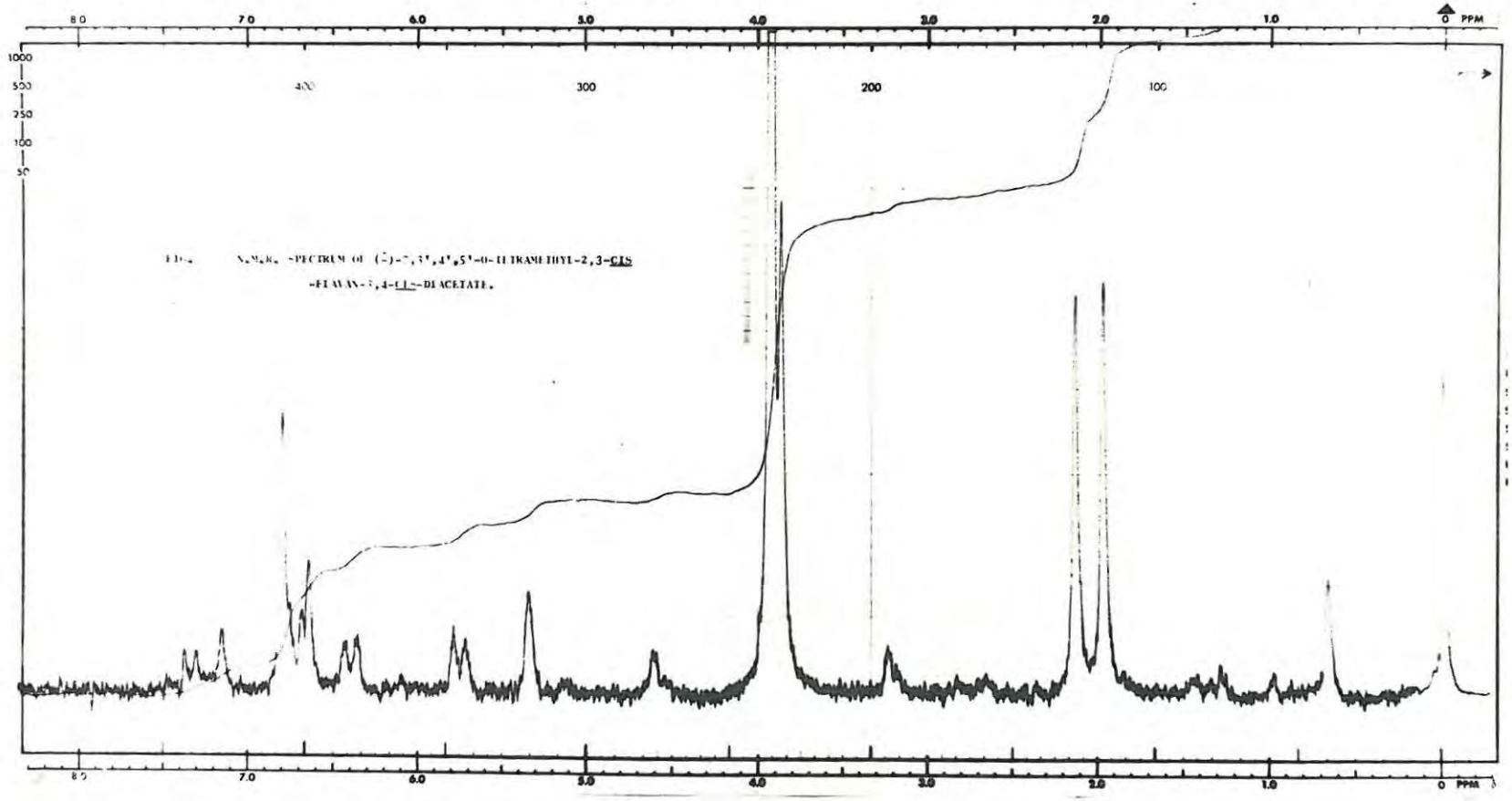
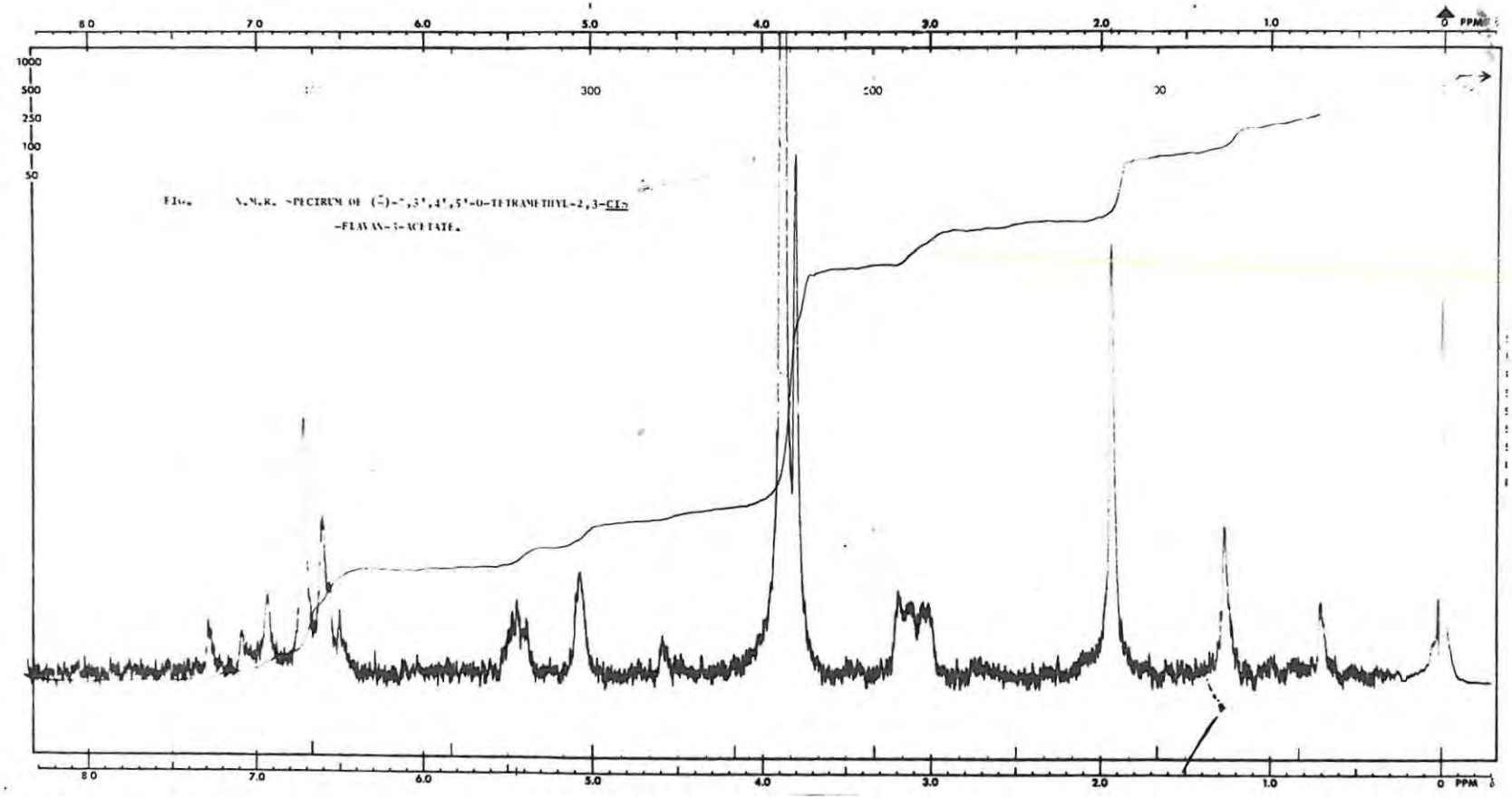


FIG. 24. N.M.R. SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL-2,3-CIS-FLAVAN-3-ACETATE



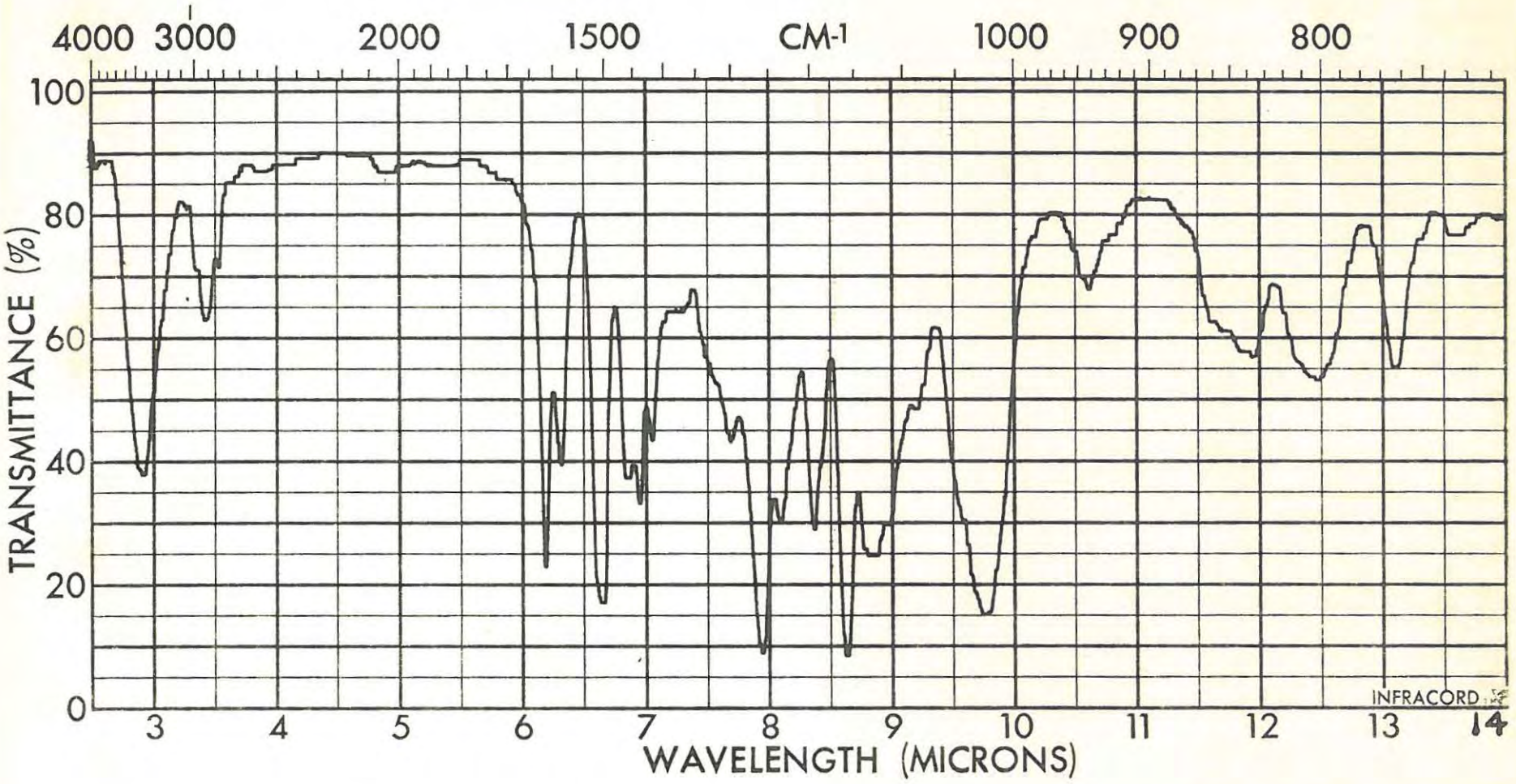


FIG. 25. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4',-O-TRIMETHYL-2,3-TRANS -FLAVAN-3,4-TRANS-DIOL.

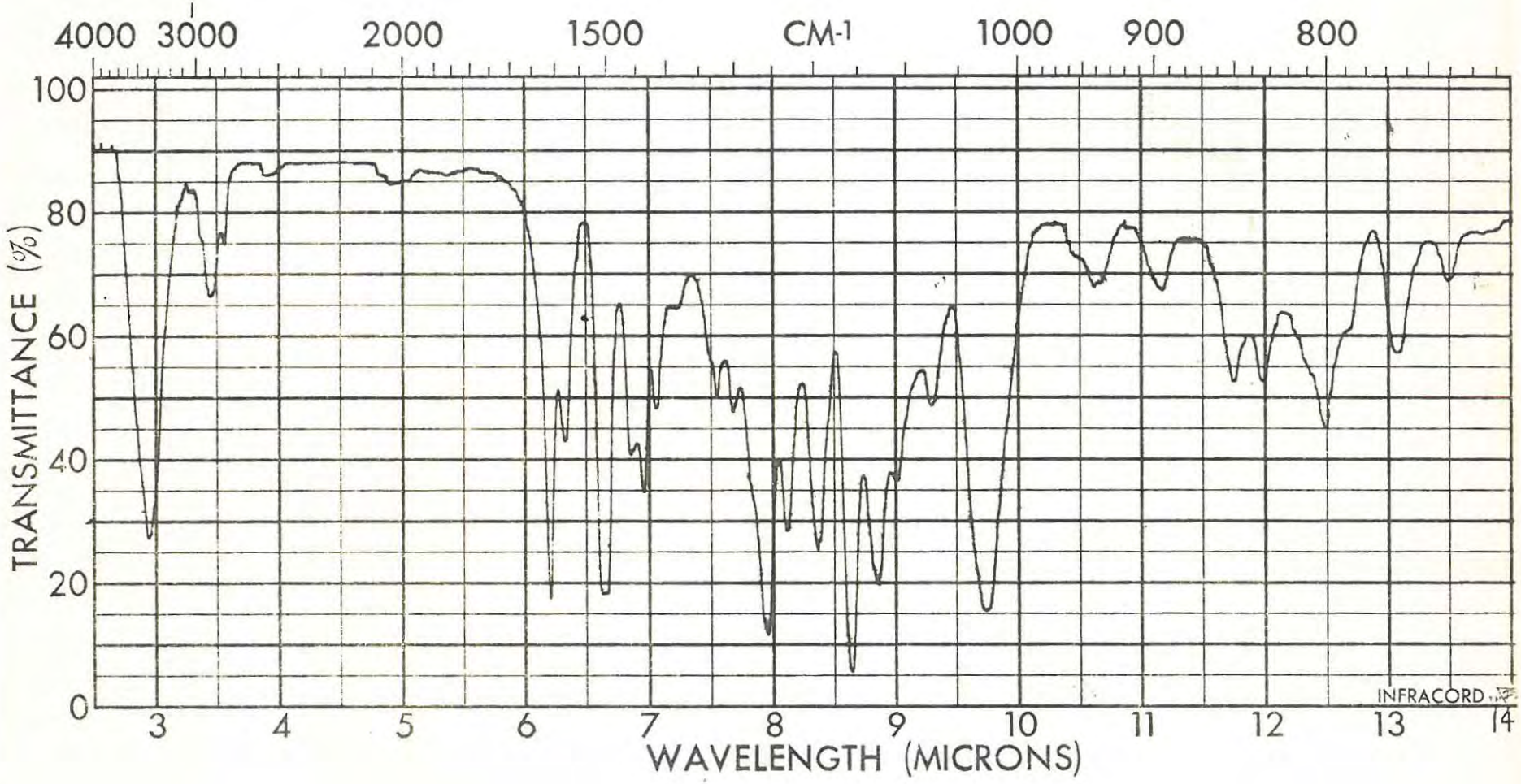


FIG. 26. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4'-O-TRIMETHYL-2,3-TRANS FLAVAN-3,4-CIS-DIOL.

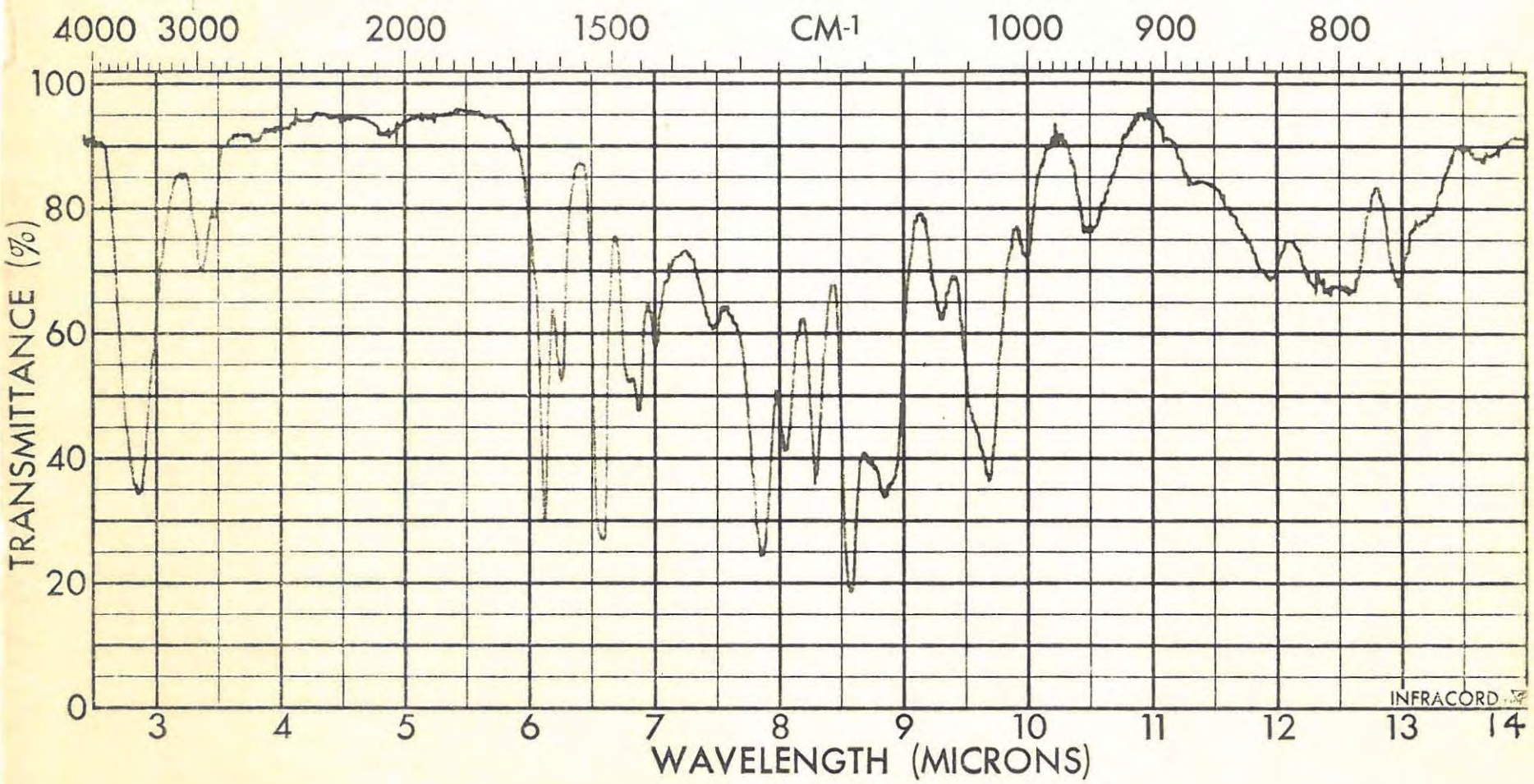


FIG. 27. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4'-O-TRIMETHYL-2,3-CIS FLAVAN-3,4-CIS-DIOL.

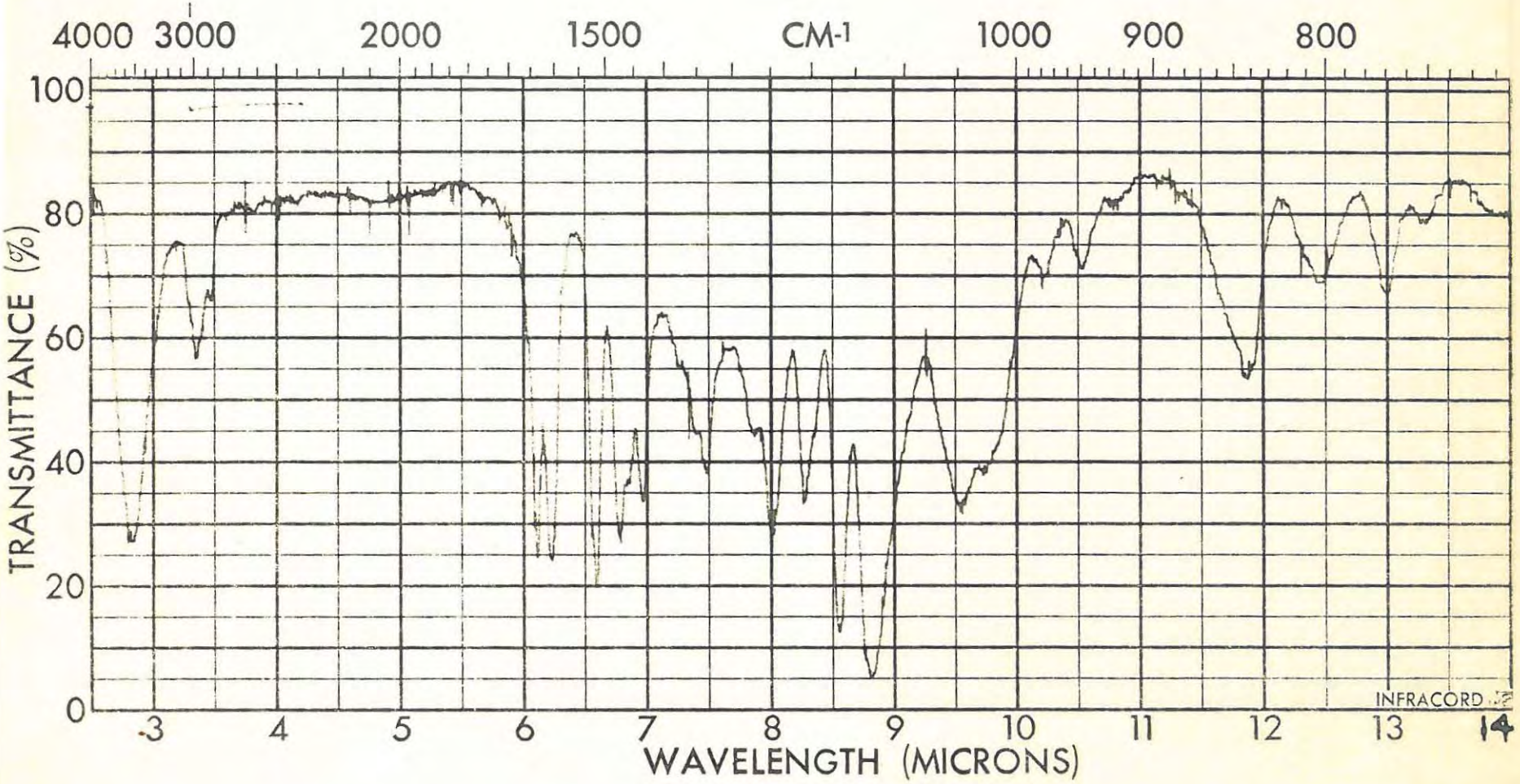


FIG. 28. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL -2,3-TRANS-FLAVAN-3,4-TRANS-DIOL.

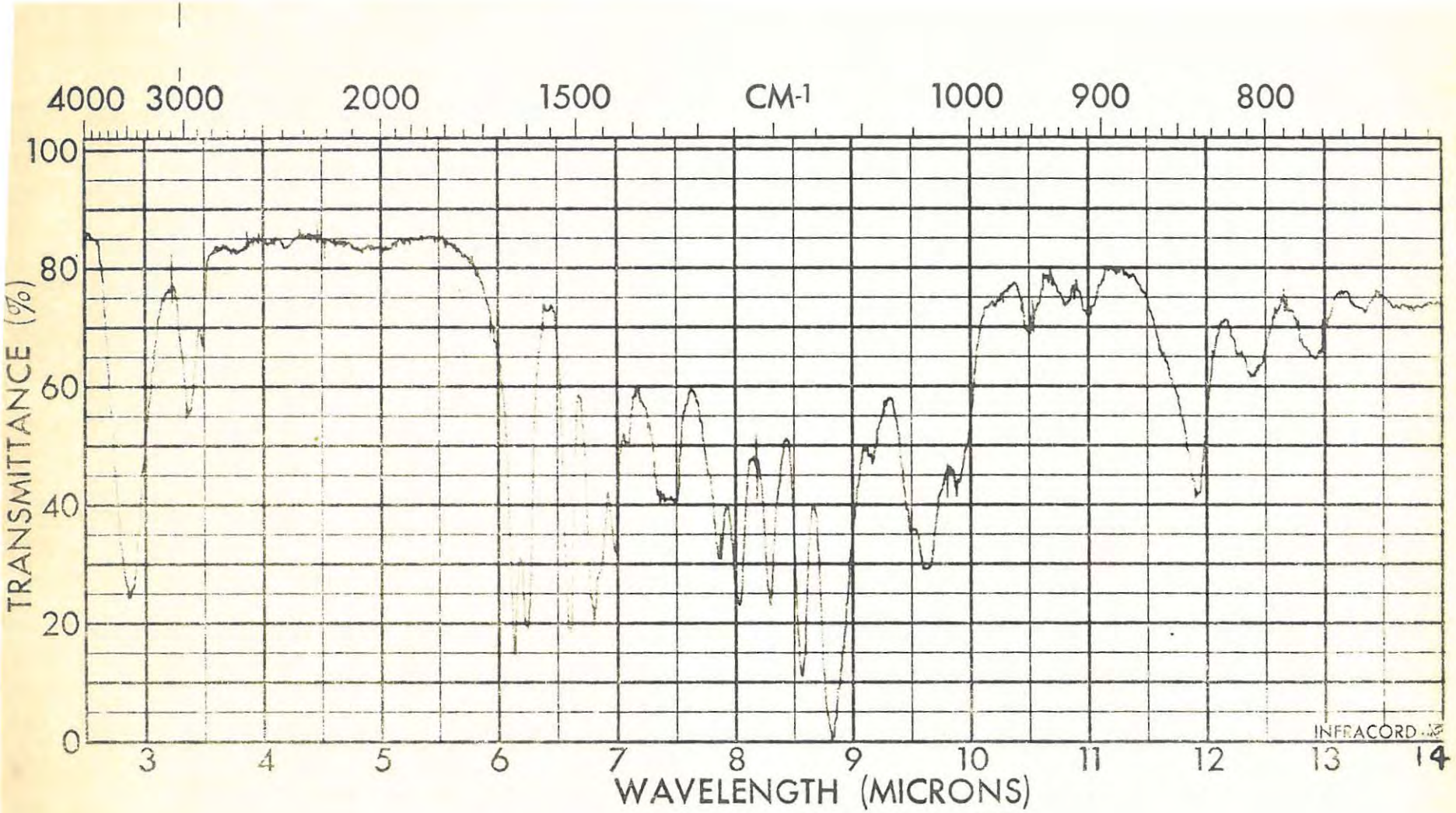


FIG. 29. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4',5'-O-TETRAMETHYL -2,3-TRANS-FLAVAN-3,4-CIS-DIOL.

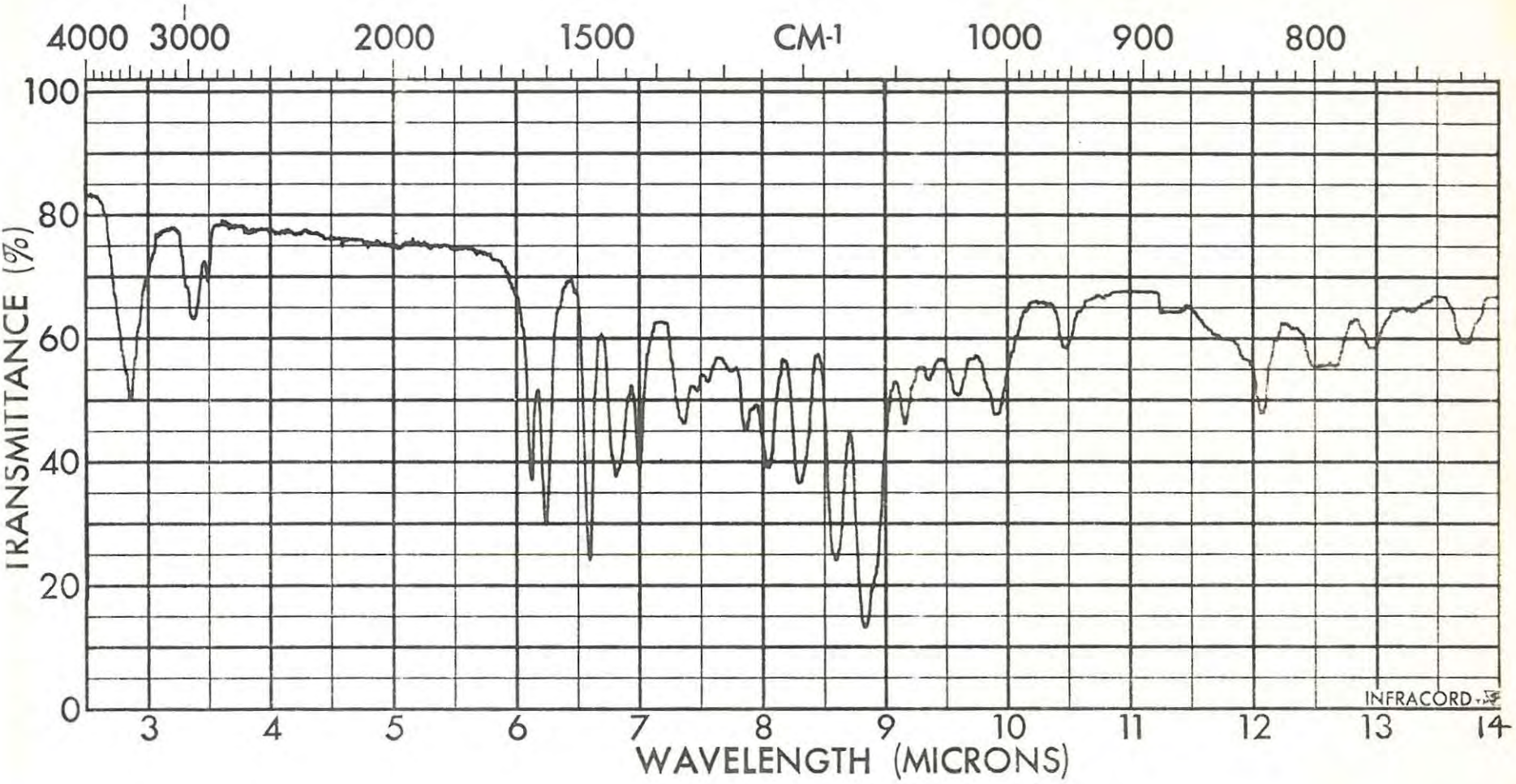


FIG. 30. INFRARED ABSORPTION SPECTRUM OF (±)-7,3',4',5-O-TETRAMETHYL -2,3-CIS-FLAVAN-3,4-CIS-DIOL

SIMPLE METHODS FOR DETERMINING THE CONFIGURATION OF THE DIOL GROUPING IN METHYLATED FLAVAN-3,4-DIOLS.

Two methods involving the use of borate-impregnated paper and standard ionophoresis apparatus were developed. These were applied successfully to the study of the configuration of flavan-3,4-diols.

Source of Compounds. Isomeric 2,3-trans-3,4-cis-, 2,3-trans-3,4-trans- and 2,3-cis-3,4-cis-flavandiol derivatives of leuco-fisetinidin and leuco-robinetinidin were obtained by the various reduction methods previously described. For this examination two additional flavandiols were isolated from natural sources.

(-)-7,8,3',4'-O-Tetramethyl-2,3-cis-flavan-3,4-cis-diol. Melacacidin, (King and Bottomley [40]), was obtained by successive separation of the cold methanol extractives of the heartwood of Acacia melanoxylon on preparative sheets of Whatman no. 3 paper with 2% (v/v) acetic acid and then water-saturated butan-2-ol. The (-)-melacacidin [(-)-7,8,3',4'-tetrahydroxy-2,3-cis-flavan-3,4-cis-diol] (Clark-Lewis and Mortimer [107]) was methylated with diazomethane, separated once more on paper sheets using 2% (v/v) acetic acid and the product then crystallized from ethanol: water (3:1 v/v), m.p. 144-145°. (Lit., [40] 145-146°).

7,8,3',4'-O-Tetramethyl-2,3-cis-flavan-3,4-trans-diol. Isomelacacidin, [7,8,3',4'-tetrahydroxy-2,3-cis-flavan-3,4-trans-diol] (Clark-Lewis and Mortimer [107]) also obtained from A. melanoxylon

during the above separations, was methylated with diazomethane. The isomelacacidin was shown to be pure by chromatography in three solvent systems, 2% (v/v) acetic acid, water-saturated butan-2-ol, and water-saturated phenol, prior to methylation.

Paper chromatography of methylated flavan-3,4-diols.

Sheets of Whatman no. 1 paper 11 in. x 18 in. were impregnated with borate by soaking for 30 min. in 0.1M sodium borate solution. The papers were dried in a warm current of air. This method of impregnation is similar to that described by Swain [161].

Chromatograms of all methylated flavan-3,4-diols were run simultaneously on single sheets by upward migration for 10 hr. (24-25 cm. migration) using water-saturated butan-1-ol and water-saturated ethyl acetate as irrigants. The same compounds were also run, for purposes of comparison, in the same solvents on unimpregnated Whatman no. 1 paper. The air dried sheets were sprayed heavily with alcoholic toluene-p-sulphonic acid (Roux [138]) and heated for 5 min. at 100°. Usually it was necessary to repeat the spraying and heating procedure before the compounds were visible as clearly-defined red areas. The R_F values (Table 18) are an average of three runs. Mixtures of isomeric trans-cis and trans-trans methylated flavan-3,4-diols were readily separated on the borate paper.

Paper ionophoresis [173] of methylated flavan-3,4-diols.

The apparatus used was the horizontal open strip type, similar to that described by Grassman and Hannig [162] in which the paper dips at each end into 0.1M sodium borate solution, a saturated atmosphere being maintained by enclosure with a plastic lid. Schleicher and Schull paper no. 2043 (4 cm. x 41 cm.) was soaked in the borate solution, carefully stretched over the supporting frame, and then placed in position with the ends dipping into the borate solution. After equilibration (1-2 hr.) the compound was applied, through a narrow slit in the lid, as a streak at the origin. A potential of 170-175 V., giving a steady current of 5 milliamps (or 0.31 milliamps/cm. width of paper for four strips) was applied for 18 hr. with a Shandon "Vokam" power supply type 2541.

After drying, the strips were sprayed with alcoholic toluene-p-sulphonic acid as before. Sharply-defined red bands were obtained (Table 19). The ionophoretic mobilities are also expressed in relation to the mobility of (+)-7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-cis-diol as unity (Table 19). The mobilities of the compounds examined were reproducible. Mixtures of isomeric trans-cis and trans-trans methylated flavan-3,4-diols were completely resolved by paper ionophoresis run under these conditions.

Table 18. Paper chromatography of isomeric flavan-3,4-diols on 0.1M sodium borate impregnated paper.

	R_F values.			
	Borate Paper		Normal Paper	
	butan-1-ol	ethyl acetate	butan-1-ol	ethyl acetate
7,3',4'-O-Trimethylflavan-3,4-diols				
2,3-cis-3,4-cis (m.p. 149°)	0.73	0.89	0.78	0.90
2,3-trans-3,4-trans (m.p.151°)	0.86	0.95	0.81	0.90
2,3-trans-3,4-cis (m.p. 185°)	0.62	0.82	0.82	0.90
7,3',4',5'-O-Tetramethylflavan-3,4-diols				
2,3-cis-3,4-cis (m.p. 165°)	0.75	0.90	0.84	0.90
2,3-trans-3,4-trans (m.p.229°)	0.87	0.95	0.85	0.90
2,3-trans-3,4-cis (m.p. 167°)	0.68	0.86	0.86	0.90
7,8,3',4'-O-Tetramethylflavan-3,4-diols				
2,3-cis-3,4-cis (m.p. 145°) (melacacidin).	0.64	-	0.79	-
2,3-cis-3,4-trans (amorphous) (isomelacacidin).	0.87	-	0.80	-

Table 19. Paper ionophoresis of isomeric methylated flavan-3,4-diols in 0.1M sodium borate.

	Mobility (cm.)	M*
7,3',4'-O-Trimethylflavan-3,4-diols		
2,3-cis-3,4-cis	+ 1.50	+ 0.48
2,3-trans-3,4-trans	- 1.85	- 0.59
2,3-trans-3,4-cis	+ 3.20	+ 1.02
7,3',4',5'-O-Tetramethylflavan-3,4-diols		
2,3-cis-3,4-cis	+ 1.80	+ 0.57
2,3-trans-3,4-trans	0.00	0.00
2,3-trans-3,4-cis	+ 3.15	+ 1.00
7,8,3',4'-O-Tetramethylflavan-3,4-diols		
2,3-cis-3,4-cis	+ 1.80	+ 0.57
2,3-cis-3,4-trans	- 2.40	- 0.76

* Mobility of compound relative to 7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-cis-diol as unity.

PART III.

SYNTHESIS OF FLAVAN-4β-OLS.

Synthesis of 2',4'-dihydroxychalcone, 7-hydroxyflavanone and 7-hydroxyflavan-4-ol.

2',4'-Dihydroxychalcone. Recrystallized resacetophenone m.p. 147° (5 g.) and freshly distilled benzaldehyde (3.7 ml.) were dissolved in alcohol (15 ml.). Nitrogen was bubbled through the solution and it was gently warmed. Potassium hydroxide (20 g. in 30 ml. water) was added and the solution refluxed for 1½ hr. The orange-yellow solution was cooled in ice and acidified with dilute hydrochloric acid. A gummy yellow precipitate formed which did not harden on standing. The solution was extracted with ether, concentrated to dryness and the residue, in benzene, treated with charcoal. Yellow needles, m.p. 146-147° (1.6 g., 20%) separated from benzene.

This chalcone has been prepared by Saiyad, Nadkarni and Wheeler [164], (m.p. 150°), Mittal [165], (m.p. 150°) and in low yield by Ellison [166], (m.p. 133°).

Better yields of chalcone were obtained by using the acid condensation method of Goel, Jain and Seshadri [167]. Resacetophenone (12 g.) was dissolved in pyridine (75 ml.) and benzoyl chloride (24 ml.) was added. After leaving overnight the solution was poured into cold water to yield dibenzoyl resacetophenone (285 g.), m.p. 81°.

The dibenzoate (14 g.) was dissolved in dry ethyl acetate (200 ml.) and dry hydrogen chloride gas was bubbled through the solution at 0° for 3 hr. After leaving the solution at 0° for 36 hr., it was neutralized with dilute sodium bicarbonate and concentrated to a small volume (20 ml.). Ethanol (50 ml.) was added and the solution refluxed with potassium hydroxide (20 g. in 300 ml.) for 3 hr. The solution was cooled in ice, acidified with dilute hydrochloric acid, and the resulting gummy precipitate separated off. It was dissolved in alcohol-benzene, (1:1, by vol.) and treated with charcoal, whereupon it crystallized in yellow needles from benzene, m.p. 147° (5.24 g., 55%). Seshadri and co-workers [167] cite 147-148°.

7-Hydroxyflavanone. 2',4'-Dihydroxychalcone (600 mg.) was dissolved in ethanol (20 ml.) and 1% aqueous sodium acetate [168] (43 ml.). The solution was refluxed for 7½ hr., then left overnight at room temperature when buff plates m.p. 176-185° (453 mg., 75%) separated out. These were recrystallized from ethanol-water (charcoal) to give pale buff plates m.p. 190-191° (300 mg.). Wheeler and co-workers [164] quote m.p. 189-190°.

7-Hydroxyflavan-4β-ol. 7-Hydroxyflavanone (440 mg.) in methanol (50 ml.), was hydrogenated over platinum oxide (225 mg.). A total of 94 mls. hydrogen (about twice the theoretical quantity) was absorbed. 7-Hydroxyflavan-4β-ol (285 mg.) was obtained as an amorphous white powder (sinters 42-46°) from methanol-water. (Found: C, 72.4; H, 6.8. $C_{15}H_{14}O_3 \cdot \frac{1}{2} H_2O$ req: C, 71.7; H, 6.0%).

4,7-Diacetoxyflavan. 7-Hydroxyflavan-4β-ol (50 mg.) was acetylated with acetic anhydride-pyridine. Clusters of slender needles (26 mg.), m.p. 128-130°, were obtained from alcohol. (Found: C, 69.9; H, 5.8; $CO.CH_3$, 26.5. $C_{19}H_{18}O_5$ req: C, 69.9; H, 5.5; $CO.CH_3$, 26.4%). Details of n.m.r.spectrum of this compound are shown in Table 22 and Fig. 31.

7-O-Methylflavan-4β-ol. This derivative was isolated as a low-melting amorphous solid which could not be crystallized.

4,7-Dibenzoyloxyflavan. 7-Hydroxyflavan-4β-ol (50 mg.) was dissolved in a minimum of pyridine (0.2 ml.) and cooled to 0°. Benzoyl chloride (0.15 ml.) was added, and the solution left overnight at room temperature. It was poured onto crushed ice when an oily solid which hardened rapidly separated. Heavy white prisms (45 mg.), m.p. 151-152°, were obtained from alcohol. (Found: C, 76.9; H, 5.2. $C_{29}H_{22}O_5$ req: C, 77.3; H, 4.9%).

Synthesis of 2'4-dihydroxychalcone, 4'-hydroxyflavanone and 4'-hydroxyflavan-4β-ol.

2',4-Dihydroxychalcone. A mixture of p-hydroxybenzaldehyde (8.6 g.) and o-hydroxyacetophenone (9 ml.) in ethanol (20 ml.) was treated at 0° with aqueous potassium hydroxide (50 g. in 75 ml. water) in an atmosphere of nitrogen. The solution was refluxed for 1½ hr. and kept at room temperature overnight out of contact with air. Water (100 ml.) was added and the solution cooled to 0°. On acidification with hydrochloric acid a yellow precipitate (12 g., 75%) formed which crystallized

from benzene in bright yellow needles m.p. 159-160°. Geissman and Clinton [169] cite a m.p. of 162° for this compound.

4'-Hydroxyflavanone. 2',4-Dihydroxychalcone (1 g.) dissolved in ethanol (20 ml.) and 1% aqueous sodium acetate (36 ml.) was refluxed for 7½ hr. After keeping overnight at room temperature, long yellow needles (922 mg., 92%) were filtered off and recrystallized from ethanol. White needles, m.p. 186°, were obtained. Geissman and Clinton [169] found m.p. 186-187° and Hattori [170] 186°.

4'-Hydroxyflavan-4β-ol. 4'-Hydroxyflavanone (500 mg.) in methanol (50 ml.) over platinum oxide (250 mg.) absorbed 91 ml. hydrogen. From the methanolic solution 4'-hydroxyflavan-4-ol (254 mg., 51%) was readily obtained as white needles, m.p. 181°. (Found: C, 73.8; H, 5.9; $C_{15}H_{14}O_3$ req: C, 74.4; H, 5.8%).

4'-O-Methylflavan-4β-ol. 4'-Hydroxyflavan-4-ol (50 mg.) in methanol (50 mg.) was treated at 0° with diazomethane to give the methyl ether (34 mg.) m.p. 150-151° (alcohol). (Found: C, 74.3; H, 6.5; OCH_3, 12.1. Calculated for: $C_{16}H_{16}O_3$: C, 75.0; H, 6.3; OCH_3, 12.1%). Wheeler and co-workers [57] cite m.p. 150-151° and Bokadia, Brown and Cummings [125] give m.p. 152-153°.

4,4'-Diacetoxyflavan. 4'-Hydroxyflavan-4-ol (30 mg.) on acetylation with acetic anhydride-pyridine gave the diacetate (39 mg.) as white needles, m.p. 117° from ethanol. (Found: C, 70.0; H, 5.7. $CO.CH_3$, 26.6.

$C_{19}H_{18}O_5$ req: C, 69.9; H, 5.5; $CO.CH_3$, 26.4%. Details of the n.m.r. spectrum of this compound are shown in Table 22 and Fig. 32.

Synthesis of 2',4',4-trihydroxychalcone, 7,4'-dihydroxyflavanone and 7,4'-dihydroxyflavan-4β-ol.

2',4',4-Trihydroxychalcone. Resacetophenone (6.25 g.) and p-hydroxybenzaldehyde (5 g.) in ethanol (12 ml.) were treated at 0° with potassium hydroxide (50 g. in 35 ml. water) in a nitrogen atmosphere. The mixture was refluxed for 45 min., kept at room temperature overnight, diluted with water (100 ml.) and cooled in ice. Acidification with dilute hydrochloric acid yielded an orange precipitate (5.5 g., 52%) which, when crystallized from alcohol-water, gave orange-red needles, m.p. 200-202°. Geissman and Clinton [169] quote 202-204° as do Nadkarni and Wheeler[171]. Roux and De Bruyn [117] cite 198-202°.

7,4'-Dihydroxyflavanone. 2',4',4-Trihydroxychalcone (940 mg.) dissolved in ethanol (30 ml.) and 1% aqueous sodium acetate (95 ml.), was refluxed for 1½ hr. The solution was kept overnight at room temperature, then extracted with an ethyl acetate-ether mixture (1:3, by vol.). The dry extract (calcium chloride) was concentrated to dryness under vacuum and the residue dissolved in an alcohol-water mixture. Crystals (582 mg., 62%) formed over 48 hr. These were recrystallized from alcohol-water to give just off-white needles m.p. 204°. The same m.p. is cited by Nadkarni and Wheeler [171] and Roux and De Bruyn [117].

7,4'-Dihydroxyflavan-4β-ol. 7,4'-Dihydroxyflavanone (400 mg.) in methanol (50 ml.) over platinum oxide (225 mg.) absorbed 73 ml. hydrogen. A white amorphous solid (270 mg.) which could not be induced to crystallize was obtained from the methanolic solution. Two-way chromatograms sprayed with 2,6-dichloroquinone chloroimide [172] showed the presence of two spots R_F (0.43) (purple) and R_F 0.64 (blue) in 2% (v/v) acetic acid. Toluene-p-sulphonic acid showed only a single typically deep mauve spot at R_F 0.43. Accordingly, the hydrogenation mixture in ethanol (40 ml.) was applied to 8 sheets Whatman no. 3 paper and developed with 2% (v/v) acetic acid by upward migration. The lower R_F band was cut out and eluted but the residue from the combined eluates failed to crystallize. This product reddened rapidly and no satisfactory C, H analysis was obtained. Although the red material (aged 1 month) still gave a typical deep mauve colour with toluene-p-sulphonic acid, two-way chromatography revealed that polymerisation had occurred since the compound no longer migrated as a single, discrete spot. Subsequent examination of the n.m.r. spectrum of the triacetate (also amorphous) showed the elimination of the C_4-hydroxyl function and generally the spectrum showed little similarity with that of the other two flavan-4β-ols, Figs. 31 and 32.

Effect of sunlight on the flavan-4β-ols.

The three flavan-4-ols, all completely white initially, were exposed to sunlight for 20 days with the following result:

4'-Hydroxyflavan-4-ol- Remained white and crystalline.

7-Hydroxyflavan-4-ol - Softened and became orange-red.

7,4'-Dihydroxyflavan-4-ol - Softened and became red rapidly.

Effect of heat.

4'-Hydroxyflavan-4-ol, 7-hydroxyflavan-4-ol and 7,4'-dihydroxyflavan -4-ol were all heated at 110° under vacuum for 2 hr. The latter two compounds became dark red within a short while whereas the 4'-hydroxy compound remained completely white.

Effect of hydrochloric acid.

The effects of this acid on the flavan-4-ols are shown below.

Table 20. Effect of hydrochloric acid on flavan-4 β-ols.

Flavan-4 β-ol (In alcoholic solution)	Addition of 1 drop conc. HCl.	Warming foregoing solution to 80°	Addition of further drop of HCl.
4'-Hydroxyflavan -4-ol	-	Yellow	Red colour developes but fades
7-Hydroxyflavan -4-ol	-	Intense yellow and formation of precipitate	Orange-yellow precipitate
7,4'-Dihydroxyflavan -4-ol	red	Intense purple-red	Intense purple-red

Reaction with spray reagents.

With toluene-p-sulphonic acid [138] and 2,6-dichloroquinone-chloroimide [172] the following colour reactions were obtained on paper chromatograms:

Table 21. Colour reactions of flavan-4β-ols.

Flavan-4-ol.	Toluene-p-sulphonic acid. (Warm to 80°)	2,6-Dichloroquinone-chloroimide
4'-Hydroxy	Yellow	Blue
7-Hydroxy	Orange-yellow	Blue
7,4'-Dihydroxy	Deep mauve	Purple

Nuclear Magnetic Resonance Spectra of Flavan-4β-ols.

(The n.m.r. spectra of the flavan-4-ols were recorded by Dr. K. Pachler, C.S.I.R., Pretoria.)

The acetates of the flavan-4-ols were found to be most suitable for examination by n.m.r. The spectra of the acetates of 4'-hydroxyflavan-4-ol and 7-hydroxyflavan-4-ol are similar, and the coupling constants for the 2- and 4-protons (2,4-cis) very nearly identical. (Table 22. Figs. 31 and 32).

Table 22. Chemical shifts and coupling constants of the protons for 4'-hydroxy- and 7-hydroxyflavan-4-ol acetates.

τ-values (p.p.m.).

Flavan-4-ol	Acetyl Me				
	4-,	7,4'	2H	3aH + 3bH	4H
4'-Hydroxy	7.91	7.72	4.78	7.1 ~ 7.7	3.79
7-Hydroxy	7.91	7.72	4.78	7.2 ~ 7.7	3.82

Coupling constants (c.p.s.).

	$J_{2,3a}$ + $J_{2,3b}$ *	$J_{3a,4}$ + $J_{3b,4}$ +
4'-Hydroxy	13.1	16.5
7-Hydroxy	13.4	16.5

*- from 2-proton

+- from 4-proton

FIG. 31. N.M.R. SPECTRUM OF 7-HYDROXYFLAVAN-4β-OL DIACETATE

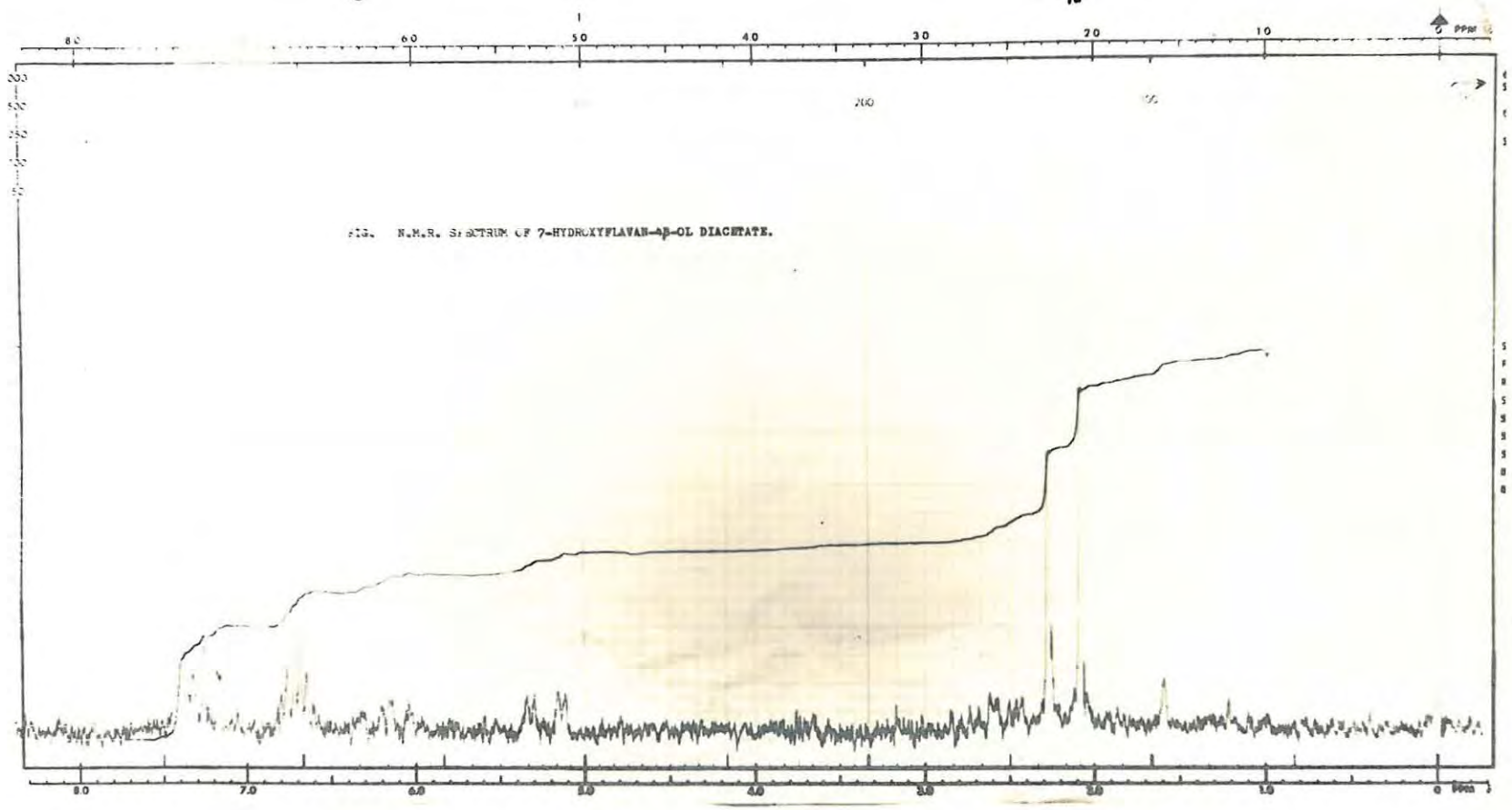
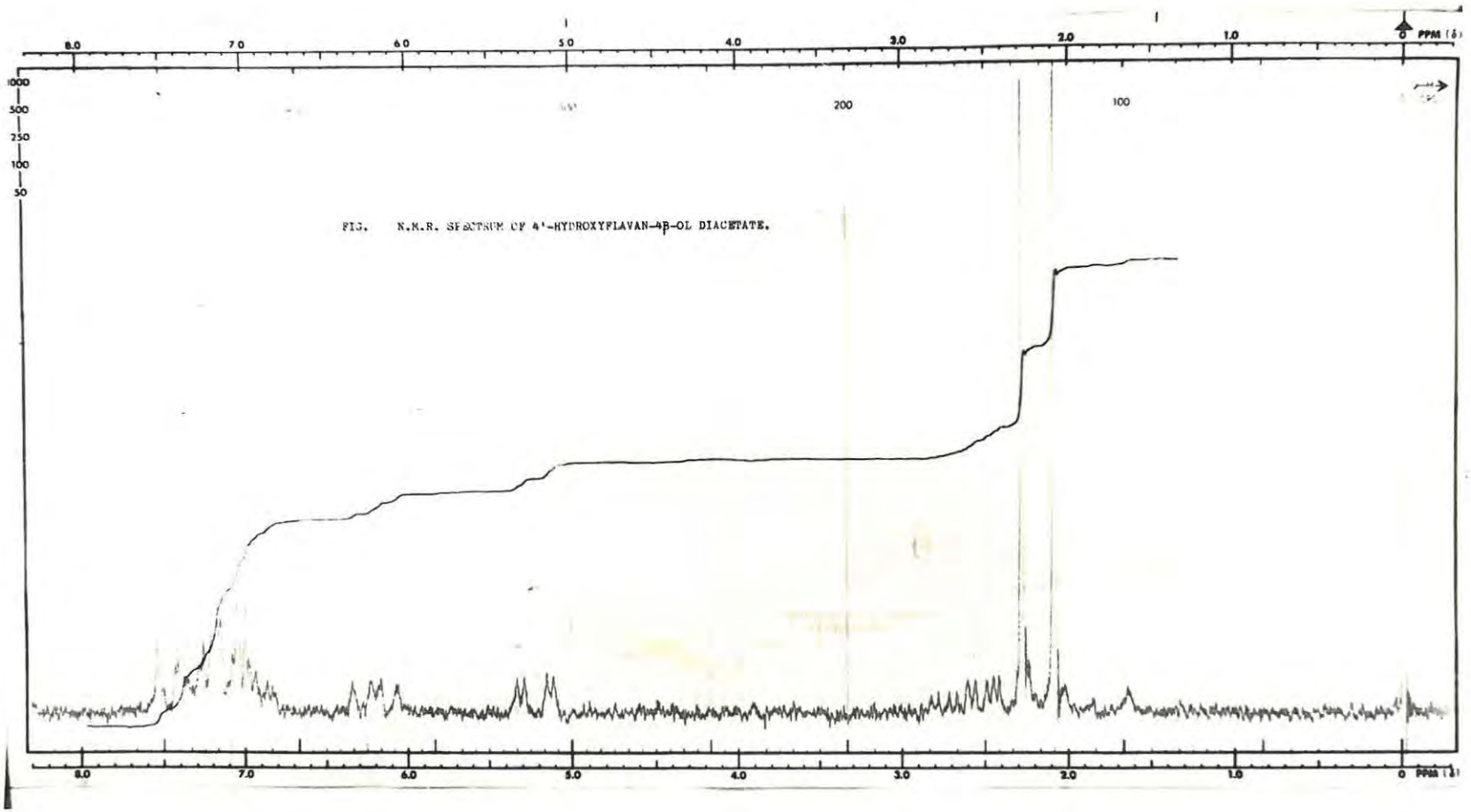


FIG. 32. N.M.R. SPECTRUM OF 4'-HYDROXYFLAVAN-4β-OL DIACETATE



DISCUSSION.

Isolation and Interrelation of Wattle Bark Components.

Black wattle (Acacia mearnsii) bark extracts are composed mainly of polymeric leuco-robinetinidin and leuco-fisetinidin tannins [138,143,174,175] accompanied by closely-related monomeric flavonoid compounds of which (-)-robinetinidol (7,3',4',5'-tetrahydroxyflavan-3-ol), (+)-catechin (5,7,3',4'-tetrahydroxyflavan-3-ol) and (+)-gallocatechin (5,7,3',4',5'-pentahydroxyflavan-3-ol) have been isolated by Roux and Maihs [133]. In addition the two flavonols, robinetin [176] (7,3',4',5'-tetrahydroxyflavone-3-ol) and fisetin [177] (7,3',4'-trihydroxyflavone-3-ol), have been identified. The presence of other flavonoid components in the bark appeared probable following the isolation of a comprehensive series of robinetinidin and fisetinidin analogues from the heartwoods of black-wattle and locust trees (Robinia pseudacacia) [63,178]. More detailed examination of fresh wattle bark extracts has now led to the isolation of the following additional flavonoid compounds: the resorcinol-pyrogallol analogues (+)-leuco-robinetinidin [(+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol], dihydrorobinetin (7,3',4',5'-tetrahydroxyflavan-3-ol-4-one), and robtein (2',4',3,4,5-pentahydroxychalcone and the resorcinol-catechol analogues (+)-leuco-fisetinidin [(+)-7,3',4'-trihydroxyflavan-3,4-diol], fustin (7,3',4'-trihydroxyflavan-3-ol-4-one), (-)-fisetinidol [(-)-7,3',4'-trihydroxyflavan-3-ol], butein (2',4',3,4-tetrahydroxychalcone) and

butin (7,3',4'-trihydroxyflavan-4-one). Robtin (7,3',4',5'-tetrahydroxyflavan-4-one), the flavanone analogue of the robinetinidin series, was not isolated. However, its occurrence in low concentration in the bark is probable since the chalcone robtein was isolated, and the ready chalcone⇄flavanone interconversion of 5-deoxyflavanones and corresponding chalcones is a well known phenomenon [179,180].

From the bark of immature wattle trees two further flavonoids, the glycosides myricitrin (3,5,7,3',4',5'-hexahydroxyflavone-3-rhamnoside) and quercitrin (3,5,7,3',4'-pentahydroxyflavone-3-rhamnoside), were obtained. These represent the only glycosides isolated hitherto from wattle bark or heartwood extracts.

The low concentration in which the new components occur in wattle bark is illustrated by the fact that they are completely absent on a two-way chromatogram of the fresh bark extract (Fig. 1). (-)-Robinetinidol, (+)-catechin and (+)-gallocatechin, on the other hand, appear as prominent spots with a "background" of high molecular weight tannins. In consequence a number of successive enrichment procedures were employed for their isolation. These included a preliminary large-scale countercurrent-separation, further enrichment through countercurrent-separation in a 160-tube Craig machine using a different solvent system, and subsequent separation by means of preparative paper chromatography employing both adsorptive and partitioning principles. This successive enrichment is illustrated by means of paper chromatograms

(Figs. 2-7) for two of the compounds isolated, fustin and (-)-fisetinidol.

The association in black wattle bark of interrelated compounds, (Table 23) belonging to two major categories, resorcinol-pyrogallol and resorcinol-catechol, parallels the existence of similarly related compounds in the heartwoods of black wattle and Robinia pseudacacia [63,178].

Table 23. Analogues of the main group of flavonoids isolated and identified in wattle bark extract.

Flavonoid type	7,3',4',5'-Tetrahydroxy compound.	7,3',4'-Trihydroxy compound.
Chalcone	Robtein	Butein
Flavanone	—	Butin
Flavonol	+Robinetin[176]	+ Fisetin[177]
Dihydroflavonol	Dihydrorobinetin	Fustin
Flavan-3-ol	+(-)-Robinetinidol[133]	(-)-Fisetinidol
Flavan-3,4-diol	(+)-Leuco-robinetinidin	(+)-Leuco-fisetinidin
Tannins	Polymeric leuco-robinetinidin tannins	Polymeric leuco-fisetinidin tannins

+ Previously identified.

The predominance of the resorcinol A nucleus in this and other Acacia spp.[133] contrasts with many fruit and forest trees, for example Prunus spp. (cf. Hergert [181]) and Pinus spp. (cf. Erdtman [182]) where

the flavonoid components are predominantly patterned on the commonly-occurring phloroglucinol A nucleus.

The pattern of phenolic hydroxylation of myricitrin and quercitrin agrees with the two known minor flavonoid groups (Table 24) present in wattle extract.

Table 24. Analogues of minor groups of flavonoids isolated and identified from black wattle bark extract.

Flavonoid type	5,7,3',4',5'-Pentahydroxy compound.	5,7,3',4'-Tetrahydroxy compound.
Flavan-3-ol	(+)-Gallocatechin[133]	(+)-Catechin[133]
Flavonol-3-rhamnoside	Myricitrin	Quercitrin
Tannin	Leuco-delphinidin[183]	—

+ Previously identified.

Apart from the interrelation of these components of wattle extract based on their patterns of phenolic hydroxylation, and their ability to be converted from one to the other, there exists a stereochemical interrelation between them. Weinges [25] has shown that (+)-dihydrorobinetin, (+)-leuco-robinetinidin and (-)-robinetinidol all have the same absolute configuration at C_2 and C_3 as (+)-catechin. (+)-Fustin, (+)-leuco-fisetinidin and (-)-fisetinidol similarly belong to the same (+)-catechin stereochemical series [26,28]. In the present

work those flavonoids, which have been isolated in sufficiently high yield for more detailed characterization, or which are amenable to examination by paper chromatography, [(+)-leuco-robinetinidin and (+)-leuco-fisetinidin], belong to the same stereoisomeric series as (+)-catechin and their counterparts in wattle and Robinia heartwoods.

Compound F.

The concentration of this compound (visible as a pale spot in Fig. 1) in the fresh bark extract varied, depending on the origin of the material. By employing the enrichment procedures outlined above, a small quantity of F, shown to be pure by chromatography, was obtained.

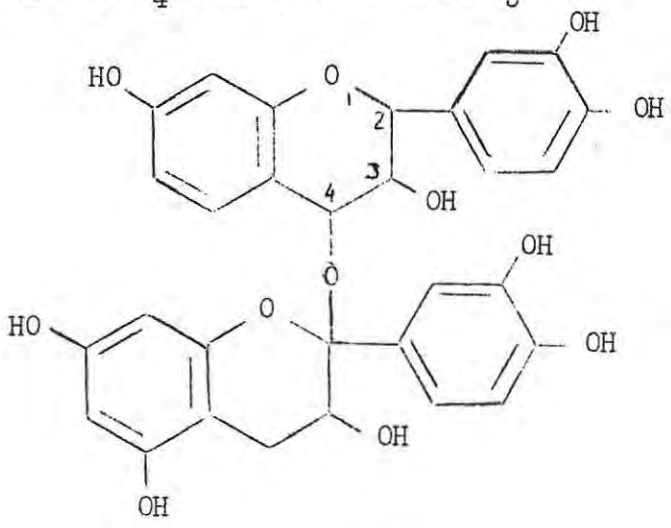
On two-way chromatograms F had R_F, 0.48 (water-saturated butan-2-ol) and 0.37 [2% (v/v) acetic acid]. This is in the same R_F region as (+)-catechin and (-)-robinetinidol are found. From its colour reactions with ferric alum (green) and ammoniacal silver nitrate (intense grey-black) the compound has a catechol B nucleus. Evidence from spraying with bis-diazotized benzidine (yellow) suggests that this is coupled with a resorcinol A nucleus, but the colour obtained with vanillin-toluene-p-sulphonic acid (bright red) also indicates a phloroglucinol A nucleus.

Micro-fusion of F yields degradation products consistent with a phloroglucinol-resorcinol A ring and a catechol B ring. F gives a positive leuco-anthocyanidin reaction [142] and paper chromatography of

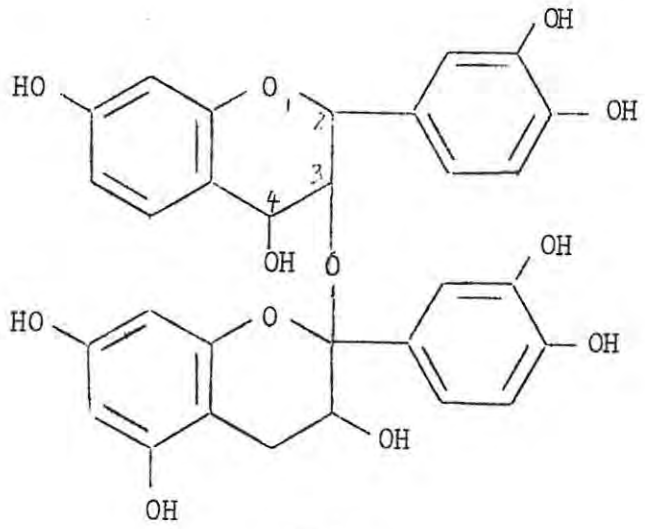
the reaction mixture in a selective solvent system [143] indicates that fisetinidin chloride (3,7,3',4'-tetrahydroxyflavylium chloride) is formed, although not in high yield. Gentle refluxing of F with dilute ethanolic hydrochloric acid yields another degradation product, which from its colour reactions on paper chromatograms (ochre with bis-diazotized benzidine, green with ferric alum and black with ammoniacal silver nitrate), as well as its position of migration in two solvent systems, is identical with (+)-catechin.

The infrared spectrum of F showed no carbonyl absorption.

Limitations of material prevented a more detailed examination of the structure of F. On the present evidence a "bis-flavonoid" structure I or II, with an acetal-type of linkage between the two halves of the molecule, is tentatively suggested for F. Models indicate that the link in the leuco-fisetinidin half of the molecule is sterically as possible at C_4(I) as it is through C_3(II).



(I)



(II)

The analytical values obtained for F and its methyl ether were in good agreement with the theoretical values.

Forsyth and Roberts [120] and Freudenberg and Weinges [121] have considered similar structures for dimeric compounds isolated from natural sources.

Stereochemistry of Wattle Tannin Precursors: (+)-Leuco-Robinetinidin and (+)-Leuco-Fisetinidin (Mollisacacidin).

(+)-Mollisacacidin, (7,3',4'-trihydroxyflavan-3,4-diol) first isolated from the heartwood of Acacia mearnsii by Keppler[65], was assigned the 3,4-cis-configuration mainly on the grounds of its methyl ether being capable of forming an acidic borate complex cf. Böeseken [184]. Subsequently Clark-Lewis and Roux [36], Roux and Paulus [63] and Clark-Lewis[110] proposed a tentative 3,4-cis-configuration for both (+)-mollisacacidin

and (+)-leuco-robinetinidin [25]. This assignment was based on the high yield of isopropylidene derivative (60-65%) obtained from the methyl ethers of the two flavan-3,4-diols, cf. Roux and Paulus [38]. The alternative 3,4-trans-configuration could not, however, be excluded entirely since Angyal and Macdonald [39] had shown in one instance that bridging of a trans-diol was possible by this method.

In this work the relative configurations at C_3 and C_4 of (+)-leuco-robinetinidin and (+)-leuco-fisetinidin were established by a) synthesis of a number of racemic diastereoisomers, b) through a comparative study of their oxidation rates and c) through a study of the n.m.r. spectra of the isomeric diols. Important conclusions on the validity of stereochemical deductions, based on the ability of flavandiols to form cyclic derivatives, resulted from these studies.

Stereospecific syntheses of racemic isomeric flavan-3,4-diols.

Catalytic reduction of the dihydroflavonols (±)-fustin and (±)-dihydrorobinetin in methanolic solution (cf. Freudenberg and Roux[61]) was stereospecific and gave only 2,3-trans-3,4-trans-flavandiols. Catalytic reduction of (±)-fustin in glacial acetic acid and also its reduction with potassium borohydride similarly yielded only the trans-trans-diol. On the other hand, reduction of the methyl ethers of (±)-fustin and (±)-dihydrorobinetin with the mixed reagent, lithium aluminium hydride-aluminium chloride (Bokadia et al.[34]), afforded the 2,3-trans-3,4-cis-diol in each instance; as a major product from (±)-O-tri-

methylfustin, but accompanied by a slight excess of the trans-trans-isomer from (±)-O-tetramethyldihydrorobinetin. By employing the oxime-amine method of Bognar and co-workers [80], the same trans-cis-diol as above was prepared from (±)-O-trimethylfustin. Apart from being more laborious, this method also gave a very low yield of diol.

The (±)-7,3',4'-O-trimethyl-2,3-trans-flavan-3,4-cis-diol [76], m.p. 185°, was characterized through preparation of its diacetate-and dibenzoate derivatives. This same diol, but of lower m.p. (180-181°), was recently obtained by Brown and MacBride [185]. Previously Chandorkar and Kulkarni [75] had synthesized a similar compound, m.p. 172°, to which they assigned the trans-trans-configuration.

(±)-7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-cis-diol, m.p. 167°, a new compound, was separated with difficulty from the isomeric trans-trans-diol. It was characterized through preparation of a diacetate.

Oxidation rates of isomeric flavan-3,4-diols.

The configuration at C_3 and C_4 of the isomeric flavan-3,4-diol racemates was determined by examining the velocity constants of the periodate and lead tetra-acetate oxidations [34] of the methyl ethers. Periodic acid had been used qualitatively by Keppler [65] for establishing the presence of an α-glycol grouping in (+)-mollisacacidin but this is the first quantitative application of this reagent to flavan-3,4-diols.

The use of these reagents for differentiating between cis- and trans-1,2-diols is well established, and both have been used extensively cf. Criegee and co-workers [33] and Duke and Bulgrin [186].

In the present work the reaction rate, as expressed by the second order velocity constant, was found to be six to nine times faster for the 3,4-cis- than for the 3,4-trans-diols (Table 13). (+)-Mollisacacidin and (+)-leuco-robinetinidin methyl ethers had oxidation rates similar to those of the corresponding 2,3-trans-3,4-trans racemates.

The above results are parallel to those of Bokadia and co-workers [34] on the tetra-acetate oxidation of 2,3-trans-flavan-3,4-diols with a low degree of substitution. While this thesis was in preparation, Clark-Lewis and Williams [187] published oxidation rates of isomeric methylated leuco-fisetinidins. These results are in essential agreement with those presented in this thesis, and published earlier as a preliminary note [76].

Nuclear magnetic resonance spectra of flavan-3,4-diols.

The assignment of a trans-configuration of substituents at C_3 and C_4 for the flavandiols was confirmed by n.m.r. spectroscopy. Comparisons of chemical shifts (Table 14) and coupling constants (Table 16) of the isomeric 3,4-diacetates (also 3,4-dibenzoates) were employed for this purpose. The derivatives of (+)-mollisacacidin and (+)-leuco-robinetinidin show the identical τ values and similar coupling

constants ($J_{2,3}$ = 8.9 - 9.1; $J_{3,4}$ = 6.9 - 7.3 c.p.s.) for the 2-, 3- and 4-protons as the corresponding 2,3-trans-3,4-trans racemates. On the other hand 2,3-trans-3,4-cis racemates show marked differences in chemical shifts (Table 14) and also have different coupling constants ($J_{2,3}$ = 10.0 - 10.3; $J_{3,4}$ = 2.9 - 3.4 c.p.s.) (Table 16).

On the assumption that the heterocyclic ring in these flavan-3,4-diols assumes the half-chair conformation and a predominantly equatorial arrangement of the C_2-phenyl group, then from the known dependence of vicinal coupling constants on the dihedral angle (Karplus [44]) as applied to flavan-3,4-diacetates (cf. Clark-Lewis and Jackman [24], Clark-Lewis, Jackman and Williams [9]) and-dibenzoates (Corey, Philbin and Wheeler [42]), the above coupling constants correlate unambiguously with the configurational assignments[trans-trans, 2(ax)H, 3(ax)H, 4(ax)H and trans-cis, 2(ax)H, 3(ax)H, 4(eq)H] already deduced from oxidation methods.

Correlation of stereochemistry with yields of cyclic derivatives.

The yields of isopropylidene derivatives (Table 25) for the two groups of isomeric 3,4-trans- and 3,4-cis-diols are high (55-86%). For the racemic leuco-robinetinidin derivatives the yield from the 3,4-trans-isomer is in fact higher than for the corresponding 3,4-cis-isomer.

Table 25. Comparison of the yields of isopropylidene derivatives of isomeric flavan-3,4-diols.

	% Yield of isopropylidene derivative.
O-Tetramethyl-2,3-trans-flavan -3,4-diols (leuco-robinetinidins)	
(±)-3,4-trans *	86
(+)-3,4-trans +	71
(±)-3,4-cis *	82
O-Trimethyl-2,3-trans-flavan -3,4-diols (leuco-fisetinidins)	
(±)-3,4-trans *	55
(+)-3,4-trans +	64
(±)-3,4-cis *	82

+ - Derivatives of natural compounds
* - Synthetic compounds.

For these highly substituted flavan-3,4-diols, ability to furnish an isopropylidene derivative in high yield does not signify the presence of a 3,4-cis-diol grouping as it was found that the two groups of isomeric 3,4-cis- and 3,4-trans-diol racemates all formed 3,4-cis-isopropylidene derivatives. Identical melting points of the respective isopropylidene derivatives (with no depression from mixed m.p.) superimposable infrared absorption spectra and the value of the coupling constants for the 2,3- and 4-protons (Table 16), all confirm the formation of 3,4-cis- derivatives. The values of the coupling constants

are in good agreement with those obtained by Corey et al.[42] for a 3,4-cis-carbonate. Furthermore, acid hydrolysis of the isopropylidene derivative of both the racemic and the optically pure O-trimethyl-2,3-trans-flavan-3,4-trans-diol yielded the racemic 3,4-cis-diol m.p. 185°. (In addition to epimerization, racemization thus appears to have occurred in the optically pure diol). Similar hydrolysis of the same derivative of O-tetramethyl-2,3-trans-flavan-3,4-trans-diol gave a product consisting predominantly of the racemic 3,4-cis isomer.

Cyclic carbonate derivatives of the 3,4-cis and 3,4-trans methylated diols of the leuco-fisetinidin series were prepared in 61% and 11% yield respectively. Although these two derivatives had slightly different melting points their n.m.r. spectra were identical and the coupling constants (Table 16) for the 2,- 3- and 4-protons ($J_{2,3}$ = 9.0 - 9.4; $J_{3,4}$ = 6.0 c.p.s.) are consistent with a 2,3-trans-3,4-cis arrangement cf. Corey, Philbin and Wheeler [42]. Stereochemical deductions, based on the widely different yields of the carbonates above, should thus be regarded with caution.

The conclusions on cyclic derivatives of highly substituted flavandiols, as outlined, are somewhat at variance with the findings of Bokadia et al.[34] who previously found that 3,4-cis-diols generally furnished a high yield of isopropylidene derivative. Also Corey, Philbin and Wheeler [42], from the n.m.r. examination of the cyclic carbonates of both cis- and trans-flavan-3,4-diol, proved the formation

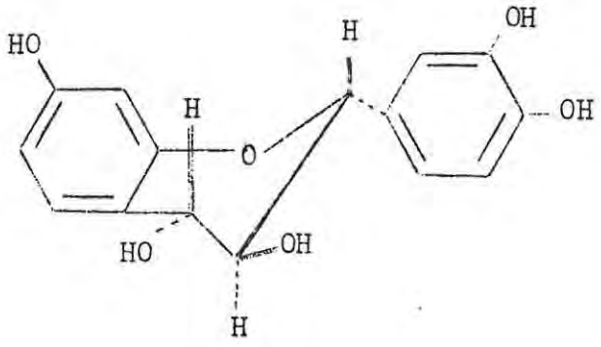
of a cyclic 3,4-trans- derivative. Both groups of workers, however, confined their researches to flavandiols with a low degree of substitution or to the completely unsubstituted flavan-3,4-diol. In no case was the strongly activating 7-hydroxyl (as is the case with the diols investigated) present. Better differentiation between 3,4-cis- and 3,4-trans-diols may possibly be effected by employing milder reaction conditions (Fujise et al.[153]) or possibly dimethoxypropane as described by Brown and MacBride [185].

The ready epimerization of the C_4-benzylhydroxyl in the methyl ethers of leuco-robinetinidin and leuco-fisetinidin under the acid conditions used for forming isopropylidene derivatives is similar to the earlier conversion of the free phenolic form of melacacidin (2,3-cis-3,4-cis) to isomelacacidin (2,3-cis-3,4-trans) as shown by Clark-Lewis and Mortimer [107]. The mechanism of such epimerization through carbonium and oxonium intermediates has been discussed by Iriarte, Ringold and Djerassi [188] for 6,7-dihydroxyoestrogens. Apparently in most cases the preferred inversion of the benzylhydroxyl is from the equatorial to the axial orientation, presuming the preferred 2(eq.): 3(eq.): 4(eq.) conformation of substituents for (+)-mollisacacidin and (+)-leuco-robinetinidin.

Absolute configuration of (+)-mollisacacidin and (+)-leuco-robinetinidin.

The foregoing establishes beyond doubt the relative configurations of substituents at C_3 and C_4 as trans for both diols. The trans-

configuration at C_2 and C_3 for leuco-fisetinidin [28] and leuco-robinetinidin [25] was established earlier by their conversion into 2,3-trans-flavan-3-ols and synthesis from the dihydroflavonols, (+)-fustin [26] and (+)-dihydrorobinetin [25], both of known 2,3-trans-configuration. From stereochemical correlations (cf. Weinges [25]) of the optically pure (+)-flavan-3,4-diols with (+)-catechin, of which the absolute configuration is known, it is now possible to establish the absolute configuration of these two natural leuco-anthocyanidins. In the R and S notation of Cahn, Ingold and Prelog [21] they are designated as 2R: 3S: 4R. For both (+)-mollisacacidin III and (+)-leuco-robinetinidin IV, the substituents at C_2, C_3 and C_4 are equatorially orientated.



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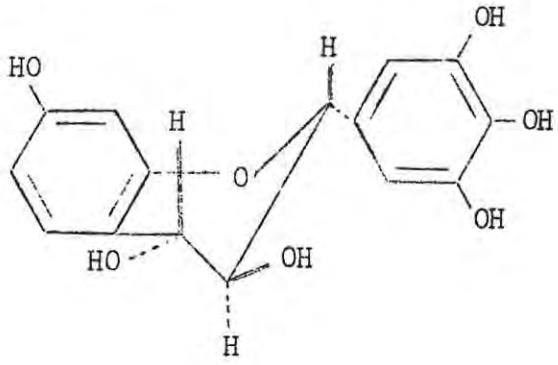


(IV)

Analogues of 2,3-cis-3,4-cis-flavandiols.

The proposed future study of the mode of link of wattle tannin prototypes through a study of oxidation rates and n.m.r. methods required the synthesis of geometrical isomers of leuco-robinetinidin and leuco-fisetinidin other than the trans-trans and trans-cis ones already described. Thus, 2,3-cis-3,4-cis-O-trimethyl-leuco-fisetinidin

was obtained by hydrogenation under pressure of (±)-7,3',4'-O-trimethylflavonol. In a similar manner, reduction of (±)-7,3',4',5'-O-tetramethylflavonol yielded the new 2,3-*cis*-3,4-*cis*-tetramethylleuco-robinetinidin. Slightly more drastic hydrogenation conditions led to the isolation of the corresponding 2,3-*cis*-flavan-3-ol. This compound which was isolated readily was obtained pure for the first time.

The stereochemistry of these synthetic flavandiols may be inferred from their method of synthesis *cf.* King and Clark-Lewis [70], but was confirmed by examination of the spin-spin coupling constants of the 2-, 3- and 4-protons of their 3,4-diacetates (Table 17). Assuming, as before, the correlation between the dihedral angle of vicinal protons and their coupling constants, [44] the coupling constants of the above analogues ($J_{2,3} \sim 1$ c.p.s. and $J_{3,4} = 3.8 - 4.2$ c.p.s.) are in agreement with a 2(*ax*), 3(*eq*), 4(*ax*) arrangement of protons, and therefore a 2(*eq*), 3(*ax*), 4(*eq*) or 2,3-*cis*-3,4-*cis*- arrangement of substituents. The values of these coupling constants are in good agreement with those obtained by Clark-Lewis, Jackman and Williams [9] for (±)-6-methyl-3',4'-O-dimethyl-2,3-*cis*-flavan-3,4-*cis*-diacetate ($J_{2,3} = 0.9$ c.p.s. and $J_{3,4} = 4.3$ c.p.s.). Similarly, the racemic 7,3',4',5'-O-tetramethylflavan-3-ol has the 2,3-*cis*- arrangement ($J_{2,3} \sim 1$ c.p.s.) (Table 17), and is therefore the epimeric racemate of the 2,3-*trans*-(-)-robinetinidol previously isolated from wattle bark by Roux and Maihs [133].

Paper chromatography and paper ionophoresis of isomeric flavan-3,4-diol methyl ethers.

Although accurate conclusions regarding the stereochemistry of the diol-grouping in flavan-3,4-diols may be drawn from comparative oxidation rates of stereoisomers, as outlined, and from the spin-spin coupling constants of the 2-, 3- and 4-protons obtained from their n.m.r. spectra (as above), additional, but less complex, alternative methods were sought.

The present work shows that correlation of both paper chromatographic and ionophoretic behaviour of methylated flavan-3,4-diols with their established stereochemistry offers simple but apparently reliable criteria for differentiating between cis- and trans-diol arrangements.

Paper chromatography.

Little or no differentiation of R_F values in water-saturated butan-1-ol and in water-saturated ethyl acetate (Table 18) is shown by the paper chromatography of isomeric 7,3',4'- or 7,3',4',5'-O-methyl-2,3-cis-3,4-cis-, 2,3-trans-3,4-cis- and 2,3-trans-3,4-trans-flavandiols [79], and of the methyl ethers of melacacidin (7,8,3',4'-O-tetramethyl-2,3-cis-flavan-3,4-cis-diol) and isomelacacidin (7,8,3',4'-O-tetramethyl-2,3-cis-flavan-3,4-trans-diol) [107]. However, on paper impregnated with 0.1M sodium borate [161, 189] all 3,4-cis-diols have appreciably lower R_F values (ΔR_F, 0.12 - 0.14 for butan-1-ol and 0.05 - 0.09 for ethyl

acetate) than the corresponding 3,4-trans-diols (Table 18). Since these methylated flavandiols contain no free phenolic hydroxyl groups, complex formation with the borate ion is restricted to the aliphatic 3,4-diol- grouping and the results suggest that this phenomenon occurs only with 3,4-diol groups having the cis-arrangement. This method therefore makes possible the separation of isomeric 3,4-cis- and 3,4-trans-diols, where these are formed simultaneously during reaction. In addition, it enables identification of methylated flavan-3,4-diols with known patterns of phenolic substitution when suitable reference compounds are employed. Thus the identity of 7,3',4'-O-trimethyl-2,3-trans-flavan-3,4- cis-diol, (obtained in low yield by the oxime-amine synthesis) was confirmed by comparing it with the trans-cis-diol of known configuration on borate impregnated paper.

The formation of an acidic borate complex by the trimethyl ether of (+)-mollisacacidin (now known to have a 3,4-trans-configuration) was regarded by Keppler [65] as indicative of the presence of a cis-glycol group in the compound, cf. Böeseken [184]. Repetition of this test by Roux and De Bruyn [117] under conditions which prevented the access of carbon dioxide (a precaution apparently not observed by Keppler), however, yielded a negative result. This is in agreement with the above findings that only 3,4-cis-flavandiols complex with borate.

Paper ionophoresis.

Foregoing evidence of complex formation of flavan-3,4-cis-diols prompted examination of the ionophoretic mobilities of the eight methylated flavan-3,4-diols in the same 0.1M sodium borate buffer. All flavan-3,4-cis-diols showed positive, although variable, mobilities (Table 19) with 2,3-trans-3,4-cis-diols having higher mobilities than isomeric 2,3-cis-3,4-cis-diols. Methylated flavan-3,4-trans-diols show either no mobility (7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-trans-diol), or a negative mobility, the latter presumably being caused by electroendosmotic flow. In view of the variable behaviour of the 3,4-trans compounds, corrections for electroendosmotic flow could not be applied and the relative mobilities M are accordingly related to that of racemic 7,3',4',5'-O-tetramethyl-2,3-trans-flavan-3,4-cis-diol.

Ionophoretic mobility in borate solution therefore apparently affords an absolute method for distinguishing between methylated flavan-3,4-cis- and 3,4-trans-diols without the use of reference compounds.

Comparison of paper chromatographic and ionophoretic behaviour of 3,4-cis-diols.

Comparison of the R_F values in butan-1-ol of geometrical isomers of two groups of analogues (7,3',4',5'-O-tetramethyl and 7,3',4'-O-trimethyl) with their ionophoretic mobilities (Tables 18 and 19) indicates that 3,4-cis-diols of lowest R_F (2,3-trans-3,4-cis)

also have the highest mobility, whereas those of intermediate R_F (2,3-cis-3,4-cis) again have intermediate mobilities. This behaviour suggests that in the trans-cis-diols stronger complexing with the borate is possible than with the cis-cis-diols.

Investigation of Structural Causes Responsible for Reddening of Wattle Bark Extracts through a Study of Flavan-4-ols.

The redness which develops when certain condensed tannins, for example those derived from wattle and quebracho, are exposed to sunlight is well known. Other vegetable extracts such as mangrove (Rhizophora mucronata), mallet (Eucalyptus astringens) and wandoo (Eucalyptus wandoo) are inherently red after commercial preparation and Hillis [190] tentatively suggested that leuco-anthocyanidins were the "precursors" of the red colour of leathers tanned with these extracts.

The condensed tannins are now known to be based on the flavonoid pattern [178], and Roux [191] has recently outlined the structural causes responsible for their reddening following the synthesis of a number of analogues of the flavan-3,4-diol prototypes of these tannins. A study of the effect of sunlight, heat and mineral acids on these analogues showed that:

a) Flavan-3,4-diols and flavan-4-ols (i.e. compounds with a hydroxyl group in the 4- position) are extremely sensitive to any of these three influences.

b) Where hydroxylation in the 4- position is absent while the remaining pattern of phenolic hydroxylation remains unaltered (flavan-3-ol and flavan analogues), reddening effects are usually entirely absent.

c) Methylation of the phenolic hydroxyls in the colour-producing flavan-3,4-diols and flavan-4-ols renders these compounds stable to sunlight.

In addition to hydroxylation at the 4- position, free phenolic hydroxyls on the benzenoid nuclei are thus necessary for reddening to occur. Since Roux and De Bruyn [117] had previously shown that 7,4'-dihydroxyflavan-4-ol reddened readily and underwent self-condensation, simultaneous phenolic hydroxylation in the 7 and 4' positions appeared sufficient to contribute colour production, and it remained to establish whether free hydroxyls in both these positions were necessary. Accordingly two new flavan-4β-ols, 7-hydroxy- and 4'-hydroxyflavan-4β-ol, were synthesized and characterized through preparation of their crystalline dibenzoate- and diacetate-, and monomethoxy- and diacetate-derivatives, respectively. In addition, the known 7,4'-dihydroxyflavan-4β-ol [117], which has not been obtained in crystalline form, was prepared.

The 4'-hydroxyflavan-4β-ol was synthesized with relative ease. In the synthesis of the 7-hydroxyflavan-4β-ol (amorphous), however, difficulty was experienced in the first stage of the synthesis,

which involved the preparation of the chalcone from the alkaline condensation of resacetophenone and benzaldehyde. Similar complications appear to have been experienced by Ellison [166] and by Mahal, Rai and Venkataraman [192], these latter workers stating emphatically that resacetophenone did not react with benzaldehyde in alkali to give the chalcone. By employing an acid condensation procedure similar to that described by Goel, Jain and Seshadri [167] good yields of the chalcone were obtained. Aqueous 1% sodium acetate (cf. Bognar, Farkas and Rakosi [168]) was found to be a very successful reagent for effecting ring closure of the chalcones to the corresponding 5-deoxy-flavanones. Yields of the three flavanones prepared varied from 62 - 92% whereas previously Roux and De Bruyn [117], using 1% sodium hydroxide to effect ring closure, had obtained only an 8 - 12% yield of 7,4'-dihydroxy-flavanone. The yields of flavan-4-ols from catalytic hydrogenation of the corresponding flavanones were about 50% in all three cases.

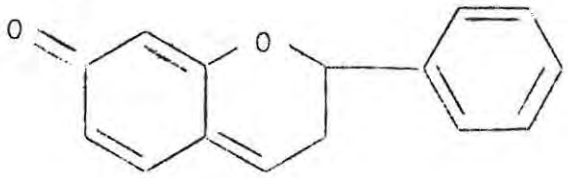
The n.m.r. spectra of the diacetates of 7-hydroxyflavan-4-ol (m.p. 128 - 130°) and 4'-hydroxyflavan-4-ol (m.p. 117°) are similar. On the assumption that $J_{2,3a}$ has the same sign as $J_{2,3b}$ and $J_{3a,4}$ as $J_{3b,4}$ [193], then for the former compound $J_{2,3a} + J_{2,3b} = 13.4$ c.p.s. and $J_{2,3a} + J_{2,3b}$ for the latter = 13.1 c.p.s. (Table 22). For both compounds $J_{3a,4} + J_{3b,4} = 16.5$ c.p.s. These values show that the 2- and 4-protons must be axial. [44,45] In addition, comparison with the coupling constants of the 2- and 4-protons from the n.m.r. spectra of the flavan-4-ol analogues of fisetin and robinetin [79] and racemic

2,4-*cis*- and 2,4-*trans*-flavan-4-ol benzoates [58,79] shows that the values of the two new flavan-4β-ols correspond to the 2,4-*cis*-arrangement.

The 7,4'-dihydroxyflavan-4-ol, initially white and migrating as a discrete spot on two-way paper chromatograms, became intensely red after one month at room temperature and appeared as a large diffuse area on chromatograms. The compound was acetylated at this stage and examined by n.m.r. spectroscopy. The spectrum, which is unlike that of the other two flavan-4-ols, shows the loss of the 4-acetyl function and it seems that this 4-ol, known to be prone to self-condensation, was no longer monomeric.

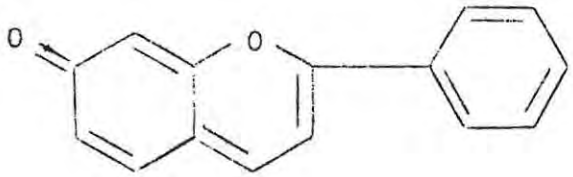
Exposure of the three flavan-4-ols to direct sunlight caused the 7-hydroxy- and the 7,4'-dihydroxyflavan-4-ol (both white initially) to turn orange-red and red respectively whereas the 4'-analogue remained completely white. The same effect was observed on heating the three compounds at 110° under vacuum. These results indicate that simultaneous substitution of hydroxy groups in the 4- and 7- positions is alone sufficient for the flavan-4-ols to redden on exposure to sunlight.

The mechanism responsible for the reddening is uncertain. Possibly a quinonemethene V is formed as an intermediate in this reaction through elimination of water between the 7- and 4-hydroxyl groups.



(V)

However, reddening associated with anthocyanidin formation VI must be regarded as equally possible. This represents a conjugated system, and



(VI)

hydroxyls in the 3',4' or 5'-positions would have an influence on the colour of the pigment formed, which is in agreement with observed facts i.e. blue, pink and orange colourations from 7,3',4',5'-tetrahydroxy-, 7,3',4'-trihydroxy- and 7,4'-dihydroxyflavan-4-ol respectively. This phenomenon parallels the situation in natural anthocyanidins where delphinidin , cyanidin and pelargonidin chlorides show a similar colour gradation.

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