GUIBOURTACACIDIN, A NEW LEUCO-ANTHOCYANIDIN FROM RHODESIAN COPALWOOD; (GUIBOURTIA COLEOSPERMA)

by

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SUMMARY.

A new leuco-anthocyanidin, (+)-7,4'-dihydroxyflavan-3:4-diol has been isolated from the heartwood of Guibourtia coleosperma.

The heartwood extractives were fractionated by enrichment procedures involving fractional precipitation and Craig partitioning, to give a high R_F low molecular weight fraction containing the above leuco-anthocyanidin. This was further fractionated by "preparative" paper chromatography.

The leuco-anthocyanidin was amorphous and present in low proportion (0.004%) in the wood. On boiling with alcoholic hydrochloric acid the compound gave an anthocyanidin which was identified as 3,7,4'-trihydroxyflavylium chloride. The degradation products formed by alkali fusion, on a micro-scale, were resorcinol and β-resorcylic acid, and also p-hydroxybenzoic acid. This indicated resorcinol and phenol A and B nuclei respectively. The formation of amorphous dimethyl ether and tetra-acetoxy derivatives indicated two phenolic and two alcoholic hydroxyl groups.

The above indicates that the compound was 7,4'-dihydroxy-flavan-3:4-diol and a molecular weight estimation showed it to be monomeric. Comparison of the infrared absorption spectra of the natural dimethyl ether with synthetic (±)-dimethoxyflavan-3:4-diol, to which a tentative 2:3-*trans*-3:4-*cis* conformation had been assigned (Phatak and Kulkarni 94), showed close identity.

Crystalline (±)-7,4'-dihydroxyflavan-3:4-diol was synthesised by catalytic hydrogenation over platinum oxide of the corresponding (±)-7,4'-dihydroxyflavanonol. The flavanonol was synthesised by sodium hyposulphite reduction of the 7,4'-dihydroxyflavonol. The infrared absorption spectra of the natural and synthetic diols were similar but not identical. Chromatographic evidence showed the apparent identity of the synthetic and natural flavan-3:4-diols, and two possible configurations were assigned for the natural flavan-3:4-diol.

The new leuco-anthocyanidin was observed to form an *O*-ethyl ether derivative on manipulation in ethanol.

A monomeric leuco-fisetinidin from the heartwood was identified, by two dimensional paper chromatography, as (+)-mollisacacidin. The condensed tannins found also in the heartwood of *G. coleosperma* appear to be polymeric

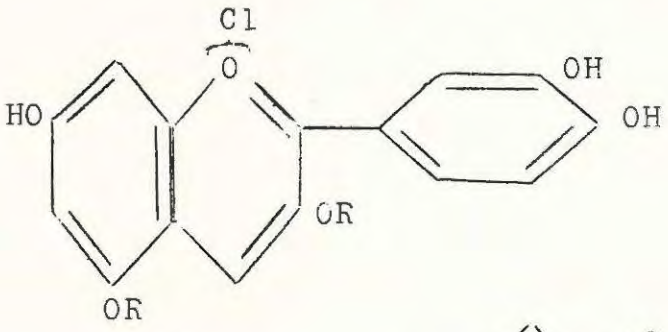
forms of leuco-fisetinidin and leuco-guibourtinidin. These polymeric tannins form a large proportion of the heartwood extractives and furnish high yields of fisetinidin and guibourtinidin chlorides on boiling with alcoholic hydrochloric acid. Examination of some other members of the Guibourtia spp. by paper chromatography showed that only G. coleosperma contained the new leuco-anthocyanidin as well as a leuco-fisetinidin. G. tessmannii and G. demeusii heartwoods contained only leuco-fisetinidin and the related polymers and a close chemical relationship to G. coleosperma is thus apparent. G. arnoldiana on the other hand is not chemically interrelated with the above members since it appeared to contain only leuco-cyanidin.

(±)-7,4'-Dihydroxyflavan-4-ol was synthesised by hydrogenation of the corresponding flavanone over platinum oxide. Observations were made regarding its reddening and ease of condensation.

CONTENTS

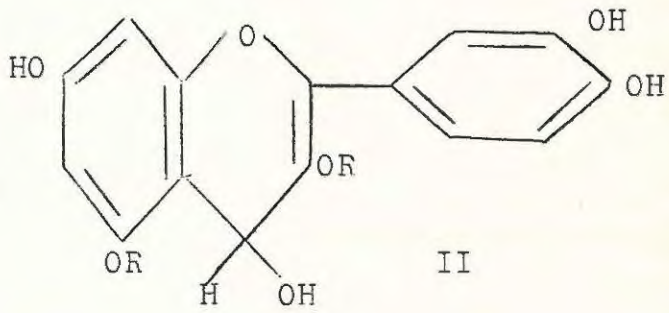
REVIEW
OF
LEUCO-ANTHOCYANIDIN CHEMISTRY.

Compounds which are converted into anthocyanidins (I) by boiling with aqueous or alcoholic hydrochloric acid are termed leuco-anthocyanins or leuco-anthocyanidins.



I

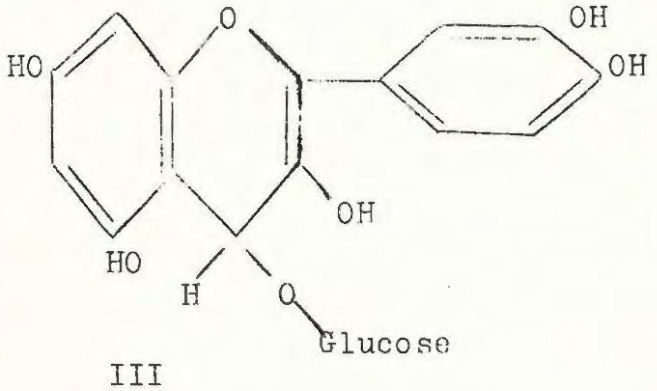
R=H, cyanidin chloride
R=glucosyl, cyanin chloride.



II

The term leuco-anthocyanin was introduced by Rosenheim (1) in 1920 to describe the supposedly glycosidic substance which he isolated from unripe purple grapes and from mature white grapes. From previous work by Willstätter he proposed that these compounds were intermediates in the synthesis of anthocyanins in the plant, during the reduction

of flavonols to anthocyanidins, and, that they were glycosides of the pseudo-bases of the corresponding anthocyanidins e.g. III.

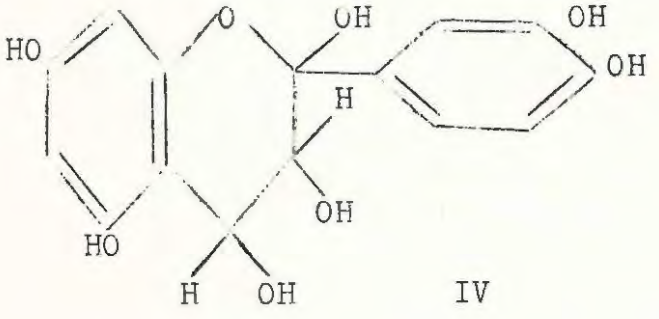


III

Robinson and Robinson (2) however, showed this was improbable because the pseudo-base (II) itself is rapidly converted into the parent anthocyanidin even by cold acid. Furthermore the individual leuco-anthocyanidins are much more stable than these pseudo-bases; cyanidin pseudo-base, for example, is rapidly converted into a product which requires boiling hydrochloric acid for its reconversion into cyanidin chloride (3).

It was not until 1933 that Rosenheim's observations were clarified. Robinson and Robinson (2) undertook a survey of leuco-anthocyanidins and showed that these were widely distributed in the plant kingdom, the majority yielding cyanidin, and the rest, with few exceptions, delphinidin. They disagreed with the name given by Rosenheim, their

contention being that "leuco" usually means the reduction of a dyestuff, whereas the leuco-anthocyanin and its anthocyanidin were probably in the same oxidation state. To avoid the difficulty contained in Rosenheim's pseudo-base theory they suggested that the leuco-anthocyanins contained the flavan-2:3:4-triol structure IV.



IV

Conversion to the anthocyanidin thus demands only dehydration, through loss of an hydroxyl at C-4 and hydrogen at C-3. They furthermore suggested that the hydroxyl groups may bear carbohydrate residues or be acylated.

Later Robinson and Robinson (4) divided the leuco-anthocyanins into three classes a) Those that are insoluble in water and the usual organic solvents.

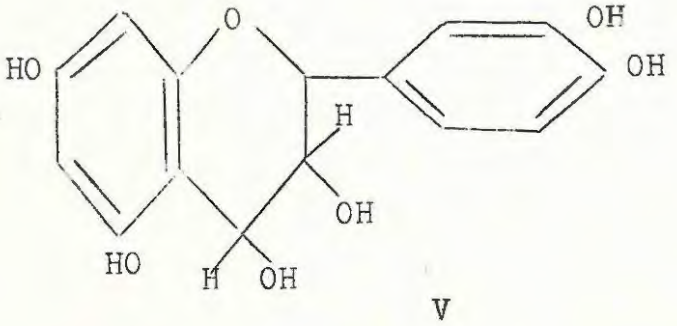
b) Those readily soluble in water but not extracted from solution by means of ethyl acetate.

c) Those capable of extraction from aqueous

solution by means of ethyl acetate. They suggested that class (b) probably consists of relatively simple glycosides or diglycosides, whereas members of class (c) are sugar-free and should be regarded as leuco-anthocyanidins.

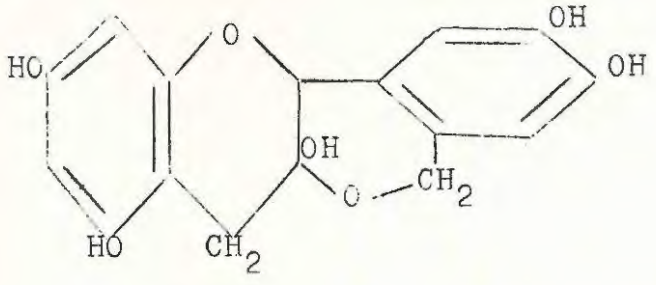
Choice between the terms leuco-anthocyanin and leuco-anthocyanidin seems to be confused and both terms have been used rather indiscriminantly. Since all the compounds of this class and known constitution so far discovered in nature are leuco-anthocyanidins (aglycones) it would seem better to use only this term. The term "proanthocyanidin" suggested by Freudenberg and Weinges (90) has not yet been adopted for general use.

Hitherto the anthocyanidins generated from leuco-anthocyanidins had been identified by means of qualitative tests and by comparisons in solution. In 1937, however, Mrs. G.M. Robinson (3) isolated cyanidin chloride in crystalline form from a leuco-anthocyanidin found in the gum of Butea frondosa. The leuco-anthocyanidin itself proved difficult to isolate but on preliminary oxidation of the gum with picric acid, and subsequent treatment with hot alcoholic 5% hydrogen chloride, a maximum yield of cyanidin chloride was obtained. This led to the recognition that the leuco-anthocyanidins have, in many cases, the state of oxidation of flavan-3:4-diols (e.g. V) rather than flavan-2:3:4-triols (e.g. IV).

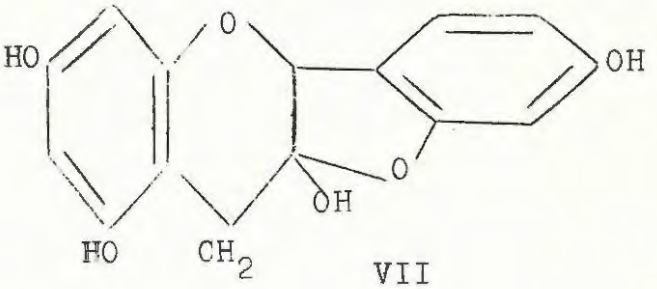


V

Previously peltogynol (4) and cyanomaclurin (6,7) had been examined by Robinson and co-workers. These compounds were found to yield anthocyanidins (although not naturally occurring ones) under certain conditions. They suggested that peltogynol had the structure VI and structure VII was proposed for cyanomaclurin.



VI



VII

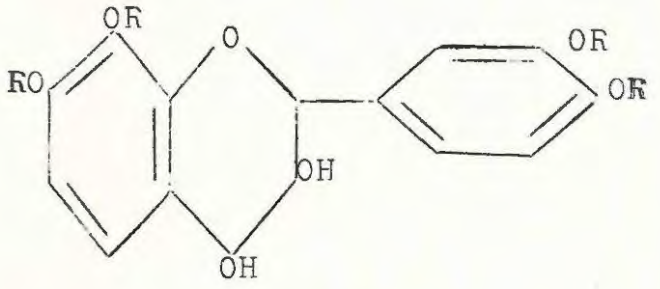
Both these compounds were described as leuco-anthocyanidins (5) although neither conform to either structure IV or V as postulated by Robinson and Robinson. Although interest was shown in the leuco-anthocyanidins as precursors of anthocyanidins in autumn leaves (8,9,10) and a number were isolated from, or demonstrated in, widely different sources (11-15), little was done to solve the problem of their structure until recently.

In 1953 Bate-Smith (16) drew attention once more to the widespread distribution of the leuco-anthocyanidins in nature, especially those yielding cyanidin and delphinidin. Later work (15,44) confirmed this observation. Bate-Smith showed, however, that they were mainly confined to "woody" plants as opposed to herbaceous plants. This confirmation of the widespread occurrence of leuco-anthocyanidins in nature caused Bate-Smith and Swain (17) to speculate on the similarity in chemical behaviour between leuco-anthocyanidins and condensed tannins. Robinson and Robinson (2) and Bate-Smith (16) have discussed the near relationship, from the chemical point of view, of the leuco-anthocyanidins and the catechins. Condensed tannins (distinct from hydrolysable tannins) are characterized by their forming a red-brown

precipitate when boiled with mineral acids. It is generally supposed that catechins (flavan-3-ols) are responsible but both Bate-Smith and Swain (17) as well as Robinson and Robinson (2) thought that leuco-anthocyanidins were responsible. Bate-Smith and Swain (17) demonstrated that leuco-anthocyanidins are easily adsorbed on hide powder, give precipitates with alkaloids, give usual phenolic colour reactions, and are markedly astringent. Furthermore the leuco-anthocyanidins from pinus and cacao give similar ultraviolet spectra in ethanol and in 0.002M ethanolic sodium ethoxide, which closely resemble that of (+) catechin. These spectra are quite different from those of other classes of flavonoid compounds, and in accord with the structures proposed by Robinson and Robinson (2,18). Bate-Smith and Swain (17) thus suggested that all these considerations taken together suggest that the leuco-anthocyanidins are closely related chemically to the catechins, and are, as well as these, to be regarded as prototypes of the condensed tannins.

Decisive chemical evidence for the structure of a naturally occurring leuco-anthocyanidin was first obtained by King and Bottomley (19,20) in elucidating the structure of melacacidin (VIII), which they isolated from the heartwood

of Australian blackwood (*Acacia melanoxylon*).

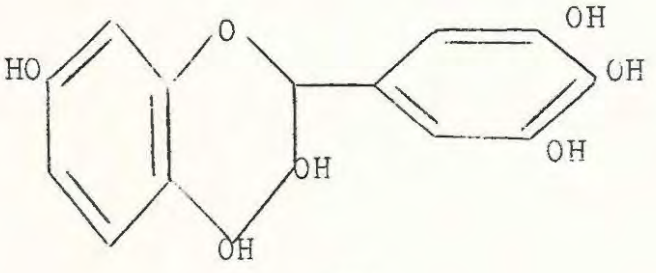


VIII R=H

Melacacidin was isolated as an amorphous compound which gave a crystalline tetramethyl derivative (VIII.R=CH_3). The tetramethyl ether formed a diacetate indicating two alcoholic groups, namely, in the 3:4 positions. From the ready production of a cyclic carbonate they concluded that the diol system has the *cis* configuration. Melacacidin on treatment with hot acid yielded a crimson solution, later (21,22) shown to contain the corresponding anthocyanidin, and a dark reddish-brown amorphous solid. They regarded the presence of this amorphous solid as being very significant and they concluded, in the light of observations by Bate-Smith and Swain (17), that the properties of so-called leuco-anthocyanidins are indistinguishable from those of condensed tannins. They implied that the hitherto vaguely defined "phlobatannins" also are derivatives of

flavan-3:4-diols.

Freudenberg and Roux (40) synthesised the first crystalline flavan-3:4-diol, leuco-robinetinidin ((±)-7,3',4',5'-tetrahydroxyflavan-3:4-diol) (IX), by hydrogenation of dihydrorobinetin with platinum oxide. Leuco-robinetinidin forms a red insoluble product, the anthocyanidin 7,3',4',5',-3-pentahydroxyflavylium chloride, with hot dilute hydrochloric acid.



IX

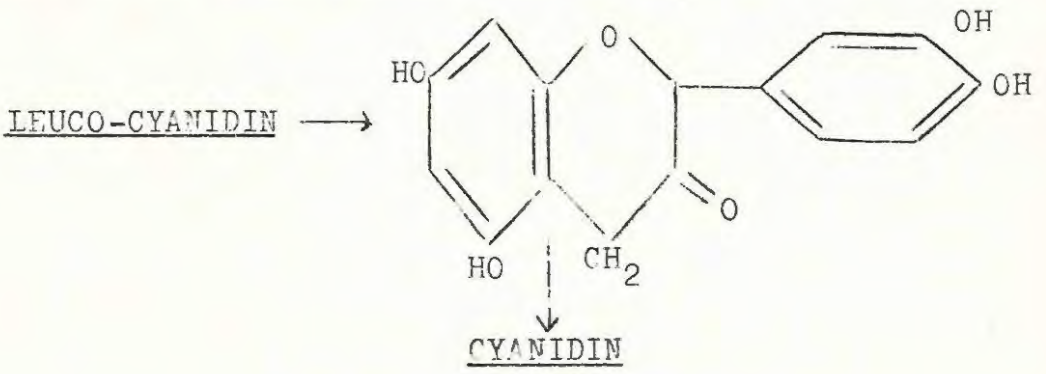
Swain (23) (1954) synthesised leuco-cyanidin by reduction of taxifolin with sodium borohydride. This amorphous leuco-cyanidin yielded cyanidin and a brown precipitate (phlobaphene) with hot acid. When the treatment was carried out under nitrogen however, only a faint pink colouration resulted indicating that oxidation is a necessary step in the conversion. Treatment with cold 2N.

hydrochloric acid gave a white precipitate soluble in alcohol which appeared to be polymeric. This polymer was non-mobile on paper chromatograms and, unlike leuco-cyanidin, markedly astringent.

Forsyth (11,12) similarly showed that in cocoa beans two compounds of the leuco-anthocyanidin type were present, both based on cyanidin. One of these was mobile on paper chromatograms and on cellulose columns, whereas the second has a high affinity for cellulose. The former appeared to be a leuco-cyanidin while the latter a complex leuco-cyanidin glycoside. The glycoside gave glucose and arabinose on hydrolysis in the ratio of 3:1.

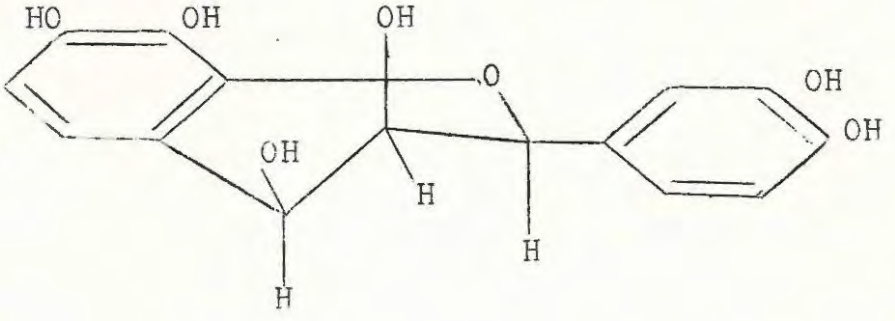
In an endeavour to trace possible intermediate stages between flavonoids and anthocyanidins, and to obtain indications of possible structures which would explain the properties of natural leuco-anthocyanidins, Bauer, Birch and Hillis (25) examined the reduction products of some flavonoid compounds. They put forward the hypothesis that natural leuco-anthocyanidins may be derivatives of 3:4 dihydroxyflavans, a possibility suggested earlier by both Bate-Smith (16) and King and Bottomley (19). They suggested that dehydration of a leuco-anthocyanidin would form a 3-ketoflavan which could then disproportionate or

oxidize to the anthocyanidin e.g.



King and Clark-Lewis (22) synthesised the tetramethyl ether of melacacidin (VIII R=CH_3) by catalytic hydrogenation of the corresponding flavonol over Raney nickel. The isolation of a single racemate where four racemic species are theoretically possible was explained by them as being due, in part, to stereospecific hydrogenation of the kind usually observed in the catalytic reduction of ethylenic bonds. Kulkarni and Joshi (27) claimed to have synthesised an identical flavan-3:4-diol (R=H) by hydrogenation of the corresponding dihydroflavonol over a platinum catalyst in acetic acid. On reduction of the same dihydroflavonol with lithium aluminium hydride a higher melting point racemate was obtained. They suggested that the higher melting point racemate was the trans isomer and that the lower melting point racemate was the cis isomer. They did not speculate

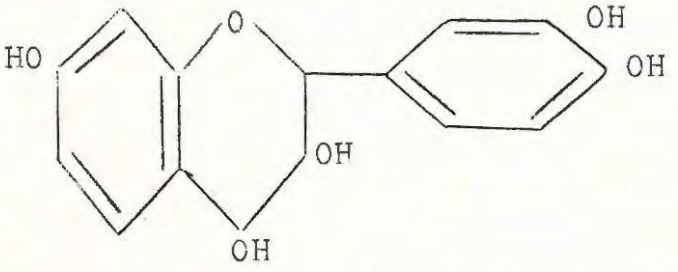
on whether melacacidin tetramethyl ether was one of the antipodes of the above racemates or of the remaining two of the four possible racemates. King and Clark-Lewis (28) assumed that, since both the synthetic and natural melacacidins formed a cyclic carbonate, the synthetic diol also possesses a <u>cis</u> configuration. This conclusion found strong confirmation from the formation of an isopropylidene derivative, since it was then considered that this derivative was not formed by a flavan-3:4-diol with a <u>trans</u> configuration. They suggested the following stereochemical formula for melacacidin (X) with the conformation 2(e):3(a):4(e).



X

1957 saw the isolation of the first crystalline leuco-anthocyanidin from natural sources. Keppler (29) isolated this leuco-anthocyanidin, which he called

mollisacacidin (XI), from the heartwood of Acacia mollisima (black wattle).



XI

Keppler postulated the structure of mollisacacidin as cis - 3:4:7:3':4'-pentahydroxyflavan which was confirmed by the formation of synthetic mollisacacidin (leucofisetinidin) by catalytic reduction of the corresponding dihydroflavonol, fustin, using the earlier method of Freudenberg and Roux (40). Both the synthetic and the natural mollisacacidin yielded the corresponding anthocyanidin, fisetinidin chloride, on treatment with 3N.HCl in isopropanol (30).

Chandorkar and Pulkarni (31) synthesised two isomeric 7,3',4'-trimethoxyflavan-3:4-diols, related to O-methyl-mollisacacidin, by the method of Pulkarni and Joshi (27).

Roux (32) obtained a monomeric substance from the wood of Schinopsis quebracho-colorado, which, from the behaviour of its derivatives, optical rotation, and absorption spectra, indicated that it had properties similar to, but not identical with, those of the 3',4',7-trihydroxyflavan-3:4-diol present in the heartwood of Acacia mollisima. He found that the tannin mixture associated with this compound was exceptionally rich in complex leuco-fisetinidin of varying molecular weight (700-2,300). Roux suggested that these results lend conclusive support to the concept of Freudenberg and Maitland (33) that flavans are basic units for condensed tannins. They also support the contention of Bate-Smith and Swain (17) that leuco-anthocyanidins in addition to catechins are prototypes of condensed tannins. Roux (34) also showed by chromatographic examination of radial samples of the wood that monomeric leuco-fisetinidin gradually disappears from the outer sapwood, where it is present in its highest concentration, towards the central pith. This work provides further presumptive evidence of the transformation of monomeric leuco-fisetinidin in the sapwood, to the various polymeric forms in the heartwood. The leucofisetinidins can thus be regarded as the possible precursor

of quebracho extract.

The first example of the occurence of an enantiomorphous pair of flavonoids in nature was discovered by Clark-Lewis and Roux (35). They found that (-)-leucofisetinidin from Schinopsis quebracho-colorado (32, 38) was enantiomorphous with mollisacacidin (29) and gleditsin (36) which are two names for a single dextrorotatory form (37) of 7,3',4'-trihydroxyflavan-3:4-diol. The enantiomorphous nature was apparent from the identity of melting point and infrared absorption of each pair of derivatives, equal but opposite rotations, and was conclusively proved by the melting point elevations of racemates from mixtures of derivatives of the enantiomorphs.

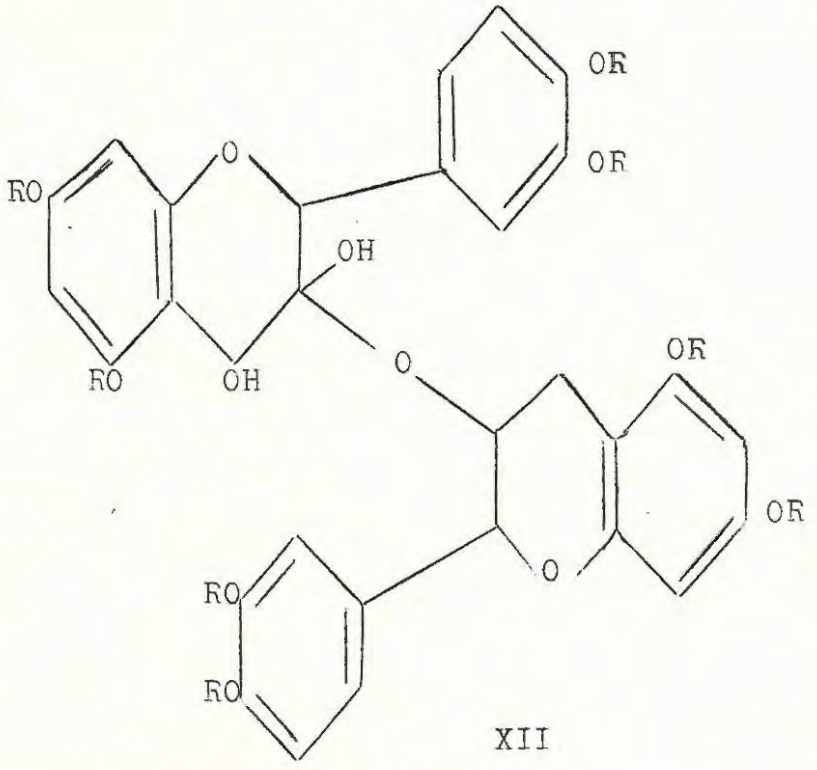
Roux and Evelyn (39) have shown black wattle bark to be relatively rich in complex leuco-robinetinidins and leuco-fisetinidins, and quebracho heartwood tannins in complex leuco-fisetinidins. They examined the heartwood extractives of 144 hardwood species and demonstrated that 33% of the samples give strongly positive reactions for leuco-anthocyanidins. The lower R_F polymeric forms predominate however, and in only 12%, mainly among the Leguminosiae, is evidence found of the presence of monomeric leuco-anthocyanidins. This work by Roux (32, 34)

and by Roux and Evelyn (39) showed the association of monomeric and complex leuco-anthocyanidins of similar structural pattern in those typical "condensed tannins" present in extractives of black wattle bark and quebracho heartwood. Chromatographic evidence illustrated the apparent conversion of monomeric into the complex polymeric leuco-anthocyanidins. Roux and Evelyn (72) provided details of the chromatographic identification, isolation and characterization of some of these new monomeric leuco-anthocyanidins. By means of a study of molecular weight, evidence was also provided to support the concept of their conversion into complex tannins during radial translocation within the plant. Roux and Paulus (98) isolated a condensed tannin from black wattle heartwood which resembled the accompanying (+)-7,3',4'-trihydroxyflavan-3:4-diol in most chemical and physical properties, but which was trimeric on a C_{15} basis. They suggested that the optically active tannin was a polymer of (+)7,3',4'-trihydroxyflavan-3:4-diol condensed possibly through the heterocyclic ring system. This work was extended when they isolated, from the same source, related tannins of progressively higher molecular weight (96). These tannins approximated in molecular weight to pentameric and decameric leuco-fisetinidins and

their properties were compared with trimeric (-)-leucofisetinidin tannin and (+)-7,3',4'-trihydroxyflavan-3:4-diol, which accompany them in the heartwood.

Weinges (1958) (41) briefly noted the presence of (+)-7,3',4'-5'-tetrahydroxyflavan-3:4-diol in Robinia pseudacacia heartwood but cited no details other than its optical rotation. He demonstrated however that (+)-leucorobinetinidin was a 2:3 trans diol. Roux and Paulus (96) extended the stereochemical identity of leuco-robinetinidin and assigned a 2:3 trans -3:4- cis diol configuration.

The structure of the complex leuco-cyanidin from fresh cacao beans (Forsyth 12,26) is still under investigation. It gives an octa-acetate and a non-phenolic octamethyl ether which does not yield an acetate when treated under mild conditions with pyridine-acetic anhydride. The octamethyl ether was converted by hot hydrochloric acid into cyanidin tetramethyl ether, and under milder conditions it gave (-)-epicatechin tetramethyl ether and a new leucocyanidin methyl ether. These facts including the intensity of the hydroxyl absorption in infrared spectra of the methyl ethers favours structure (XII).



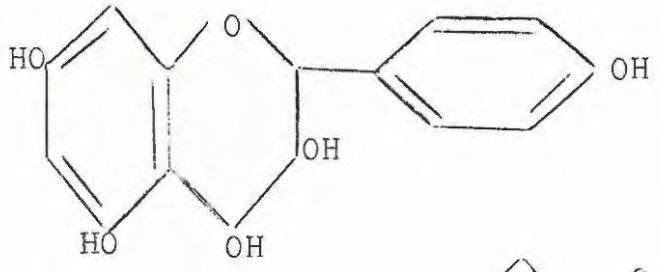
XII

Peltogynol (4) was one of the first leuco-anthocyanidins to be isolated, characterized and investigated structurally. Chan, Forsyth and Hassall (42,43) modified the structure of peltogynol which relates it to the flavan-3:4-diols. They considered that the structure proposed by Robinson and Robinson (4) was at variance with the hypothesis that leuco-anthocyanidins are derivatives of flavan-3:4-diols. They accordingly studied some further reactions of peltogynol and suggested structure (XIII).

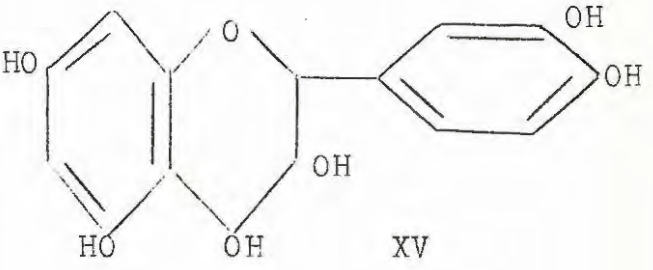
anthocyanidin peltogynidin on treatment with acid. However, unlike peltogynol, the isomer B turns red through formation of peltogynidin when heated near the melting-point in air. This reaction involves trans elimination of the 4-(axial)-hydroxyl group with a 3-(axial)-hydrogen atom so that peltogynol B is a cis-diol derivative. Peltogynol B trimethyl ether is also oxidized to peltogynone trimethyl ether. However, reduction of peltogynone trimethyl ether with sodium borohydride gives exclusively peltogynol and not the B isomer. Thus peltogynol and peltogynol B differ only in their mode of attachment of the 4-hydroxyl group. Peltogynol may therefore be regarded as a flavan-trans-3:4-diol derivative, and peltogynol B as the corresponding flavan-cis-3:4-diol derivative (38,39).

Although the majority of naturally occurring leuco-anthocyanidins have been shown to yield cyanidin and a lesser number delphinidin, when boiled with hydrochloric acid (2,44), it is only very recently that the first leuco-anthocyanidins corresponding to common anthocyanidins were isolated and characterized. The first leuco-cyanidin was that obtained from cacao beans (26). Ganguly and Seshadri (45) isolated and characterized a leuco-pelargonidin (XIV) from the gum of Eucalyptus

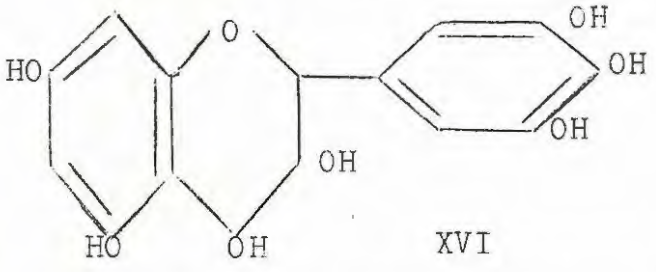
calophylla. It gave a laevorotary methyl ether suitable for characterization and leuco-pelargonidin was considered to be a flavan-3:4-diol. Ganguly and Seshadri (45) also succeeded in isolating a leuco-cyanidin (XV) from Butea frondosa gum, which G.M. Robinson (3) had earlier shown to yield cyanidin under certain conditions.



XIV



XV



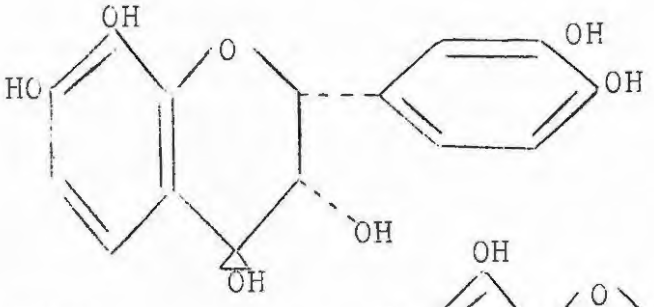
XVI

Laumas and Seshadri (46) identified a leuco-cyanidin in Tamarind seed testa and they considered it a stereoisomer of the one found in Butea frondosa (45). The leuco-anthocyanidin of Karada bark, Cleistanthus collinus, was isolated

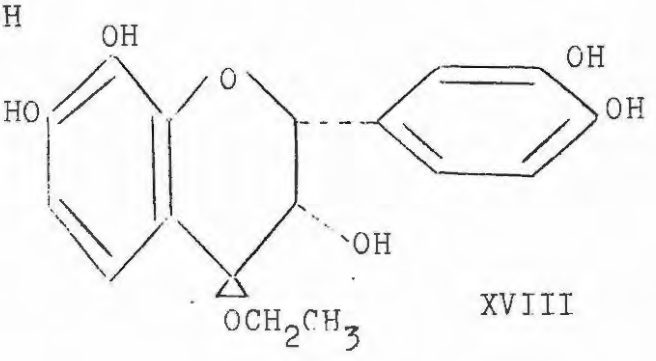
by Ganguly, Seshadri and Subramanian (47) and shown to be a (-)-leuco-delphinidin (XVI), 5,7,3',4',5'-pentahydroxy-flavan-3:4-diol. An isomeric dextrorotatory form was isolated from the gum of Eucalyptus pilularis kino and also from Emblica officinalis. Leuco-delphinidins have also been isolated from cottonseed hulls (48) and from oak bark (49).

Clark-Lewis and Mortimer have isolated a new leuco-anthocyanidin, isomelacacidin, which was found, with its 4-epimer melacacidin, in the heartwood of three species of Acacia (50). Crude extractives from these Acacia heartwoods were shown by paper chromatography to contain melacacidin and two other monomeric leuco-anthocyanidins, isomelacacidin and O-ethyl-isomelacacidin. Isomelacacidin behaves as a reactive p-hydroxybenzyl alcohol and readily forms an ethyl ether by reaction with ethanol, and this distinction from melacacidin facilitates separation. Isomelacacidin (XVII) has not yet been induced to crystallise and its structure was inferred largely from O-ethyl-isomelacacidin, which was characterized by its tetramethyl ether and sulphone derivatives. The anthocyanidin formed from O-ethyl-isomelacacidin was chromatographically indistinguishable from the 3,7,8,3',4'-pentahydroxyflavylium chloride similarly derived from melacacidin. Consequently they

considered the structure of O-ethyl-isomelacacidin to be XVIII the alternative being with the ethoxy group in the 3-position. They excluded the alternative however because of the extreme lability of the ethyl group.



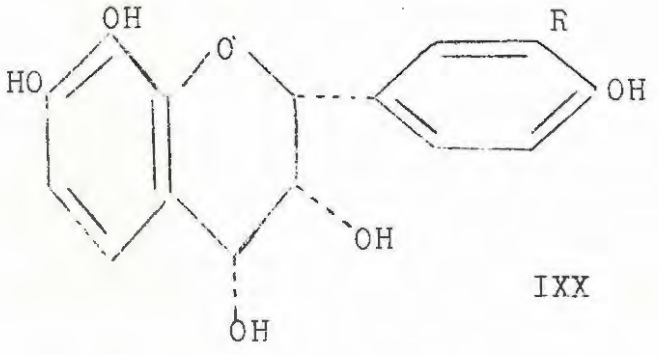
XVII



XVIII

Clark-Lewis and Mortimer indicated that the 4-hydroxyl group in isomelacacidin is axial and that the 2 (eq), 3 (ax), 4 (ax) conformation is preferred since it permits maximum resonance stabilization of the 4-carbonium ion. They explained the comparatively unreactive nature of the 4-epimer, melacacidin, as being a consequence of its conformational stability, since the 2 (eq), 3 (ax), 4 (eq) conformation of melacacidin is unfavourable for resonance stabilization of the 4-carbonium ion.

Clark-Lewis, Katekar and Mortimer (51) isolated another new leuco-anthocyanidin, teracacidin (IXX) from the heartwood of *Acacia intertexta*. They found it to be a (-)-7,8,4'-trihydroxy-2,3 *cis* flavan-3:4-*cis* diol, an analogue of melacacidin. The wood also contains a very small proportion of isoteracacidin and (+)-pinitol. Teracacidin and isoteracacidin appear to be related to each other in the same way as melacacidin and isomelacacidin and the epimers thus differ only in configuration at the 4- position.



IXX

Noteworthy is the fact that teracacidin is the first natural representative of flavonoids with the 7,8,4'-pattern of phenolic hydroxylation. The structure of teracacidin was established by oxidation and degradative studies and the leuco-anthocyanidin properties of teracacidin support the flavan-3:4-diol formulation. The

structure was confirmed by synthesis of the 2:3-*cis*-3:4-*cis* racemate of 7,8,4'-trimethoxyflavan-3:4-diol by catalytic hydrogenation of the 7,8,4'-trimethoxyflavonol. The product gave an infrared absorption indistinguishable from that of the laevorotary teracacidin trimethyl ether, but markedly different from the absorption of the 2:3-*trans*-3:4-*cis* racemate prepared from *trans*-dihydro-7,8,4'-trimethoxy-flavonol by reduction with sodium borohydride or by hydrogenation over palladium. Teracacidin (IXX R=H) is thus stereochemically identical with melacacidin (IXX R=OH). The stereochemical representations are supported by the similarity in molecular rotations of the methyl ethers and sulphones of the teracacidin series with those of the melacacidin series.

Since peltogynol and peltogynol B occur in *Peltogyne porphyrocardia* and teracacidin and isoteracacidin are found together in *Acacia intertexta*, Clark-Lewis and co-workers considered that it was possible for flavan-3:4-diols to occur frequently, or even generally, as mixtures of 4-epimers. Such epimers clearly could arise in the plant through non-specific reduction of a dihydro-flavonol although, from the ease of epimerization, iso-melacacidin might also be formed from melacacidin.

Paper Chromatography of Flavonoid Compounds.

Bate-Smith and Westall (58) investigated the chromatographic behaviour of some naturally occurring C_{15} compounds. They found a close relationship between R_F value and chemical constitution, especially in respect of the nature and number of the particular substituent groups, namely hydroxy-, methoxy-, and carboxy derivatives. The more highly hydroxylated substances had in general lower R_F values than the less hydroxylated. They demonstrated that in the solvent system butan-1-ol: acetic acid: water (4:1:5^v/v) the weight of hydroxylation dominated all other factors in its effects on R_F value, but, that methylation of these hydroxyl groups reverses the effect of hydroxylation, so that the greater the number of methoxyl groups, the higher is the R_F value. The increase in R_F value brought about by methylation, however, is rather less than the decrease caused by hydroxylation. Another factor influencing the migration of flavonoid substances on paper chromatograms is their stereochemical configuration.

Roberts and Wood (79) first showed that the optical antipodes of catechin and gallocatechin and their epimers could be resolved using water as irrigant.

Roux, Maihs and Paulus (80) showed that the optical antipodes of fisetinidol and of the flavan-3:4-diols, leuco-fisetinidin and leuco-robinetinidin, may similarly be separated in water or 2% acetic acid, as may also the 2,3-dihydroflavonols, and, that this sometimes occurs with a reversal in the sequence of migration of the (+) and (-) forms. They found that amongst the flavan-3-ols, those of 2,3-*trans* configuration of substituent groups always have an appreciably higher R_F than the related epimer of 2,3-*cis* configuration. Comparison of the 2,3-dihydroflavonols with their stereochemically related flavan-3-ols (catechins), shows that introduction of a carbonyl group in the 4-position reduces the R_F considerably. This lower R_F of the dihydroflavonols may be due to the equatorial arrangement of the 2-phenyl group and also of the 3-hydroxyl group which would confer a nearly planar structure to 2,3-dihydroflavonols, resulting, as in the (-)-epicatechins, in a reduced R_F in water. Hydroxylation in the 4-position produces a smaller increase in R_F than originally anticipated (91). This effect may be expected as introduction of an aliphatic hydroxyl on the heterocyclic ring should contribute to the solubility of the C_{15} unit as a whole. However, the introduction of aromatic hydroxyls in position

5 and 5' (phenolic hydroxyl) produces large and very small reductions in R_F values respectively. Substitution of hydroxyls (aliphatic) in the 3-position in both flavones and flavans produces large R_F increases in 2% acetic acid contrasting with the small increase accompanying hydroxylation (aliphatic) in the 4-position.

Selective spray reagents used in the flavonoid field are of considerable importance as diagnostic agents. Ammoniacal silver nitrate reagent, due to Partridge (52), is very widely used in chromatography. It is very useful for detecting strongly reducing organic compounds such as carbohydrates, aldehydes and phenols, and was introduced into the flavonoid field by Bate-Smith (58,59). Ortho-dihydroxy and ortho-trihydroxy phenolic groups reduce silver nitrate instantly in the cold and show up as brown or black spots. On the other hand mono- and meta-hydroxy phenolic groups reduce silver nitrate very slowly and consequently give either no reaction or a very weak reduction.

Bis-diazotized benzidine spray reagent, due to Lindstedt (60), is extremely selective and differentiates between a phloroglucinol nucleus (amber), and a resorcinol nucleus (yellow). Roux and Maihs (61) correlated the colour reactions, of various phenolic bodies, given with

bis-diazotized benzidine and they found that they are highly indicative of variations in the distribution of phenolic hydroxyl groups.

A spray reagent of particular diagnostic value in the flavonoid field is toluene-p-sulphonic acid, a reagent due to Roux (57). It renders possible the direct and easy recognition of leuco-anthocyanidins. Leuco-anthocyanidins not hydroxylated in the 5- position (leuco-robinetinidin, leuco-fisetinidin and melacacidin) give scarlet to pink spots, depending on the anthocyanidin formed, while leuco-anthocyanidins hydroxylated in the 5- position (leuco-cyanidin, leuco-pelargonidin) show up as yellow red spots after prolonged heating.

2:6-Dichloroquinonechloroimide spray reagent, due to Bray, Thorpe and White (71), was used in the detection of hydroxybenzoic acids. It was found to be rather unspecific, however, since it gave a blue colour with all the acids tested with one exception. Gierer (81) used it to determine the presence of p-benzyl alcoholic groupings in lignin but also found it to be unspecific.

EXPERIMENTAL AND RESULTS.

General Analytical and Preparative Techniques.

Determination of melting points. These were done in an insulated, electrically operated melting point apparatus which incorporates a coarse and a fine adjustment for both a rapid and a slow increase in temperature.

All melting points are uncorrected.

Determination of optical rotations. Optical rotations were measured with a Bellingham and Stanley Model A polarimeter with a glass circle and micrometer drum, reading to 0.01°. The material, normally about 20 mg., was accurately weighed and dissolved in 2.5 ml. of a suitable solvent. The rotation was measured in a 2 dm. Zeiss polarimeter tube and a minimum of ten readings taken. From this data the specific rotations and standard deviations were calculated.

Determination of U.V. and visible range spectra. The U.V. and visible range spectra were measured with a Beckmann Model DU Photoelectric Quartz Spectrophometer with photomultiplier. The sample was dissolved in ethanol and the

solution suitably diluted to enable the bulk of the optical density readings over the most accurate range of the scale (δ 0.1 to 0.5). The measurements are made in silica cells against a blank containing the pure solvent. In the far ultraviolet range (200 - 240 $m\mu$) water must be used as a solvent due to absorption by ethanol in that range.

Determination of infra-red spectra. Infrared spectra measurements were supervised by Dr. J.R. Nunn and Dr. P.F. Enslin of the National Chemical Research Laboratory, C.S.I.R., Pretoria. The spectra were determined from K Br discs over the range 2.5 - 15 μ using a Perkin Elmer Infracord spectrophotometer.

Microanalysis. Analysis of carbon, hydrogen, methoxyl and acetyl were by K. Jones of the National Chemical Research Laboratory, C.S.I.R., Pretoria.

Paper chromatographic techniques.

Solvents and apparatus. For two dimensional paper chromatography water saturated butan-1-ol, the first direction, 2% aqueous acetic acid for the second direction, and Whatman N^{o} 1 chromatographic paper (18$\frac{1}{4}$" by 11$\frac{1}{4}$") were

used. Up to eight chromatograms were suspended on stainless steel frames which were inserted into stainless steel trays containing the solvents. The chromatograms were developed by upward migration.

For "preparative" paper chromatography, as described by Nordström and Swain (65), Roux (39, 54) and Brownell, Hamilton and Casselman (55), Whatman No.3 chromatographic paper, 18½" by 22½", was used. For ascending chromatography in 2% acetic acid the paper was used without prior washing, as minor impurities migrated with the solvent front and did not interfere. However, for descending "preparative" paper chromatography in partitioning solvents, butan -1- ol: acetic acid: water (6:1:2 v/v)(B.A.W.), the paper sheets were prewashed for twelve hours with distilled and deionized water.

All chromatographic operations were carried out at 20° in a constant temperature room.

Selective Spray Reagents Used.

Ammoniacal silver nitrate. This reagent (52) consists of an approximately 1% solution of $AgNO_3$ in distilled water to which 6 N. ammonium hydroxide is added until the silver oxide formed just dissolves.

Chromatograms are sprayed in a fume cupboard using an all glass spray gun. They were washed with distilled water in a stainless steel tray, and fixed with a 0.01% sodium thiosulphate solution. The thiosulphate is removed by washing with tap water (20 minutes) and the chromatograms finally allowed to dry in the tray.

Bis-diazotized benzidine. This reagent (60) consists of two solutions: (A) Benzidine (5g) stirred with concentrated hydrochloric acid (14 ml) and the suspension dissolved in water (980 ml), and (B) 10% aqueous sodium nitrite. Two parts of (B) are added to three parts of (A) and the reagent is used immediately after mixing.

After spraying the chromatograms, 1 to 3 minutes is allowed for full colour development before washing with tap water. By washing development of a yellow background is avoided.

Toluene-p-sulphonic acid. This reagent (57) is a 3% solution of toluene-p-sulphonic acid in absolute ethanol. The chromatograms are sprayed lightly and then baked in an oven for five to ten minutes at 80° to 100°.

2:6 dichloroquinonechloroimide. This reagent (71) was used as a 1-2% solution in ethanol, sprayed on lightly, and the chromatogram fumed with ammonia.

THE EXTRACTION AND FRACTIONATION OF TANNINS FROM THE HEARTWOOD OF GUIBOURTIA COLEOSPERMA.

Strips of wood, suitable for parquet block making, but which had not been kiln-dried, were converted into small turnings with a large drill. Such disruption of the cell walls facilitates extraction. The wood turnings (13 Kg) were extracted in 5 litre flat bottomed flasks at room temperature.

Selection of solvent. A small quantity of wood drillings (5 g) was extracted with methanol and examined by two dimensional paper chromatography. The presence of a high proportion of low R_F polymeric material was shown by spraying with silver nitrate. A similar extraction was carried out using a mixture of ethyl acetate and ethanol (19:1 v/v), and examined by two dimensional paper chromatography. Spraying with silver nitrate showed a much lower proportion of polymeric background trail, and since the primary interest is in the higher R_F fractions this solvent was selected for extraction.

The wood drillings were given four successive extractions, and the solvent evaporated under vacuum in a rotary evaporator at 65° to give 404g. of brown-red solids.

Ethylacetate is, however, also a good solvent for

natural fats and waxes. The solids were therefore finely powdered and freed of waxes by Soxhlet extraction, with petroleum (b.p. 58°-65°), prior to fractionation of the polyphenolic constituents.

This de-waxing process was repeated six times in all. After each extraction the material was dried, dissolved in methanol, and the methanol evaporated under reduced pressure. This exposes a fresh surface each time and ensures complete removal of waxes.

Enrichment of the high R_F constituents. Two dimensional chromatograms of the ethyl acetate: ethanol extracts showed the presence of a low proportion of low R_F tannins and a high proportion of tannins of intermediate R_F. The toluene-p-sulphonic acid spray reagent indicated the presence of two leuco-anthocyanidins, one at R_F 0.53 (2% aqueous acetic acid) and the other at R_F 0.55. Further enrichment was thus essential to enable the separation of high R_F leuco-anthocyanidins. Precipitation with chloroform was used.

Conditions of precipitation. The correct proportion of chloroform to be used was determined by the following empirical procedure.

Varying quantities of chloroform as below, were

added to three flasks each containing 13.5g. extractives dissolved in 100 ml. ethanol.

Sample A = 25 ml. $CHCl_3$.
" B = 35 " "
" C = 45 " "

Each precipitate was filtered rapidly (Whatman No.541) and the filtrates examined by two dimensional paper chromatography. The chromatograms, on spraying with ammoniacal silver nitrate (52) showed that the filtrate from sample C contained a negligible amount of polymeric background trail compared with samples A and B. The proportion of chloroform used for C was therefore satisfactory, and the total extractives were treated in this way. The precipitates were retained (183g.) and the combined filtrates evaporated to dryness at 65° (221 g.) (Precipitate A).

Separation of the soluble components was attempted by means of "preparative" paper chromatography. The tannins (1g.) were dissolved in 25 ml. methanol and the solution streaked onto the origin of 5 sheets of Whatman No.3 paper (200 mg. per sheet) and the chromatograms developed by the ascending technique in 2% aqueous acetic acid. Development of the chromatograms was slow and uneven, due, apparently to

the presence of an insoluble substance remaining on the origin.

Removal of water insoluble portion. Precipitate A (1g) in 5 ml. ethanol, was treated with water (10 ml.), added slowly and with continuous shaking, until a precipitate formed. The filtrate was evaporated to dryness under reduced pressure, and 200 mg. applied to one sheet of Whatman No.3. Development in 2% acetic acid was rapid and even. The total precipitate A was accordingly treated in 10g. amounts by dissolving in a minimum ethanol and adding water (100 ml.) as before. The solutions were filtered through Whatman No.541 paper, the precipitates retained and dried (101g.), and the filtrates evaporated under reduced pressure as before (precipitate B (120g.)).

The separation of the leuco-anthocyanidin from precipitate B. The separation of the leuco-anthocyanidins by "preparative" paper chromatography at this stage was found to be too laborious and the Craig countercurrent partitioning method (56) was used for further enrichment.

The Craig machine used was a fully automatic model, with 160 tubes, built by "Glasapparatebau Gottingen, Helmut Rettberg". Each tube holds 50ml. upper and lower phase.

First Craig Separation.

Sixteen litres of the partitioning mixture,

butan-2-ol : benzine : water (4:1:5^{v}/v), was prepared. Precipitate B (120g.) was warmed (60^{o}) with the lower phase of the partitioning mixture (400 ml.), with shaking. Insolubles, which separated as a brown sticky residue on cooling, were filtered off and dried (60g.). The filtrate was introduced into the first eight tubes of the Craig machine. The remaining 152 tubes were filled with the lower phase and the automatic feeding flask with upper phase of the partitioning mixture. The operation was started with a three minute shaking period followed by a fifteen minutes rest period, during which the two phases separated. This was followed by a transfer operation, transferring the upper phase of each tube into the next tube while the automatic feeding flask refills the upper phase of the first tube. After 160 transfers the machine was stopped and the contents of every fifth tube examined by two dimensional paper chromatography.

(1) Tubes 75-89 contained a leuco-anthocyanidin of R_F 0.53 (2% acetic acid) and 0.62 (water saturated butan-1-ol). This leuco-anthocyanidin gave a grey spot with ammoniacal silver nitrate and a reddish pink with toluene-p-sulphonic acid.

(2) Tubes 90-109 contained what appeared to be a less complex mixture of the leuco-anthocyanidins found in

fraction (1).

(3) Tubes 110-129 showed the presence of a leuco-anthocyanidin of R_F 0.55 (2% acetic acid) and 0.74 (water saturated butan-1-ol). The leuco-anthocyanidin did not reduce ammoniacal silver nitrate and produced an orange-pink colour with toluene-p-sulphonic acid.

(4) Tubes 130-145 contained what appeared to be a more complex mixture containing the same leuco-anthocyanidin found in (3).

The tubes were grouped together as above, and the organic phase separated. The aqueous phase was extracted exhaustively with ethyl acetate and the extracts added to the organic phase. These were evaporated to dryness as before.

Yields

(1) Tubes 75-89 = 2.4 g.
(2) " 90-109 = 5.1 g.
(3) " 110-129 = 9.95g.
(4) " 130-145 = 6.45g.

Fractionation of the Craig Fractions by "Preparative" Paper Chromatography.

Fraction (3) (9.95g.) was dissolved in 250 ml. methanol and streaked on the origin of 50 sheets Whatman No.3 chromatographic paper (200 mg. per sheet). These were developed

by the ascending method in 2% aqueous acetic acid.

The sheets were dried in a current of air and examined under ultraviolet light. The leuco-anthocyanidin band was located at R_F 0.61 by ammoniacal silver nitrate (weak grey) and toluene-p-sulphonic acid (orange-pink) spray reagents. The bands were cut and the strips eluted with 70% ethanol. The combined eluates were concentrated under reduced pressure at 65°.

The off white amorphous powder was examined by two dimensional paper chromatography and found to consist mainly of a single component together with a component which ran to a slightly higher R_F in both aqueous and partitioning solvents. Both components give a similar colouration (orange-pink) with toluene-p-sulphonic acid spray reagent. The powder, in methanol (100 ml.), was streaked on 20 sheets of prewashed Whatman No.3 paper and the chromatograms developed by the descending method in partitioning solvent (B.A.W. 6:1:2 v/v). The sheets were dried and the leuco-anthocyanidin fraction identified as before, bands cut and eluted and the eluate evaporated to dryness, under reduced pressure, at 60°. A light coloured amorphous powder resulted (0.55g.) which was easily soluble in both water and alcohol. It was examined by two dimensional paper chromatography using the

following spray reagents.

(1) Ammoniacal silver nitrate (52). The reduction with silver nitrate was very weak and a light grey spot was only apparent when the substance was run at relatively high concentrations. This indicates the possibility of a single phenolic hydroxyl group on both A and B rings, since mono- and meta-hydroxy phenolic groups reduce silver nitrate very slowly and consequently give either no reaction or a very weak reduction.

(2) Bis-diazotized benzidine (60). With this reagent a very pale yellow develops after ten minutes. The yellow colour indicates monohydroxy phenolic-nuclei and the slow development again points to the presence of a single phenolic hydroxyl on the B ring (61).

(3) Toluene-p-sulphonic acid. A relatively strong orange-pink develops indicating that the substance is probably a leuco-anthocyanidin. This would also suggest that it is not hydroxylated in the 5 position since such leuco-anthocyanidins show up as yellow red spots and only after prolonged heating.

(4) 2:6 dichloroquinonechloroimide. After fuming with ammonia this reagent gives a purple, which fades rapidly to a light brown colour.

On all these two dimensional chromatograms a

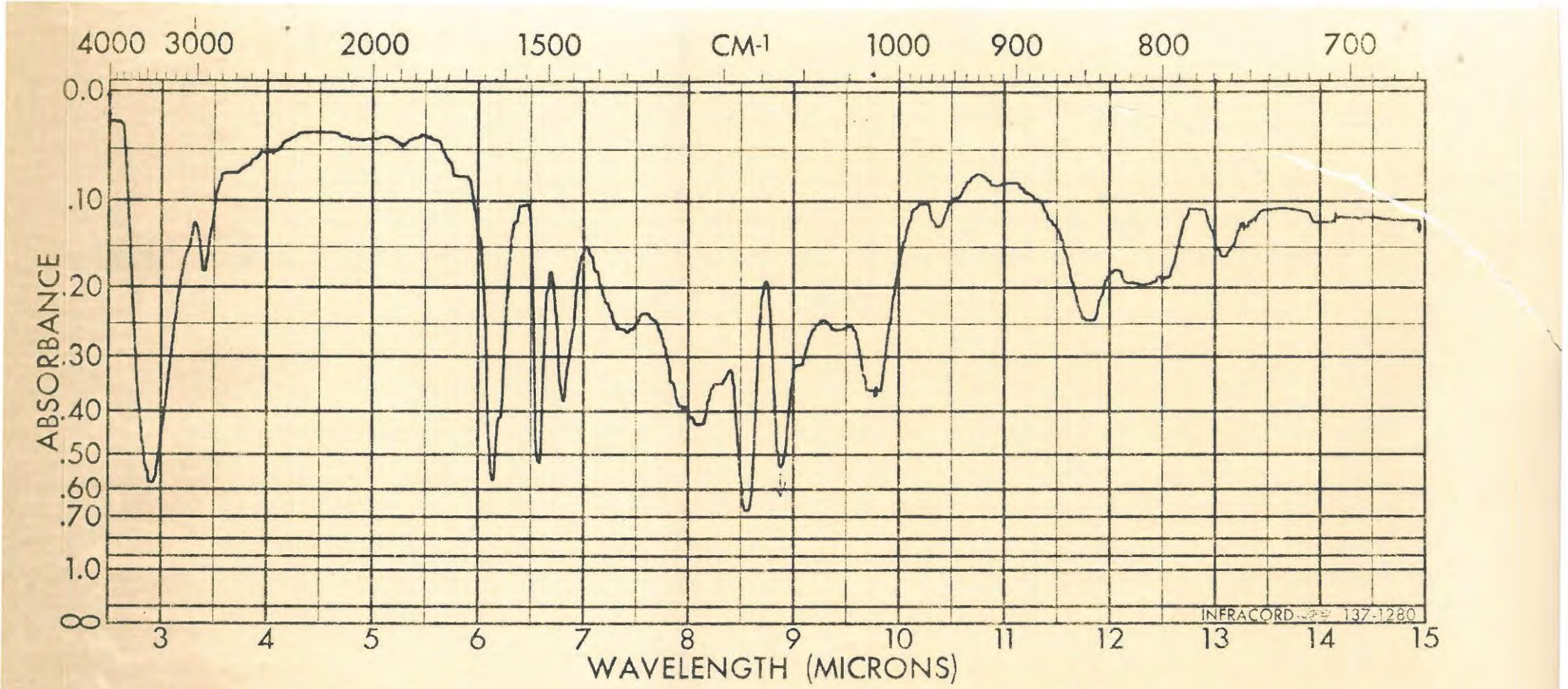


FIGURE 1. INFRARED ABSORPTION SPECTRUM OF GUIBOURTACACIDIN (FIRST SAMPLE)

second spot, which reacted in an exactly similar way to all the spray reagents but with the exception that it gave no colour with 2:6 dichloroquinonechloroimide, appeared at a slightly higher R_F in both aqueous and partitioning solvents.

Second Craig Separation.

The leuco-anthocyanidin (0.39g.) was separated as before from 9 Kg. of wood drillings.

Percentage yield of leuco-anthocyanidin from first Craig separation, based on the total wood drillings extracted = 0.0042%. Percentage yield of leuco-anthocyanidin from second Craig separation = 0.0043%.

Investigation of the Leuco-anthocyanidin.

The compound refused to crystallize from water, ethanol-water or acetone. It sinters at 127°, reddens at 150° and decomposes at 170°. Found: C,65.42; H,5.82. Calculated for $C_{15} H_{14} O_5$: C,65.69; H,5.14%.

The ultraviolet and visible range spectra were examined in ethanol, λ max. 280 mμ. In accordance with predicted behaviour there was no shift to longer wavelength on the addition of ethanolic aluminium chloride. The leuco-anthocyanidin (8.40 mg./100 ml.) in ethanol gave an absorption density, 0.598 at 280 mμ. $E^{1\%}_{1cm}$ 71.2, and εmax. 1935.

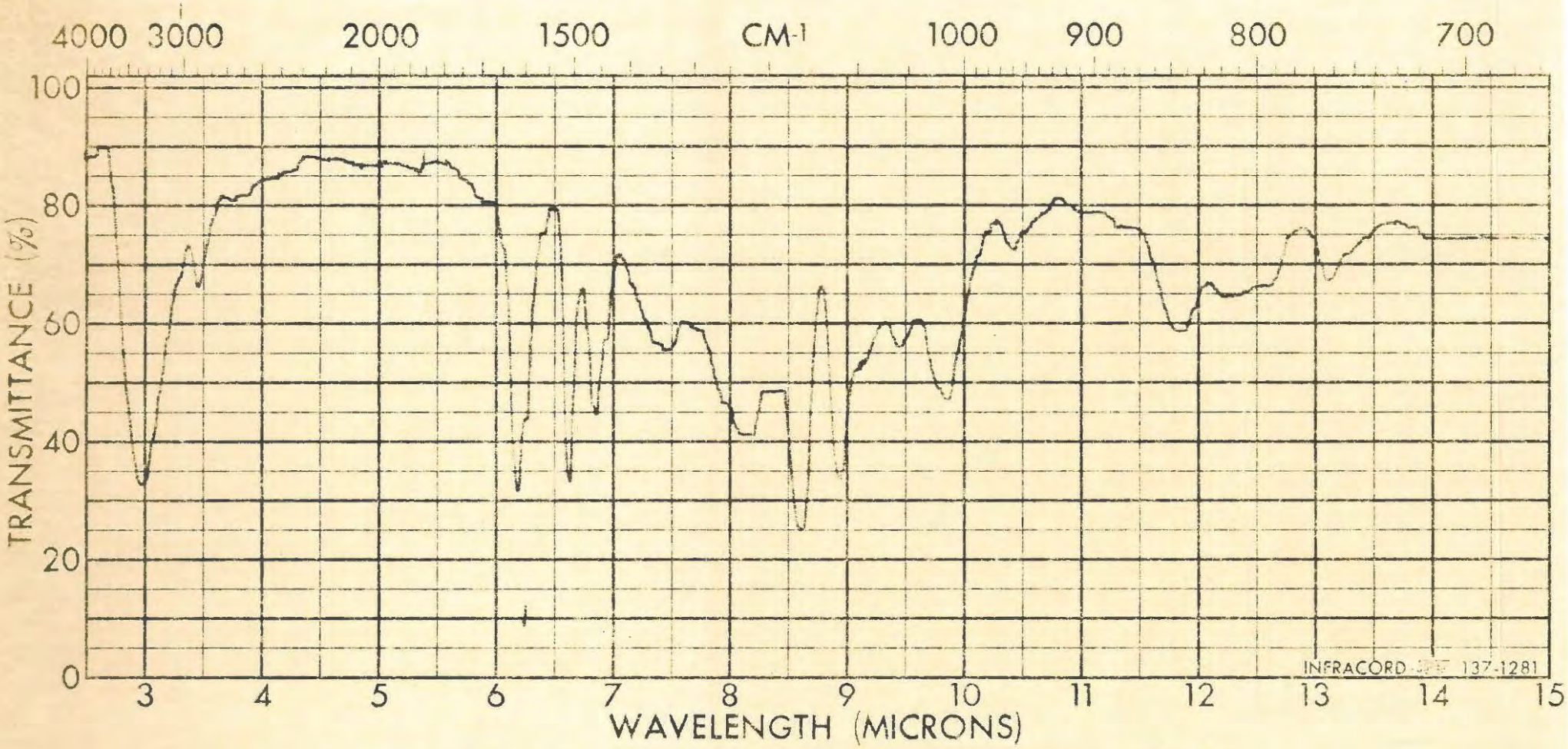


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FIGURE 2. INFRARED ABSORPTION SPECTRUM OF GUIBOURTACACIDIN (SECOND SAMPLE)

The infrared spectra of the leuco-anthocyanidin samples from the two separate preparations, were identical over the range 2.5-15μ (Figure 1 and 2). The infrared spectrum showed the following absorption : 3450 cm. $^{-1}$ (S) (-OH stretching frequency); 2940 cm. $^{-1}$ (W) (C-H stretching frequency); 1525 cm. $^{-1}$ (S), 1505 cm. $^{-1}$ (S) and 1475 cm. $^{-1}$ (m) (C=C skeletal in-plane vibrations); 1350 cm. $^{-1}$ (W) (-OH deformation); 1030 cm. $^{-1}$ (m) (-OH deformation secondary aliphatic OH); 1130 cm. $^{-1}$ (S) (aryl-alkyl ether).

Formation of the anthocyanidin from the leuco-anthocyanidin. The reaction was carried out according to the method of Pigman, Anderson, Fischer, Buchanan and Browning (30). The compound (6 mg.) was weighed into an 8 ml. pressure tube and dissolved in 5 ml. of a mixture of isopropyl alcohol : 3N.HCl (4:1 ^{v}/v). The tube was heated on a waterbath for one hour and the yellow-orange pigment formed examined by paper chromatography on Whatman No.1 paper.

The pigment was compared with the following synthetic anthocyanidins:- 3,7,4'-trihydroxyflavylium chloride (85), 3,7,3',4'-tetrahydroxyflavylium chloride (fisetinidin chloride) (86), 3,7,3',4',5'-pentahydroxy-flavylium chloride (robinetinidin chloride) (40) and with

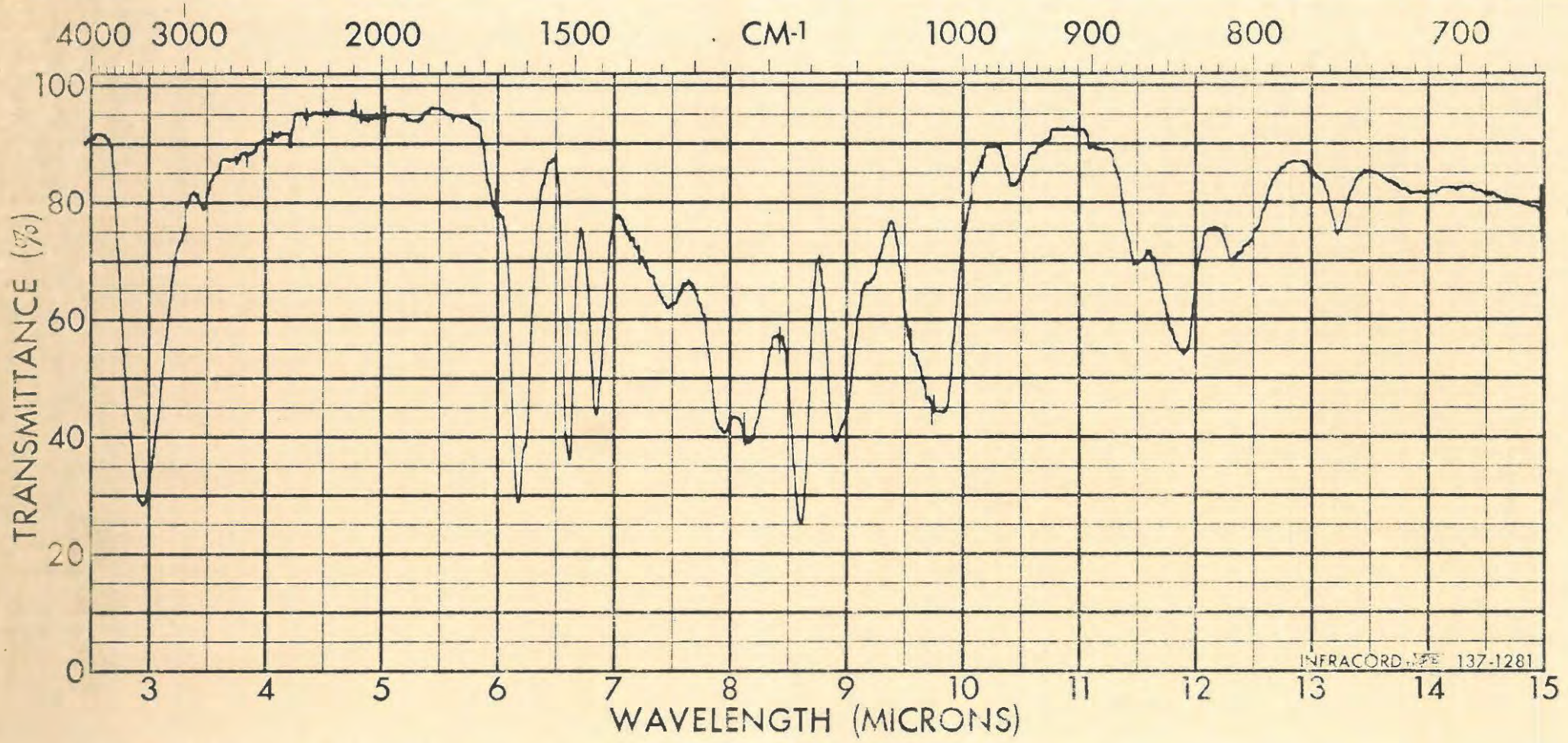

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FIGURE 2a. INFRARED ABSORPTION SPECTRUM OF SYNTHETIC GUIBOURTACACIDIN.

3,5,7,4'-tetrahydroxyflavylium chloride (pelargonidin chloride). Pelargonidin chloride was obtained from flowers of Pelargonium spp. by boiling with 3N.HCl for 20 minutes, extracting with amyl alcohol (5ml.), and spotting on the chromatogram. These compounds were compared because of their similarities, in R_F and absorption spectra, with the generated pigment.

The chromatogram was developed one dimensionally, by the descending technique, in a mixture of 90% formic acid: 3N.HCl (1:1 v/v), and the R_F values determined. The colours of the anthocyanidin were examined under ultraviolet light and the change in colour noted. The anthocyanidin spots were then cut out and the light absorption measured from the paper chromatogram by the method of Bradfield and Flood (69) before and after applying a few drops of ethanolic aluminium chloride (5% w/v) (62, 63). The results are tabulated in Table I.

TABLE I.

Anthocyanidin chlorides	R_F	λ max. mμ.	λ max. after $AlCl_3$ addition	Colour in visible light	Colour in U.V. light.
Generated pigment	0.60	490	490	orange-yellow	intense yellow
3,7,4' trihydroxy flavylium chloride.	0.60	490	490	orange-yellow	intense yellow
Fisetinidin chloride	0.43	520	545	pink	orange
Robinetinidin chloride	0.26	532	560	pink-purple	deep pink
Pelargonidin chloride	0.33	530	530	reddish pink	orange red

From the table it is obvious that the pigment generated and the synthetic anthocyanidin 3,7,4'-trihydroxy-flavylium chloride are identical. Both have the same R_F, λ max. and there is no shift in wavelength on addition of ethanolic aluminium chloride, pointing to the absence of ortho-hydroxy groups on the A or B rings. This anthocyanidin is readily recognised by its characteristic and distinctive bright yellow fluorescence under ultraviolet light. In ethanol both synthetic 3,7,4'-trihydroxyflayylium chloride and the anthocyanidin from the flavan-3:4-diol have a λ max. of 512 mμ.

Quantitative estimation of anthocyanidin generated. The leuco-anthocyanidin (4.37 mg.) was dissolved in 25 ml. propan-2-ol : 3N.HCl (4:1 $^{v}/_{v}$) in a volumetric flask. 5 ml. of this mixture was pipetted into a pressure tube and the anthocyanidin generated on a water bath for one hour. The contents of the tube was transferred quantitatively to a 100 ml. volumetric flask by washing with ethanol and the absorption density measured at 512 mμ. (= 0.399, 0.394). Synthetic 3,7,4'-trihydroxyflavylium chloride (1.7 mg.) was similarly dissolved in 5 ml. of the propan-2-ol : 3N.HCl (4:1 $^{v}/_{v}$), diluted to 500 ml. with ethanol, and the absorption density measured as above (= 0.480).

From the above the percentage yield of the anthocyanidin from the flavan-3,4-diol was calculated (32.36% and 31.93%). By comparison the percentage yields of anthocyanidin from (+)-leuco-fisetinidin and (+)-leuco-robinetinidin (23.8% and 26.9% respectively), were lower (39).

Microdegradation of the leuco-anthocyanidin by KOH fusion. Information regarding the A and B nuclei of a C_{15} compound may be obtained from alkali fusion using an improved micro-degradation technique by Roux (54).

2 to 4 mg. of the compound was weighed into a 15 x 1 cm. hard glass tube and fused for 90 seconds with

molten KOH. The soluble degradation products were separated by the bicarbonate technique into phenols and phenolic-carboxylic acids. These were identified on paper chromatograms by comparison with resorcinol, phloroglucinol, β -resorcylic acid, gallic acid, protocatechuic and p-hydroxy benzoic acids as references. The chromatograms of the phenolic portion were developed by the ascending technique in butan-1-ol : acetic acid : water (4:1:5 $^{v}/v$), and the phenolic acids in 2% acetic acid.

The degradation products of the leuco-anthocyanidin were found to be resorcinol and β-resorcyclic acid (A nucleus), and p-hydroxy benzoic acid (B nucleus). The degradation products ran to the same R_F values as the reference compounds and gave the same colour reactions with the spray reagents used. Resorcinol showed a claret maroon spot (R_F 0.86, R_F of degradation product 0.87) with bis-diazotized benzidine (60). The acids were identified with diazotized p-nitroaniline spray reagent (71). This reagent, after overspraying with ethanolic caustic soda, gave a clear rose red colour with p-hydroxy benzoic acid (R_F 0.59, R_F degradation product 0.57) and a deep purple with β -resorcylic acid (R_F 0.49, R_F degradation product 0.47). The deep purple fades to an orange brown after a short while.

Optical rotation. $[\alpha]_D^{21}$ + 11.7° ± 1.7° in acetone : water (1:1 v/v) (c = 0.66).

Methylation of the leuco-anthocyanidin. Diazomethane in ether (250 ml.), generated from 20g. nitrosomethylurea, was added to a methanolic solution (50 ml.) of the leuco-anthocyanidin (100 mg.), and the reaction was allowed to proceed for 36 hours at -15°. The excess diazomethane was removed with acetic acid, the solution concentrated under reduced pressure to 2 ml., and poured into water (10 ml.). The white flocculent precipitate formed was allowed to stand overnight and sucked off.

This methylated derivative proved difficult to crystallise. After 3 weeks, however, a crystalline material was obtained from ethanol/water.

Found: C, 59.36, 59.26; H, 5.67, 5.34; $(OCH_3)_2$, 14.05, 12.95. Calculated $C_{17}H_{18}O_5$: C, 67.54; H, 6.0; $(OCH_3)_2$, 20.53%.

From the analytical values it is obvious that this methylated derivative was probably impure.

A second methylation attempt on the leuco-anthocyanidin (200 mg.) refused to crystallize from ethanol/water.

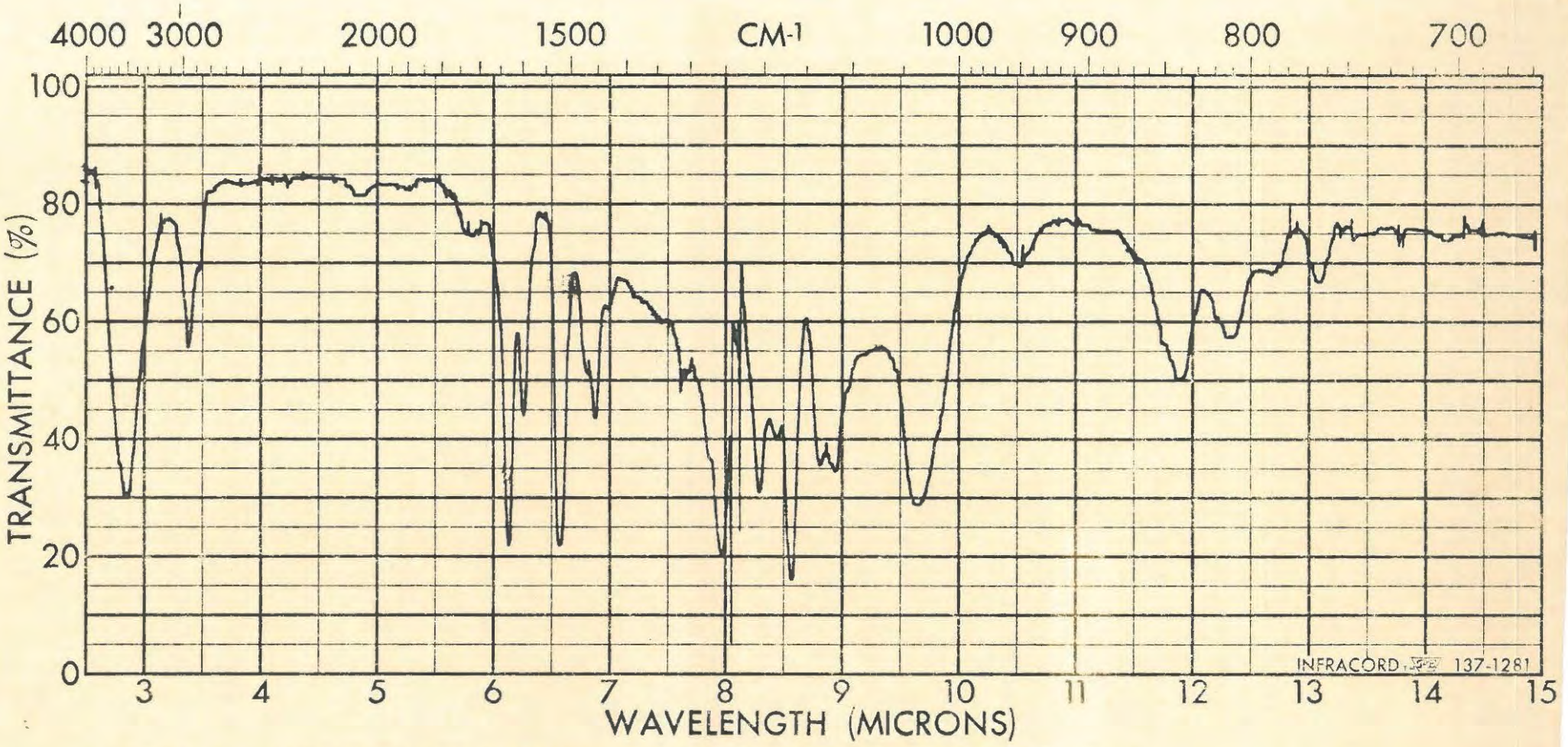

FIGURE 3. INFRARED ABSORPTION SPECTRUM OF METHYLATED GUIBOURTACACIDIN.

In order to free the methylated derivative of suspected impurities the products of both methylations were combined and separated by "preparative" paper chromatography on 10 sheets of Whatman No.3 paper. Development was by the ascending technique in 10% acetic acid, which was found to give better separation, with less trailing, than either 2% or 5% aqueous acetic acid. The methylated product was located (R_F 0.68), on a test strip sprayed with toluene-p-sulphonic acid, as a pink band. These bands were cut and eluted with 70% ethanol and the eluates concentrated under reduced pressure. The purified methylated compound failed to crystallise and was accordingly dissolved in ethanol (2 ml.) and filtered into dust free water (10 ml.). A white precipitate was filtered off after a few days (26 mg.) (m.p . 56°).

Found:- C, 66.70 ; H, 6.85 ; $(OCH_3)_2$ 19.03

Calculated for $C_{17}H_{18}O_5$:- C, 67.54 ; H, 6.0 ; $(OCH_3)_2$, 20.53%.

The above methylated leuco-anthocyanidin (1 mg.) was dissolved in 5 ml. propan-2-ol: 3N.HCl (4:1 v/v) and heated, in a pressure tube, for one hour on a waterbath. The dimethyl anthocyanidin (λ max. 500 mμ) and the unmethylated anthocyanidin (λ max. 512 mμ) appear to have

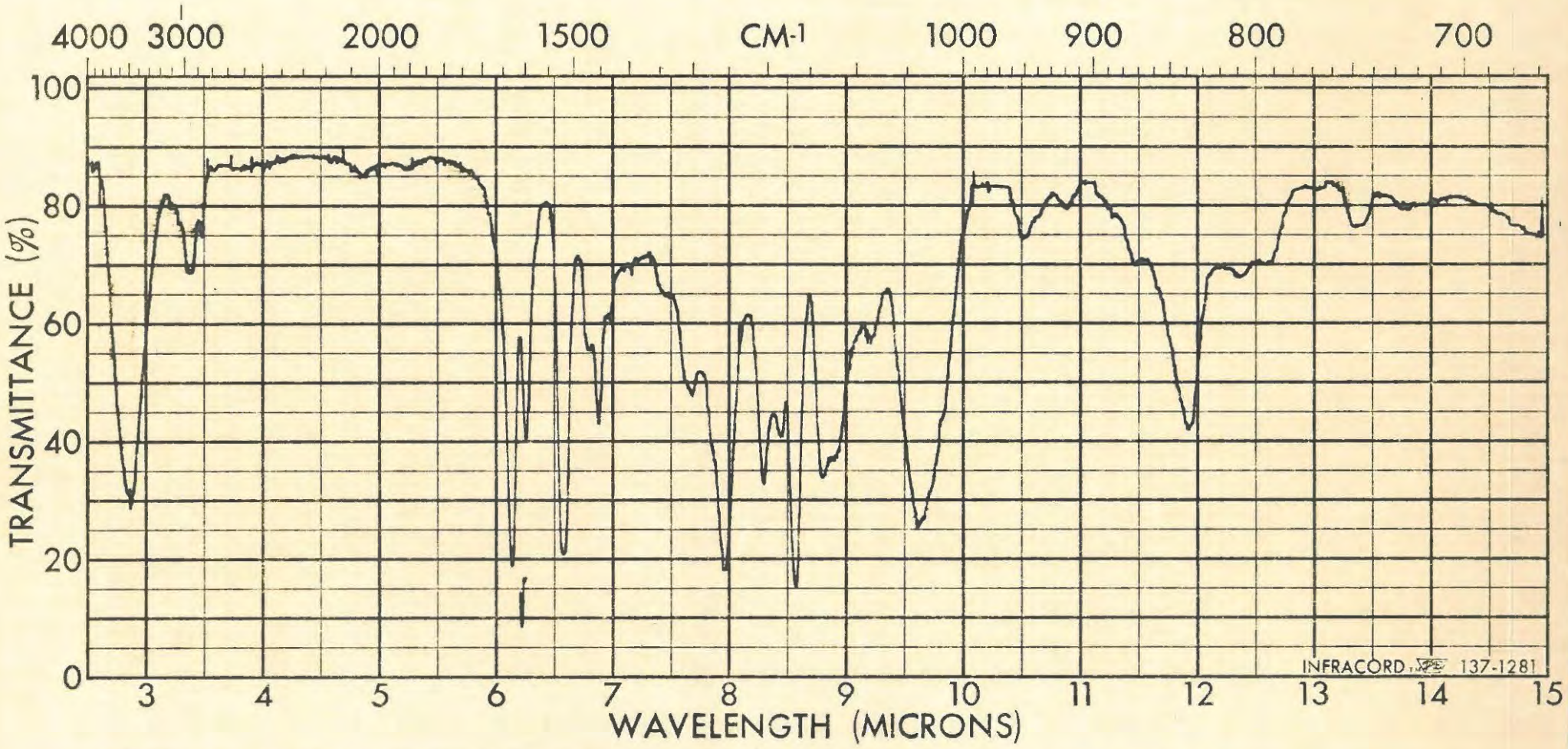

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FIGURE 4. INFRARED-ABSORPTION SPECTRUM OF (±)-7,4'-DIMETHOXYFLAVAN-3,4-DIOL

the same colour, but the R_F of the dimethyl anthocyanidin (in 90% formic acid : 3N.HCl (1:1 v/v)) is higher (0.78) than the R_F of 3,7,4'-trihydroxyflavylium chloride (0.60). This increase in R_F is consistent with the methylation of two hydroxyl groups as methylation of phenolic hydroxyl groups reverses the effect of hydroxylation (58). Therefore the anthocyanidin from the methylated flavan-3:4-diol may be presumed to be the 7,4'-dimethyl derivative of 3,7,4'-trihydroxyflavylium chloride.

Optical rotation of methylated leuco-anthocyanidin.

$[\alpha]_D^{21}$ + 9.2° ± 0.7° in symm. tetrachloroethane (C = 0.6)

The infrared absorption spectra of the methylated leuco-anthocyanidin was compared with that of a synthetic sample of 7,4'-dimethylflavan-3:4-diol (94), Figures 3 and 4. They were virtually superimposable over the higher frequency range (4000 cm^{-1}-1000 cm^{-1}) but there were minor differences over the lower frequency range (1000 cm^{-1}-700 cm^{-1}) particularly at 765 cm^{-1}.

Acetylation of the leuco-anthocyanidin. The compound (51 mg.) dissolved in 0.2 ml. pyridine was treated with acetic anhydride (0.3 ml.). This was allowed to stand at room temperature for 4 hours and poured into water (10 ml.). The milky

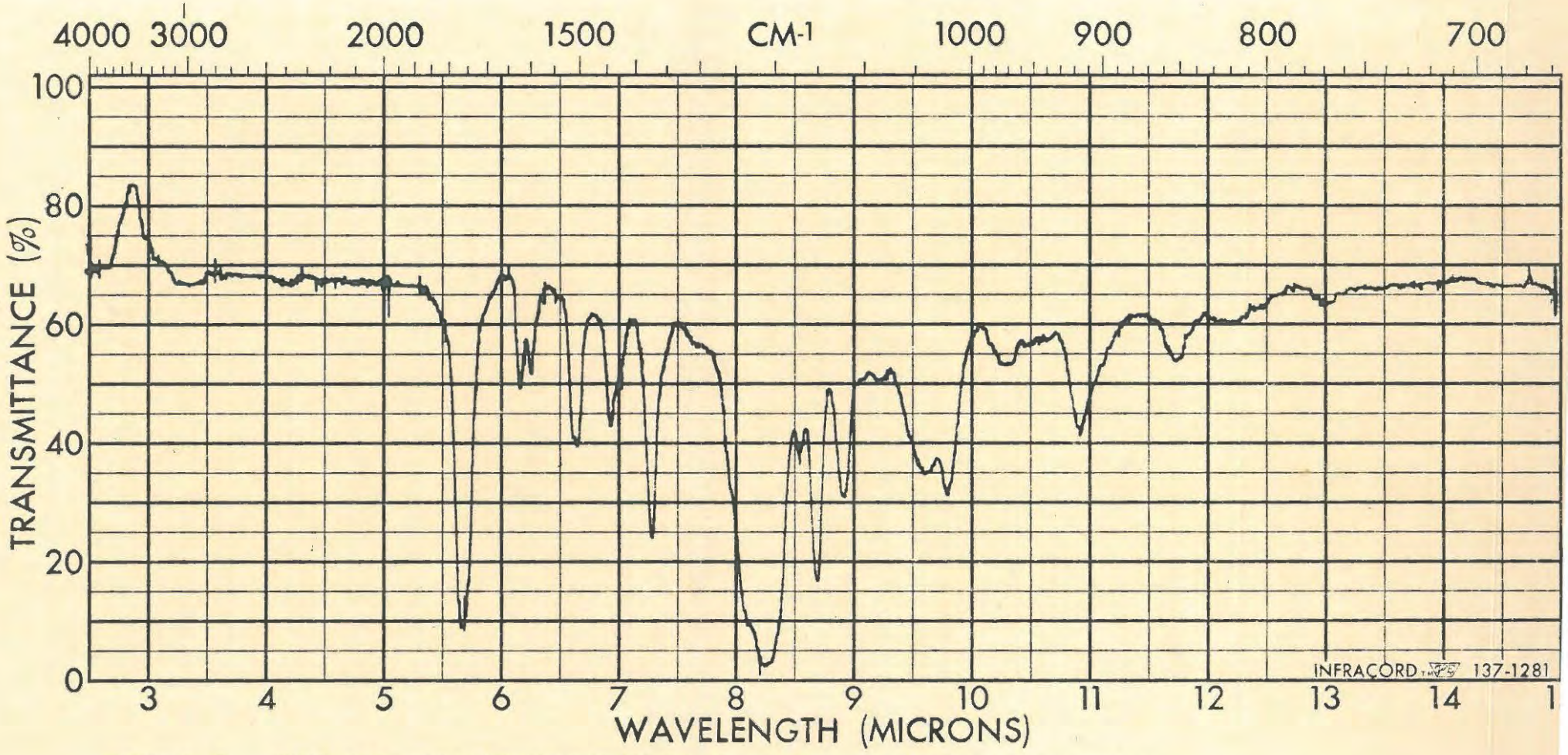

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FIGURE 5. INFRARED ABSORPTION SPECTRUM OF ACETYLATED GUIBOURTACACIDIN

colloidal solution formed was stirred with a small glass rod, and the product allowed to harden overnight. The acetylated product (42.5 mg.) was filtered off and dried under vacuum over $CaCl_2$, m.p. 63°. Attempts to crystallise the compound were unsuccessful. It settled as a sludge from ethanol.

Found:- C, 63.36: H, 5.54; $(COCH_3)_4$, 36.2.
Calculated for $C_{23}H_{22}O_9$: C, 63.44; H, 5.01; $(COCH_3)_4$, 38.92%.

Optical rotation.

$[\alpha]_D^{21}$ + 40.4° ± 0.1° in symm. tetrachloroethane (C = 0.6).

Comparison of R_F values of the amorphous leuco-anthocyanidin with (+)-leuco-fisetinidin , (+)-7,3',4'-trihydroxyflavan-3,4-diol, from Acacia mearnsii (29) (35) and (+)-leuco-robinetinidin, (+)-7,3',4',5'-tetrahydroxyflavan-3,4-diol from Robina pseudacacia (40) (41).

These were spotted on sheets of Whatman No.1 chromatographic paper and examined by one dimensional chromatography in aqueous (2% acetic) and partitioning (B.A.W.) solvents. Development in aqueous solvent was by the ascending technique and by the descending technique

in partitioning solvent. The chromatograms were sprayed with toluene-p-sulphonic acid reagent (57). Leuco-robinetinidin gave a scarlet colour, leuco-fisetinidin a reddish pink and the new leuco-anthocyanidin an orange pink. In these chromatograms the new leuco-anthocyanidin was always accompanied by a low proportion of a forward running component which gave the same orange-pink with the toluene-p-sulphonic reagent. This associated compound could apparently not be eliminated by repeated chromatographic separation in spite of marked differences in R_F compared with the flavan-3,4-diol. R_F values are tabulated in Table II.

Table II.

	Ascending R_F (2% acetic).	Descending R_F (B.A.W. 6:1:2 v/v)
Leuco-robinetinidin	0.51	0.51
Leuco-fisetinidin	0.53	0.70
Leuco-anthocyanidin	0.55	0.83
Associated spot.	0.66	0.92

Formation of the O-ethyl ether. The spontaneous generation of the forward running component during handling in methanolic or ethanolic solution was analogous to the similar behaviour of isomelacacidin (50) and isoteracacidin (51). The following

experimental procedure was therefore adopted.

The leuco-anthocyanidin (10 mg.) was refluxed with ethanol (10 ml.) and acetic acid (0.1 ml.). After two hours the reaction product was examined by one dimensional ascending paper chromatography in a partitioning solvent (B.A.W. 4:1:5 $^{V}/v$) and the chromatograms sprayed with toluene-p-sulphonic acid. The reaction was not complete and consequently refluxed for a further two hours. The product was similarly examined and the reaction was still not quantitative since the lower R_F component was still present, although the O-ethyl ether was now the more prominent component. On spraying a similar chromatogram with 2:6 dichloroquinonechloroimide it was observed that the O-ethyl ether gave no reaction but the lower R_F component gave a purple colour. (R_F values are cited in Table III). The anthocyanidin generated by the O-ethyl derivative was found to be identical to that from the leuco-anthocyanidin.

Hydrolysis of the O-ethyl ether. The O-ethyl derivative formed above was evaporated to dryness under reduced pressure, and refluxed with 0. 1 N. acetic acid (5 ml.) for twenty minutes. This was examined similarly by one dimensional ascending chromatography, in 2% aqueous acetic

acid, using the natural leuco-anthocyanidin as the reference compound (Table III).

Table III.

R_F values of O-ethyl ether derivative and the hydrolysed product.

	R_F values (Ascending)	
	2% Acetic.	B.A.W. 4:1:5 v/v (Upper layer)
Natural leuco-anthocyanidin	0.55	0.77
O-ethyl ether derivative	0.66	0.85
Hydrolysed (0.1N. Acetic) product	0.60	

Optical rotation of hydrolysed product.

$[\alpha]_D^{21}$ + 8.8° ± 0.9° in ethanol (C = 0.6)

$[\alpha]_D^{21}$ + 4.8° ± 0.3° in acetone:water (1:1 v/v) (C=0.5)

From the table (III) it may be seen that the hydrolysed product has an R_F intermediate between the natural leuco-anthocyanidin and its O-ethyl ether derivative. The optical rotation is of lower value than that estimated for the natural leuco-anthocyanidin.

Potentiometric test for the presence of cis-hydroxyl groups.

The trimethyl ether of (+)-leuco-fisetinidin (20 mg.) was dissolved in 50% aqueous ethanol (50 ml.). The pH was determined = 7.4 and adjusted to 9.93 by the addition of dilute sodium carbonate solution. Sodium borate (27 mg.) was dissolved in 50% aqueous ethanol (100 ml.) and the pH determined = 10.18. Aliquots of this sodium borate solution were added, from a burette, to the solution (50 ml.) of the methylated (+)-leuco-fisetinidin. Following each addition the pH was determined.

A reduction in pH from 9.93 to 9.14 was observed (0.8 ml. sodium borate) after which the pH increased to 9.63 (5 ml. sodium borate). This could point to the formation of an acidic borate complex and the presence of cis-hydroxyl groups. However, when the above reaction was repeated under conditions which prevented the access of CO_2 by employing an enclosed beaker and a hydrogen bubbler, no reduction in pH was observed.

This test, due to Böeseken (89), was used by Feppler (88) for (+)-mollisacacidin ((+)-leuco-fisetinidin) with positive results. However, since the possibility of CO_2 affecting the pH was not considered by Feppler the test appears to be of doubtful validity.

Molecular weight determination by depression of the freezing point of camphor (Rast's Method) (87). Camphor (124.2 mg. m.p. 181°) was intimately mixed and melted with benzoic acid (12.1 mg.). The average m.p. (6 readings) was found to be 151°, a depression of 30°. The molecular depression constant K was calculated (376).

Similarly the acetylated leuco-anthocyanidin (5.84 mg.) was mixed with camphor (72.04 mg.). The average m.p. (6 readings) was found to be 174.5°, a depression of 6.5°. From the above the molecular weight of the acetylated leuco-anthocyanidin (four acetyl groups) was estimated.

Found: Molecular weight, 468.9.
Calculated molecular weight for $C_{23}H_{22}O_9$, 442.4.

Chromatographic examination of certain other Guibourtia Species.

Fine drillings (5 g.) from the heartwoods of G. arnoldiana, G. demeusei and G. tessmannii were extracted with methanol (30 ml.). The wood drillings were separated by filtration through Whatman No.541, and the methanol evaporated under reduced pressure. The extracts were examined by two dimensional paper chromatography and by generating anthocyanidins with propan-2-ol : 3N.HCl (4:1 v/v) at 100°. The anthocyanidins formed were examined by one dimensional descending chromatography in 90% formic acid: 3N.HCl (1:1 v/v).

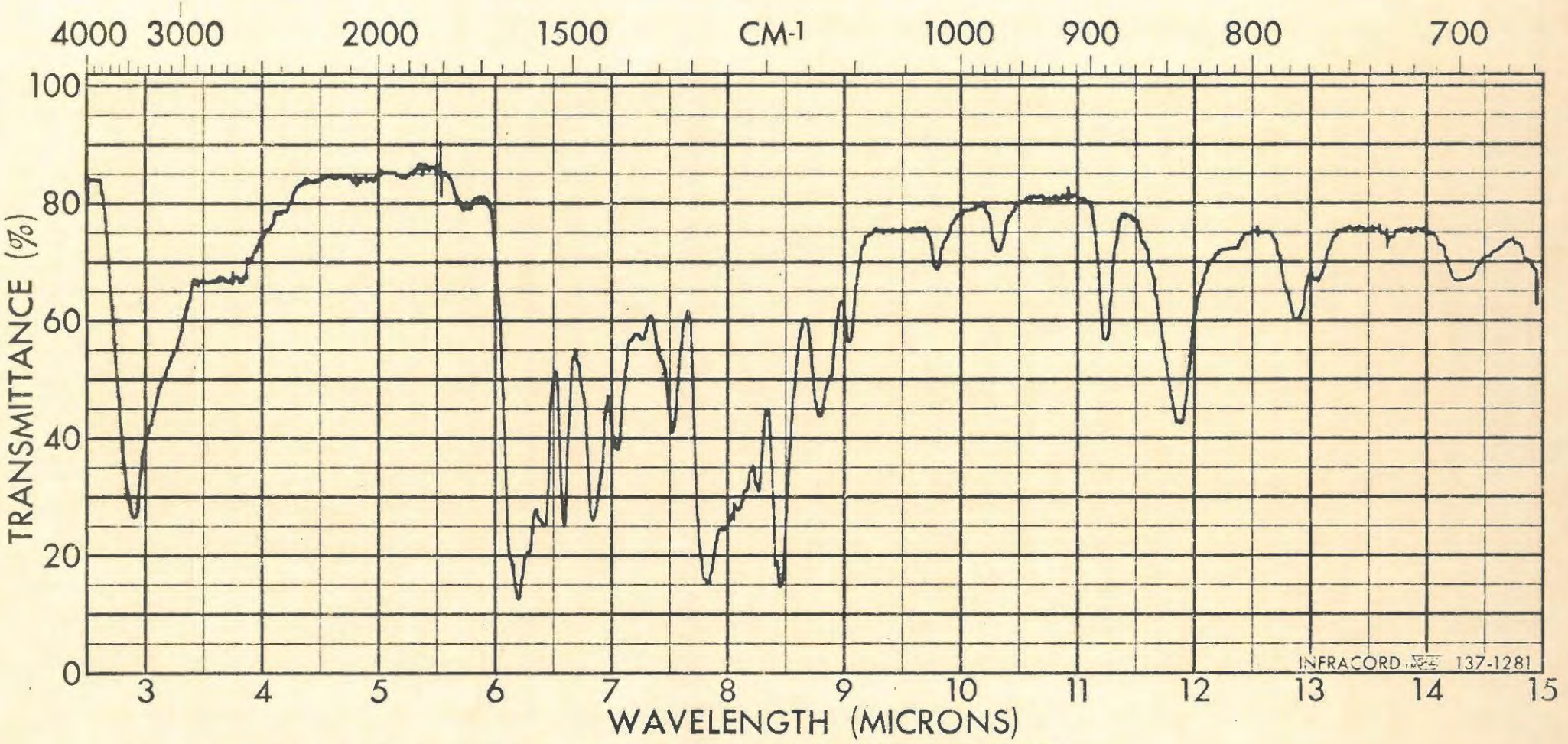

FIGURE 6. INFRARED ABSORPTION SPECTRUM OF 7,4'-DIHYDROXYFLAVONOL

G. demeusei and G. tessmannii contained polymeric (low R_F) and monomeric (R_F 0.53 in 2% acetic acid, red-pink with toluene-p-sulphonic acid) leuco-fisetinidins, which yield fisetinidin chloride (R_F 0.43). G. arnoldiana differed in that it appeared to contain leuco-cyanidins only, in low proportion, which gave cyanidin chloride (R_F 0.20).

Synthesis of (±)-7,4'-dihydroxyflavan-3:4-diol.

(±)-7,4'-Dihydroxyflavan-3:4-diol was synthesised by hydrogenation of the corresponding dihydroflavonol, (±)-7,4'-dihydroxyflavanonol. The (±)-7,4'-dihydroxyflavanonol was synthesised by sodium hyposulphite reduction of 7,4'-dihydroxyflavonol.

Synthesis of 7,4'-dihydroxyflavonol. Robinson and Shinoda (78) synthesised the flavonol kaempferol by the method indicated by Allan and Robinson (77). Utilizing this method the 7,4'-dihydroxyflavonol was synthesised.

ω-Methoxyresacetophenone was prepared according to the method of Slater and Stephen (75). Anisic anhydride was prepared (76) by refluxing anisic acid (20 g.) with acetic anhydride (40 g.) for 20 hours. The acetic acid formed and the excess acetic anhydride was removed by vacuum

distillation. The residue crystallized from ethyl acetate in needles (15 g.) m.p. 98°.

ω -Methoxyresacetophenone (2.5g.) was condensed with anisic anhydride (10 g.) and sodium anisate (5 g.) under reduced pressure at 170° - 180° for three hours. The product was hydrolysed by refluxing (30 minutes) with 10% alcoholic KOH (100 ml.). The alcohol was removed by vacuum distillation and the residue dissolved in water (50 ml.). The brown solution was saturated with carbon dioxide and the 7-hydroxy-3,4',-dimethoxyflavone precipitated (4.15 g.). Demethylation with HI was accomplished under reflux for 30 minutes in a stream of carbon-dioxide. The mixture was added to dilute sulphurous acid and the resulting 7,4'-dihydroxyflavonol crystallised from a mixture of 50% acetic acid: ethanol (19:1 v/v) (50 ml.). Shiny, reddish brown needles were obtained (470 mg.) m.p. 306° - 309° (sintered at 286°). λ max. (ethanol) at 259 mμ, 319 mμ and 359 mμ. After addition of sodium ethoxide (64, 65, 66) λ max. at 290 mμ, 335 mμ and 420 mμ. Found: C, 66.5 ; H, 3.8. Calculated for $C_{15} H_{10} O_5$: C, 66.67 ; H, 3.73%.

The 7,4'-dihydroxyflavonol (50 mg.) was acetylated with pyridine (0.2 ml.) and acetic anhydride

(0.3 ml.). Yield = 42.5 mg. m.p. 159°. Found: C, 63.30; H, 4.19; $(COCH_3)_3$ 32.4%. Calculated for $C_{21}H_{16}O_8$: C, 63.63; H, 4.07 : $(COCH_3)_3$, 32.58%.

The above flavonol was synthesised by Rao and Seshadri (97), their 'resokaempferol', but it had a different m.p. (280°) although the m.p. of the acetyl derivative (159-160°) was identical to that above.

The method of Algar and Flynn (24), which involves oxidation of the corresponding chalkone with alkaline peroxide, was also used in an attempt to prepare the 7,4'-dihydroxyflavonol, but proved unsuccessful.

Synthesis of (±)-7,4'-dihydroxyflavanonol. The method due to Pew (82) as improved by Shimizu and Yoshikawa (83), was used. The experimental conditions were first tested on a related flavonol.

Reduction of robinetin (7,3',4',5'-tetrahydroxyflavonol to dihydrorobinetin ((±)7,3',4',5'-tetrahydroxyflavanonol).

Robinetin (0.35 g.) was intimately mixed with boric acid (0.14 g) and sodium carbonate (3.5 g.) and the mixture dissolved with warming, in water (50 ml.). Sodium hyposulphite (7 g. 85% pure) was added and the solution immersed in a boiling water bath for 20 minutes. The mixture was diluted with water (60 ml.) and acidified with

dilute hydrochloric acid. The mixture was filtered and the filtrate extracted with four 50 ml. portions of ethyl acetate. The extract was concentrated under reduced pressure and examined by one dimensional ascending chromatography in 2% acetic acid, but the conversion to dihydrorobinetin proved unsuccessful.

The reduction was repeated using the modified method of Geissman and Lischner (84). This involves conducting the reaction in an inert atmosphere of nitrogen or hydrogen. Under these conditions the formation of dihydrorobinetin from robinetin was successful. The ethyl acetate extracts were concentrated to 20 ml. under reduced pressure and separated by "preparative" paper chromatography, by the ascending technique in 2% acetic acid, on four sheets of Whatman No.3 paper. Dihydrorobinetin was located (R_F 0.35) by spraying a test strip with ammoniacal silver nitrate, bands were cut and eluted with 70% ethanol. The eluates were concentrated to a small volume (5 ml.) under reduced pressure and dihydrorobinetin crystallized as granules from water (26 mg., m.p. 225° - 231°). A mixed m.p. with authentic (±) dihydrorobinetin from Robinia pseudacacia gave no depression.

Reduction of 7,4'-dihydroxyflavonol to (±)-7,4'-dihydroxyflavanonol.

7,4'-Dihydroxyflavonol (0.45 g.) was treated in exactly the same way as above with boric acid (0.18 g.), sodium carbonate (4.5 g.) and sodium hyposulphite (10.7 g.). Separation was attained by "preparative" paper chromatography, by the ascending technique in 2% acetic acid, on four sheets of Whatman No.3 paper. The (±)-7,4'-dihydroxyflavanonol was located (R_F 0.49) by spraying a test strip with toluene-p-sulphonic acid (pink), bands were cut and eluted with 70% ethanol. The eluates were concentrated to a small volume (5 ml.) under reduced pressure and the compound crystallized as colourless needles from water. (6 mg.) (m.p. 212° - 215° after sintering strongly at 205°).

Due to the low yield it was not possible to form derivatives.

Synthesis of (±)-7,4'-dihydroxyflavan-3:4-diol.

(±)-7,4'-dihydroxyflavanonol (6 mg.) was hydrogenated for six hours over a platinum oxide catalyst (100 mg.) in methanol (50 ml.). The catalyst was removed by filtration and the filtrate evaporated to dryness under vacuum at 60°.

The product was dissolved in ethanol (5 ml.) and examined by one dimensional paper chromatography both

in 2% acetic acid (ascending) and in a partitioning solvent (B.A.W. 6:1:2^{V}/v) (descending). The natural leuco-anthocyanidin was compared on the same chromatograms. After spraying with toluene-p-sulphonic acid, both the natural and synthetic flavan-3:4-diols gave identical orange-pinks. The natural leuco-anthocyanidin showed a slightly higher R_F in 2% acetic acid (0.55, 0.55, 0.55, 0.56) than the synthetic compound (0.52, 0.52, 0.52, 0.51, 0.52). However, in partitioning solvent the R_F values were similar (0.83, 0.83, 0.83, 0.83 for the natural compound and 0.81, 0.82, 0.82, 0.83 for the synthetic). 3,7,4'-Trihydroxyflavylium chloride was generated from both leuco-anthocyanidins when boiled with propan-2-ol: 3N.HCl (4:1^{V}/v) as before, both giving an orange yellow pigment of identical R_F (0.60) and characteristic bright yellow fluorescence under ultraviolet light. A bright green yellow fluorescent spot was observed at R_F 0.15 (90% formic acid: 3N.HCl (1:1^{V}/v)). When compared by one dimensional descending paper chromatography, in the above solvent, with synthetic 7,4'-dihydroxyflavonol they were both identical in R_F (0.15) and green yellow fluorescence.

The remaining ethanolic solution of (±)-7,4'-dihydroxyflavan-3:4-diol was evaporated to dryness over

$CaCl_2$ in a vacuum desiccator, dissolved in water (1 ml.) and kept at 0° for one week. Colourless needles crystallised (1 mg.) which decomposed at 170° (after sintering at 105°).

The infrared absorption spectrum of the synthetic (±)-7,4'-dihydroxyflavan-3:4-diol was similar to that of the natural compound. There were, however, small but significant differences. Several differences in peak height may be due to the greater purity of the synthetic sample (Figures 1, 2,2a).

Synthesis of (±)-7,4'-dihydroxyflavan-4-ol. (±)-7,4'-dihydroxyflavan-4-ol was prepared by hydrogenation of the 7,4'-dihydroxyflavanone, obtained by ring closure of the corresponding chalkone, 2:4-dihydroxy-phenyl-4-hydroxystyryl ketone.

Synthesis of 2:4-dihydroxy-phenyl-4-hydroxystyryl ketone. 2:4-dihydroxy-phenyl-4-hydroxystyryl ketone was prepared according to the method of Nadkarni and Wheeler (74).

A mixture of p-hydroxybenzaldehyde (5 g.) and resacetophenone (6.25 g.) in ethanol (12 ml.) was treated at 0° with aqueous potassium hydroxide (50 g. in 35 ml. water) in an atmosphere of hydrogen. The mixture was refluxed for 30 minutes and kept at room temperature overnight out of contact with air. Water (50 ml.) was

added and the mixture cooled (0°) and acidified with dilute hydrochloric acid. The resulting precipitate (2.9 g.) crystallised from dilute alcohol in brown-yellow clusters m.p. 198° - 202°.

Synthesis of 7,4'-dihydroxyflavanone. Geissman and Clinton (53) prepared flavanones by both acid and alkali catalysed isomerization of the chalkone. Both methods were attempted and in each case the flavanone was formed in low yield (8-12%), but the alkaline isomerization was found to be the simpler method.

2:4-hydroxy-phenyl-4-hydroxystyryl ketone (1 g.) was dissolved in slightly more than the amount of 1% sodium hydroxide solution equivalent to all the free hydroxyl groups (25 ml.). Ethanol (4 ml.) was added, the solution refluxed for one-half hour and allowed to stand overnight. After acidification with acetic acid the solution was evaporated to dryness under reduced pressure. The product was suspended in water, boiled and filtered hot to remove the insoluble chalkone. The solution was decolourised with animal charcoal, and on filtration a cream precipitate of the flavanone was formed (35 mg. m.p. 204°). The chalkone precipitate was resuspended in water (5 ml.) boiled, filtered and decolourised as above

in order to recover more flavanone. The flavanone was recrystallized from water and the yield from the chalkone (6 g.) was 640 mg. of 7,4'-dihydroxyflavanone.

Synthesis of the (±)7,4'-dihydroxyflavan-4-ol.

7,4'-Dihydroxyflavanone (600 mg.), after drying in a vacuum pistol (110°) for two hours, was hydrogenated over a platinum oxide catalyst (400 mg.). The catalyst was removed by filtration and the methanol evaporated to dryness under reduced pressure (60°). A white microcrystalline powder resulted which was examined by two dimensional paper chromatography. An impurity was located at R_F 0.61 (2% acetic acid) which gave a blue colour with 2:6 dichloroquinone-chloroimide but no reaction with toluene-p-sulphonic acid spray reagent. The (±)-7,4'-dihydroxyflavan-4-ol was located at R_F 0.35 (2% acetic acid, strong mauve with toluene-p-sulphonic acid).

The powder was dissolved in methanol (50 ml.) and separated by "preparative" paper chromatography on 10 sheets of Whatman No.3 paper. The chromatograms were developed in 2% acetic acid (ascending). Bands were cut and eluted with 70% ethanol. The flavan-4-ol eluates were concentrated to a small volume (5 ml.) under vacuum, and a fine white sludge separated. The solution was evaporated

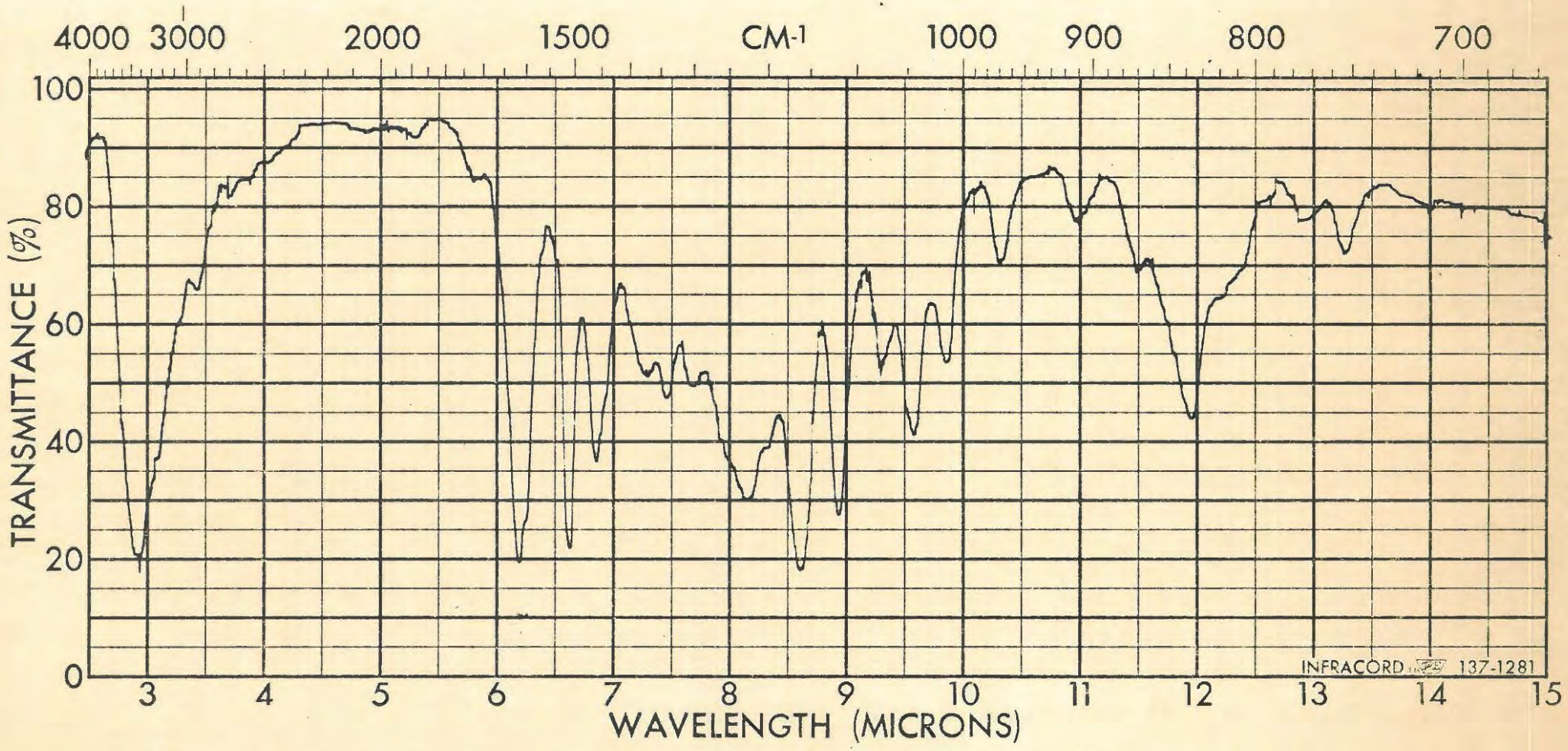

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FIGURE 7. INFRARED ABSORPTION SPECTRUM OF 7,4'-DIHYDROXYFLAVAN-4-OL

to dryness over $CaCl_2$ in a vacuum desiccator. (Found: C, 65.9 ; H, 5.8. Calculated for $C_{15}H_{14}O_4.H_2O$: C, 65.2 ; H, 5.8%). The compound showed a tendency to redden but it still gave the flavan-4-ol test (purple with concentrated hydrochloric acid).

The infrared absorption spectra of (±)-7,4'-dihydroxyflavan-4-ol (Figure 7) and of natural (+)-guibourtacacidin (Figure 1 and 2) were superimposable over the range 2.5 - 9μ, but were widely different over the range 9 - 15μ, especially at 1000-1100 cm^{-1} where absorption due to secondary hydroxyl groups occur.

DISCUSSION.

Guibourtia coleosperma commonly known as "Rhodesian copalwood", or alternatively as "Mtjibi" or "Mu Zaule", is an evergreen tree conspicuous in the deciduous forests, and more or less confined to the southern parts of tropical Africa.

The extractives of African Guibourtia spp., known before as Copaifera (92), were previously examined by Roux (93) using two dimensional paper chromatography. Two leuco-anthocyanidins were identified, a leuco-fisetinidin which had the same position on two-way chromatograms as (+)-7,3',4'-trihydroxyflavan-3:4-diol from Acacia mearnsii (29), and a new leuco-anthocyanidin for which he proposed the possible structure 7,4'-dihydroxyflavan-3,4-diol (guibourtacacidin). This substance appeared to be the third member of the "resorcinol series" of flavan-3:4-diols of 2,3-trans-3:4 cis configuration, the others being (+)-leuco-robinetinidin (41) and (+)-leuco-fisetinidin (29,32). Phatak and Kulkarni (94) consequently synthesised a crystalline dimethyl ether of guibourtacacidin by reduction of the corresponding flavanonol, to which they tentatively assigned the 2:3 trans-3:4 cis configuration.

Roux's findings were based on chromatographic evidence only, and the present work describes the isolation and characterization of the new leuco-anthocyanidin from Guibourtia coleosperma.

The leuco-anthocyanidin is present in low proportion in the heartwood, and migrated as a single spot on two dimensional chromatograms. Isolation was achieved by preliminary enrichment techniques to remove both polymeric tannins and a water insoluble substance by fractional precipitation. Further enrichment of the high R_F material was by Craig separation and "preparative" paper chromatography.

The compound was non-crystalline and isolated in 0.0042% yield from the wood. Characterization was complicated by its amorphous nature and low yield. Microdegradation gave resorcinol, β-resorcylic acid and p-hydroxybenzoic acid showing the presence of resorcinol and phenol A and B nuclei. Absence of ortho-dihydroxy groups was confirmed by the lack of reduction with ammoniacal silver nitrate spray reagent. With alcoholic hydrochloric acid the compound gave an orange-yellow anthocyanidin which was proved to be identical with synthetic 3,7,4'-trihydroxyflavylium chloride (85) by chromatographic and light absorption methods.

This anthocyanidin was formed in greater yield than the anthocyanidins due to leuco-fisetinidin or leuco-robinetinidin. The above suggests a flavan-3:4-diol structure.

Analysis gave an empirical formula, $C_{15}H_{14}O_5$. Diazomethane methylation and acetylation procedures gave amorphous dimethyl ether and tetra-acetoxy derivatives respectively, indicating the presence of two phenolic and two alcoholic hydroxyl groups. The above evidence indicates a 7,4'-dihydroxyflavan-3:4-diol structure and a molecular weight estimation on the acetylated derivative showed the compound to be monomeric.

Further evidence for this structure was provided by the similar, but not superimposable, infrared absorption curves of the O-dimethyl derivative with the (±)-7,4'-dimethylflavan-3:4-diol synthesised by Phatak and Kulkarni (94) (Figures 3 and 4). Additional confirmation was provided by the synthesis of crystalline (±)-7,4'-dihydroxyflavan-3:4-diol. 7,4'-Dihydroxyflavonol prepared by the method of Rao and Seshadri (97), was converted in low yield to (±)-7,4'-dihydroxyflavanonol by reduction with sodium hyposulphite. The (±)-7,4'-dihydroxyflavanonol was hydrogenated to (±)-7,4'-dihydroxyflavan-3:4-diol with platinum oxide as catalyst. The synthetic and natural flavan-3:4-diols

had identical melting points and similar, but not identical, infrared absorption spectra (Figures 1,2,2a). Chromatographic comparison of the natural and synthetic compounds showed that except for slight R_F differences in 2% acetic acid, the compounds were identical. However the synthetic flavan-3:4-diol did not form an O-ethyl derivative and, unlike (±)-leuco-fisetinidin and (±)-leuco-robinetinidin, was not resolved into the (+) and (-) epimers by paper chromatography in 2% aqueous acetic acid. The anthocyanidin formed by the synthetic diol was identical to that obtained from the natural compound. Chromatographic comparisons of (+)-7,4'-dihydroxyflavan-3:4-diol, (+)-7,3',4'-trihydroxyflavan-3:4-diol and (+)-7,3',4',5'-tetrahydroxyflavan-3:4-diol shows a regular increase of R_F with decrease in the number of substituent hydroxyl groups (Table II). This regular pattern is more apparent in aqueous solvent (2% acetic acid) where they all occupy an intermediate R_F range (0.51-0.55). In partitioning solvent (B.A.W. 6:1:2 v/v), however, the R_F interval is greater and less regular, due possibly to a telescoping effect of the compounds on the solvent front.

Comparisons of specific rotations shows that (+)-7,4'-dihydroxyflavan-3:4-diol has a much lower rotation

($[\alpha]_D^{21}$ + 11.7°) than either (+)-7,3',4'-trihydroxyflavan-3:4-diol ($[\alpha]_D^{21}$ + 30.7°) or (+)-7,3',4',5'-tetrahydroxyflavan-3:4-diol ($[\alpha]_D^{21}$ + 33.9°). The lower rotation may be due to partial racemization. Both (+)-7,3',4'-trihydroxyflavan-3:4-diol and (+)-7,3',4',5'-tetrahydroxyflavan-3:4-diol have been synthesised (73,96) by hydrogenation of natural (+)-2:3-trans dihydroflavonols, and both have been tentatively assigned a 2:3-trans-3:4-cis configuration by formation of isopropylidene derivatives. An attempt to establish the cis or trans configuration of the 3:4-diol grouping in (+)-7,4'-dihydroxyflavan-3:4-diol, by the formation of an acidic borate complex (88,89) was inconclusive since this test, which has been used previously for (+)-7,3',4'-trihydroxy-flavan-3:4-diol now appears to be of doubtful validity. The formation of an isopropylidene derivative could not be attempted due to the amorphous nature of the methylated derivative.

Examination of the wood drillings, cold extracted with methanol, by two dimensional chromatography did not show significant amounts of the O-ethyl derivative. However, during the subsequent enrichment procedures with repeated manipulation in alcohols, the O-ethyl derivative did not appear to form as readily as with leuco-anthocyanidins isomelacacidin (50) and isoteracacidin (51), since pro-longed refluxing in ethanolic acetic acid was necessary

and even then the conversion was not quantitative. An O-ethyl derivative was similarly formed by (+)-leucofisetinidin but also not very readily. The hydrolysis of the O-ethyl derivative with 0.1N acetic acid was complete after refluxing for twenty minutes and a compound of R_F 0.60 (2% acetic acid) intermediate between the parent compound (0.55) and its O-ethyl derivative (0.66) was formed. This compound also had a lower specific rotation than the natural diol. The significance of this difference is at present not apparent but may be due to easy epimerization of the 4-hydroxyl group.

From the above evidence it would appear that there are two possible alternatives for the configuration of the natural compound. A 2:3-*trans*-3:4-*cis* configuration is one possibility (XX) and the second possibility is the 2:3-*cis*-3:4-*trans* configuration (XXI) similar to that found in isomelacacidin (50) and isoteracacidin (51). The latter configuration appears to be the stronger possibility due to:

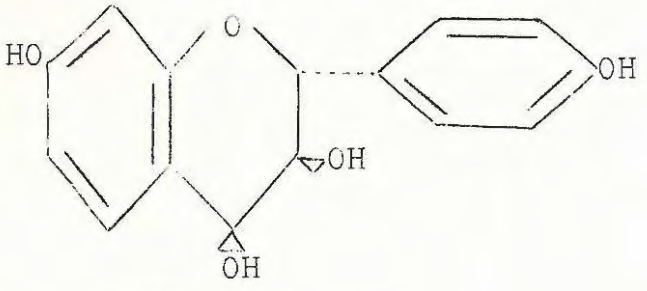
(1) The amorphous nature of the natural compound compared with the ease of crystallization of the synthetic compound.

(2) Differences in the infrared absorption spectra of the synthetic (2:3-*trans*-3:4-*cis* configuration)

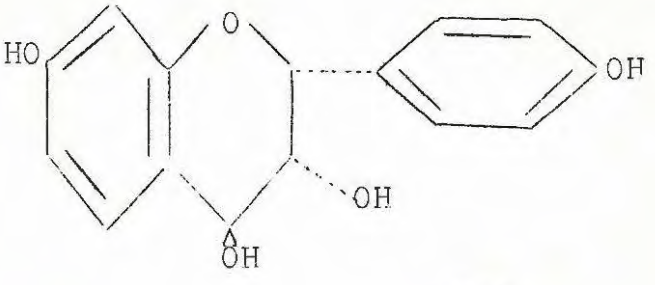
and natural diols.

(3) The ease of spontaneous formation of the 4- ethyl ether of the natural compound compared with the synthetic compound and

(4) the small but definite differences between the infrared absorption spectra of the dimethyl ether derivative and the synthetic (±)-7,4'-dimethoxyflavan-3:4-diol of 2:3-trans-3:4-cis configuration. The natural dimethyl ether is also non-crystalline whereas the synthetic compound was crystalline. A 2:3-cis-3:4-cis configuration as in melacacidin would not be applicable since such a structure will not form ethers.



XX



XXI

A leuco-fisetinidin is also present in the heartwood of G. coleosperma and chromatographic evidence indicates that it is (+)-mollisacacidin ((+)-7,3',4'-trihydroxyflavan-3:4-diol).

Condensed tannins form a large proportion of the heartwood extractives of G. coleosperma. These tannins appear to be complex polymeric forms of leuco-fisetinidin and leuco-guibourtinidin since on boiling with alcoholic hydrochloric acid they furnish high yields of both fisetinidin (86) and guibourtinidin (85) chlorides. Examination of some other members of the Guibourtia spp. by paper chromatography showed that only G. coleosperma contained the new leuco-anthocyanidin as well as a leuco-fisetinidin. G. demeusei and G. tessmannii heartwoods contained only leuco-fisetinidins, accompanied by polymeric leuco-fisetinidins of low R_F, and a close chemical relationship to G. coleosperma is thus apparent. G. arnoldiana on the other hand was found to be chemically unrelated to the above members since it appeared to contain only a leucocyanidin.

In order to investigate the influence of the 4- hydroxyl group in the molecule the flavan-4-ol was synthesised. (±)-7,4'-Dihydroxyflavan-4-ol was synthesised

by hydrogenation of the corresponding synthetic flavanone. The compound refused to crystallise and underwent easy self condensation with marked reddening, as occurs with (±)-7,3',4'-trihydroxyflavan-4-ol (98) and (±)-7,3',4',5'-tetrahydroxyflavan-4-ol (100). The tannins of G. coleosperma, which constitute a major portion of the extract, also exhibit this characteristic red colour. The absence of ortho-hydroxy groups in the new synthetic flavan-4-ol would suggest that these are not necessary for colour formation, but that, as suggested by Roux and Paulus (98), the hydroxyl group in the 4- position is responsible for redness inherent in condensed tannins of the leuco-anthocyanidin type. The infrared absorption spectra of the flavan-4-ol and natural (+)-guibourtacacidin were similar over the higher frequency range (4000-1100 cm^{-1}) but, as was to be expected, differed widely over the lower frequency range (1100-700 cm^{-1}).

The isolation of the leuco-anthocyanidin, (+)-7,4'-dihydroxyflavan-3:4-diol, from a member of the Leguminosiae lends further emphasis to the almost exclusive occurrence amongst the Leguminosiae and two genera of the Anacardiaceae, of almost all those C_{15} 'flavonoid' compounds of the resorcinol series which have hitherto been isolated and identified in nature (Roux and Maihs 99).

REFERENCES.

The abbreviated titles of Journals conform to those published in the "Handbook for Chemical Society Authors" 1960, p.203-220.

Note: Journal of the Society of Leather Trade Chemists will be abbreviated J.S.L.T.C.

1. Rosenheim,O., Biochem. J., 1920, 14, 178.
2. Robinson,G.M. & Robinson,R., Biochem. J., 1933, 27, 206.
3. Robinson,G.M., J.Chem.Soc., 1937, p.1157.
4. Robinson,G.M. & Robinson,R., J.Chem.Soc., 1935, p.744.
5. Robinson,R., Nature, 1936, 137, 172.
6. Charlesworth,E.M., Chavan,J.J. & Robinson,R., J.Chem.Soc., 1933, p.370.
7. Appel,H. & Robinson,R., J.Chem.Soc., 1935, p.752.
8. Bancroft,W.D. & Rutzler,J.E., J.Amer.Chem.Soc., 1938,60,2738.
9. Bancroft,W.D. & Rutzler,J.E., J.Amer.Chem.Soc., 1938,60,2945.
10. Rutzler,J.E., J.Amer.Chem.Soc., 1939, 61, 1160.
11. Forsyth,W.G.C., Nature, 1949, 164, 25.
12. Forsyth,W.G.C., Biochem. J., 1952, 51, 511.
13. Williams,A.H., Chem. and Ind., 1953, p.540.
14. Wieser,M.E., Pollard,A. & Williams,A.H., Chem. & Ind.,1953, p.1260.
15. Bate-Smith,E.C. & Lerner,N.H., Biochem. J., 1954, 58, 126.
16. Bate-Smith,E.C., J. Exp. Botany, 1953, 4, 1.
17. Bate-Smith,E.C. & Swain,T., Chem. and Ind., 1953, p.377.
18. Robinson,G.M. & Robinson,R., J.Amer.Chem.Soc., 1939,61,1605.
19. King,F.E. & Bottomley,W., Chem. and Ind., 1953, p.1368.
20. King,F.E. & Bottomley,W., J.Chem.Soc., 1954, p.1399.
21. Bottomley,W., Chem. and Ind., 1954, p.516.
22. King,F.E. & Clark-Lewis,J.W., Chem. and Ind., 1954, p.757.
23. Swain,T., Chem. and Ind., 1954, p.1144.
24. Algar,J. & Flynn,J.P., Proc.Roy.Irish Acad., 1934, B.42, 1.
25. Bauer,L., Birch,A.J. & Hillis,W.E., Chem. & Ind., 1954, p.433.
26. Forsyth,W.G.C., Nature, 1953, 172, 726.
27. Kulkarni,A.B. & Joshi,C.G., Chem. and Ind., 1954, p.1456.
28. King,F.E. & Clark-Lewis,J.W., J.Chem.Soc., 1955, p.3384.
29. Keppler,H.H., J.Chem.Soc., 1957, p.2721.
30. Pigman,W., Anderson,E., Fischer,E., Buchanan,M.A. & Browning, B.L., Transactions of the American Pulp and Paper Industry, 1953, 36, 4.

31. Chandorkar,K.R. & Kulkarni,A.B., Current Sci.(India), 1957, 26, 354.
32. Roux,D.G., Chem. and Ind., 1958, p.161.
33. Freudenberg,K & Maitland,P., Annalen , 1934, 510, 193.
34. Roux,D.G., Nature, 1958, 181, 1454.
35. Clark-Lewis,J.W. & Roux,D.G., Chem. and Ind., 1958, p.1475.
36. Mitsumo,M. & Yoshizaki,M., J.Pharm.Soc.(Japan), 1957, 77, 557.
37. Clark-Lewis,J.W. & Mitsumo,M., J.Chem.Soc., 1958, p.1724.
38. Freudenberg,K. & Roux,D.G., Annalen., 1958, 613, 56.
39. Roux,D.G. & Evelyn,S.R., Biochem. J., 1958, 69, 530.
40. Freudenberg,K. & Roux,D.G., Naturwiss., 1954, 41, 450.
41. Weinges,K., Annalen., 1958, 615, 203.
42. Chan.W.R., Forsyth,W.G.C. & Hassall,C.H., Chem.& Ind.,1957,p261.
43. Chan.W.R., Forsyth,W.G.C. & Hassall,C.H., J.Chem.Soc.,1958,p3174.
44. Bate-Smith,E.C., Biochem. J., 1954, 58, 122.
45. Ganguly,A.K. & Seshadri,T.R., J.Sci.Ind.Research(India), 1958, 17B,168; Tetrahedron,1959, 6, 21.
46. Laumas,K.R. & Seshadri,T.R., J.Sci.Ind.Research(India), 1958, 17B, 44.
47. Ganguly,A.K., Seshadri,T.R. & Subramanian,P., Tetrahedron, 1958, 3, 225.
48. Chander,K. & Seshadri,T.R., J.Sci.Ind.Research(India), 1957, 16A, 319.
49. Hathway,D.E., Biochem. J., 1958, 70, 34.
50. Clark-Lewis,J.W. & Mortimer,P.I., J.Chem.Soc., 1960, p.4106.
51. Clark-Lewis,J.W. Katekar,G.F. & Mortimer,P.I., J.Chem.Soc., 1961, p.499.
52. Partridge,S.M., Biochem. J., 1948, 42, 238.
53. Geissmann,T.A. & Clinton,R.O., J.Amer.Chem.Soc.,1946, 68, 697.
54. Roux,D.G., J.Amer.Leather Chemists' Assoc., 1958, 53, 384.
55. Brownell,H.H., Hamilton,J.G. & Casselman,A.A., Analyt.Chem., 1957, 29, 550.
56. Craig,L.C., Fortschr.Chem.Forsch., 1949, 1, 292.
57. Roux,D.G., Nature, 1957, 180, 973.
58. Bate-Smith,E.C. & Westall,R.G., Biochim et Biophys. Acta, 1950, 4, 427.
59. Bate-Smith,E.C., Biochem.Soc.Symposia, 1949, 3, 62.
60. Lindstedt,G., Acta Chem. Scand., 1950, 4, 448.
61. Roux,D.G. & Maihs,A.E., J. of Chromatog., 1960, 4, 65.
62. Geissmann,T.A., Jorgenson,E.C. & Harborne,J.B., Chem. and Ind., 1953, p.1389.
63. Swain,T., Chem. and Ind., 1954, p.1480.
64. Mansfield,G.H., Swain,T. & Nordström,C.G., Nature,1953,172,23.
65. Nordström,C.G. & Swain,T., J. Chem.Soc., 1953, p.2764.
66. Harborne,J.B., Chem. and Ind., 1954, p.1142.
67. Roux,D.G., Nature, 1957, 179, 305,
68. Harborne,J.B., J. of Chromatog., 1958, 1, 473.

69. Bradfield,A.E. & Flood,A.E., J. Chem.Soc., 1952, p.1740.
70. Roux,D.G., J.S.L.T.C., 1952, 36, 393.
71. Bray,H.G., Thorpe,W.V. & White,K., Biochem.J., 1950, 46, 271.
72. Roux,D.G. & Evelyn,S.R., Biochem. J., 1958, 70, 344.
73. Roux,D.G. & Paulus,E., Biochem. J., 1960, 77, 315.
74. Nadkarni,D.R. & Wheeler,T.S., J.Chem.Soc., 1938, p.1320.
75. Slater,W.F. & Stephen,H., J.Chem.Soc., 1920, p.309.
76. Liebermann,C., Ber., 1883, 21, 3372.
77. Allan,J. & Robinson,R., J.Chem.Soc., 1924, p.2192.
78. Robinson,R. & Shinoda,J., J.Chem.Soc., 1925, p.1973.
79. Roberts,E.A.H. & Wood,J.E., Biochem. J., 1953, 53, 332.
80. Roux,D.G., Maihs,E.A. & Paulus,E., J. of Chromatog.,1961, 5, 9.
81. Gierer,J., Acta Chem. Scand., 1954, 8, 1319.
82. Pew,J.C., J.Amer.Chem.Soc., 1948, 70, 3031.
83. Shimizu,M. & Yoshikawa,T., J.Pharm.Soc.Japan, 1952, 72, 331.
84. Geissmann,T.A. & Lischner,J., J.Amer.Chem.Soc., 1952, 74, 3001.
85. Freudenberg,K., Farimullah & Steinbrunn,G., Annalen, 1935, 518, 37.
86. Pratt,D.D. & Robinson,R., J.Chem.Soc., 1925, p.1136.
87. Mann,F.G. & Saunders,B.C., Practical Organic Chemistry, p.342.
88. Keppler,H.H., Ph.D. Thesis, Univ. of Natal, 1956.
89. Böeseken,J., Rec. Trav. Chim., 1921, 40, 553.
90. Freudenberg,K. & Weinges,K., Annalen, 1958, 613, 61.
91. Roux,D.G. & Evelyn,S.R., J. of Chromatog., 1958, 1, 537.
92. Léonard,J., Bull.Jard.Bot.Brux., 1949, 19, 100.
93. Roux,D.G., Nature, 1959, 183, 890.
94. Phatak,D.M. & Kulkarni,A.B., Current Sci.(India),1959, 28, 328.
95. Roux,D.G., Maihs,E.A. & Paulus,E., Biochem. J., 1961, 78, 834.
96. Roux,D.G. & Paulus,E., Biochem. J., 1962, 82, 324.
97. Rao,P.V. & Seshadri,T.R., Proc.Indian Acad.Sci.,1948, 28A, 96.
98. Roux,D.G. & Paulus,E., Biochem. J., 1961, 80, 476.
99. Roux.D.G. & Maihs,E.A., Biochem. J., 1960, 74, 44.
100. Roux.D.G. & Paulus,E., Biochem. J., 1962, 84, 416.

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