The Constitution and Synthesis of Leucoanthocyanidins.

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[Reprint Order No. 6350.]

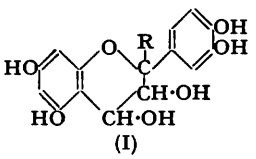
The catalytic reduction of 7 : 8 : 3′ : 4′-tetramethoxyflavonol gives a (±)-7 : 8 : 3′ : 4′-tetramethoxyflavan-3 : 4-diol to which a 2(e) : 3(a) : 4(e) conformation for the substituents at the 2 : 3 : 4-positions is attributed. It is further shown that the synthetic diol is the racemic modification of (−)-tetra-*O*-methylmelacacidin, thus completely identifying the phlobaphen–anthocyanidin-forming compound, melacacidin, a natural product probably typical in structure of the majority of leucoanthocyanidins.

LEUCOANTHOCYANIDINS are colourless phenolic substances which are converted into anthocyanidins by the action of aqueous or alcoholic acid, and the term leucoanthocyanin was introduced by Rosenheim (*Biochem. J.*, 1920, **14**, 178) to describe a supposedly glycosylated member of the series found in the young leaves and stems of the grape (*Vitis vinifera*). Investigations since Rosenheim's discovery of the first leucoanthocyan(id)in have been largely concerned with their distribution in the plant kingdom, and the extensive surveys of Robinson and Robinson (*Biochem. J.*, 1931, **25**, 1687; 1932, **26**, 1647; 1933, **27**, 206) have established their almost ubiquitous occurrence among the higher plants. Leucoanthocyanidins are invariably detected by converting them into the corresponding anthocyanidins, the products being identified either by means of colour reactions and partition methods (Robinson and Robinson, *loc. cit.*) or by the more recent paper-chromatographic technique (Bate-Smith, *J. Exp. Bot.*, 1953, **4**, 1; *Biochem. J.*, 1954, **58**, 122; Bate-Smith and Lerner, *ibid.*, p. 126). However, the formation of anthocyanidins from their leucocompounds demands rather more vigorous conditions than are normally required for the regeneration of flavylium salts from the related carbinols; consequently, despite the evidently close relationship of the two series, the leucoanthocyanidins cannot be identical with the ordinary *pseudo*-bases. Robinson and Robinson (*loc. cit.*) accordingly proposed a structure for leucocyanidin, *i.e.*, (I; R = OH), which takes the form of a hydrated carbinol at the same oxidation state as that of the corresponding flavylium salt. A modified expression (I; R = H) was tentatively suggested by Bate-Smith (*J. Exp. Bot.*, 1953, **4**, 1), but no evidence was available with which to decide between the alternative formulæ.

The chemical investigation of leucoanthocyanidins is obstructed by difficulties arising from their amorphous character and from their occurrence as mixtures with other ill-defined substances of comparable physical properties. Moreover, the crude materials are slowly oxidised in the air and resemble the catechins (II) in their tendency to form intractable condensation polymers (" phlobaphens "). Definite indications as to the nature of leucoanthocyanidins were first obtained by King and Bottomley (*Chem. and Ind.*, 1953, 1368; *J.*, 1954, 1399) through the isolation from Australian blackwood (*Acacia melanoxylon*) of a new phenolic compound (melacacidin), $C_{15}H_{14}O_7$. Unlike the associated phenolic materials, melacacidin proved to be somewhat soluble in boiling ether and was therefore without difficulty extracted from the wood in a relatively pure condition. Although amorphous it readily formed crystalline derivatives, *e.g.*, a tetramethyl ether, which was identified as the flavandiol (III; R = Me). Apart from its uninvestigated prototype, flavan-3 : 4-diol (Mozingo and Adkins, *J. Amer. Chem. Soc.*, 1938, **60**, 669), no other flavan-3 : 4-diol was then known. The true character of melacacidin (III; R = H) was evident on treating it with boiling hydrochloric acid which afforded a deep cherry-red solution due to an anthocyanidin, shown by paper chromatography (King and Clark-Lewis, *Chem. and Ind.*, 1954, 757; see also Bottomley, *ibid.*, p. 516) to be 3 : 7 : 8 : 3′ : 4′-pentahydroxyflavylium chloride (IV) (Robinson and Vasey, *J.*, 1941, 660).

The structure of (−)-tetra-*O*-methylmelacacidin has since been confirmed by comparison of its properties with those of a synthetic (±)-7 : 8 : 3′ : 4′-tetramethoxyflavan-3 : 4-diol (III; R = Me) (King and Clark-Lewis, *loc. cit.*). The flavandiol was prepared by hydrogenation of 7 : 8 : 3′ : 4′-tetramethoxyflavonol (V) over Raney nickel, which

afforded a single crystalline racemate, m. p. 135—136° alone or mixed with the natural (—)-melacacidin tetramethyl ether, m. p. 146°. The identification of this racemate as (±)-tetra-*O*-methylmelacacidin, which was inherently probable from the m. p. observations, was confirmed by measurements of the infrared absorption, carbon tetrachloride solutions of the synthetic material and of (—)-melacacidin tetramethyl ether having coincident spectra. We are indebted to Dr. F. B. Strauss for these determinations and for his opinion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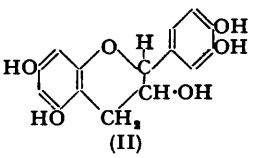
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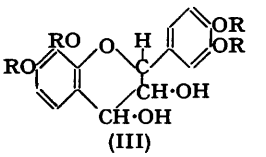
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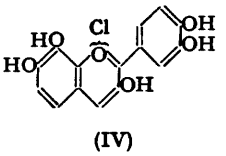
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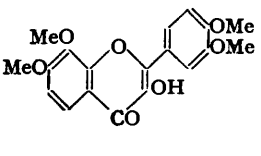
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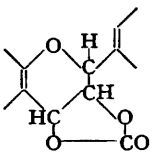
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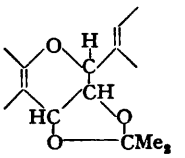
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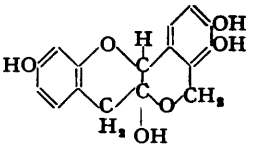
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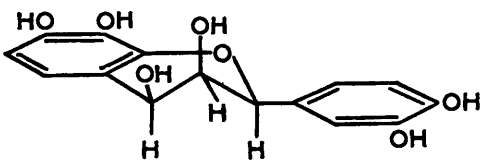
(VIII) Melacacidin

as to the relation of the two samples. The synthetic compound (III; R = Me) formed a diacetate, m. p. 157—158°, which had mixed m. p. 167—168° with the (—)-diacetate, m. p. 194°. A cyclic carbonate (VI), m. p. 205° (decomp.), was obtained by treating the (±)-diol with carbonyl chloride and triethylamine in toluene, and a mixture of this derivative with (—)-tetra-*O*-methylmelacacidin carbonate, m. p. 209° had m. p. 201—202° (decomp.). From the formation of a cyclic carbonate it may be assumed—as with the natural compound (King and Bottomley, *loc. cit.*)—that the synthetic diol also possesses the *cis*-configuration. This conclusion receives strong confirmation from the formation of an *iso*propylidene derivative (VII) of the racemic flavandiol since it is known that cyclic acetals are not normally obtained from *trans*-α-diols (" Progress in Stereochemistry," ed. W. Klyne, Butterworths, 1954, Vol. I, p. 55).

Since the catalytic reduction of ethylenic bonds invariably proceeds by *cis*-addition, it is possible to deduce the complete conformational structure of the flavandiol prepared by hydrogenation of 7 : 8 : 3′ : 4′-tetramethoxyflavonol (V) which accordingly results in a product wherein the 2-aryl and the 3-hydroxyl substituent are in the *cis*-configuration. The 3-hydroxyl group having already been shown to be a constituent of a *cis*-3 : 4-diol, it follows that all three substituents (aryl : hydroxyl : hydroxyl) at the adjacent asymmetric centres are *cis*-related, and in view of the spectroscopic evidence already quoted this must also be true of the tetra-*O*-methylmelacacidin and therefore of melacacidin.

Two principal conformations, 2(a) : 3(e) : 4(a) and 2(e) : 3(a) : 4(e), are thus possible for the natural flavandiol (III; R = H) and its compounds, which in their stereochemistry present a problem similar to that discussed in connexion with the catechins (King, Clark-Lewis, and Forbes, *J.*, 1955, 2948). Of the alternatives, the 2(e) : 3(a) : 3(e) disposition (VIII) is intrinsically the more probable since both the largest substituent, *i.e.*, the dihydroxyphenyl, and the numerically larger number of groups other than hydrogen are equatorially situated. In this energetically favoured arrangement the 4-hydroxyl group has the equatorial conformation which is, in fact, the configuration normally resulting from the slow catalytic reduction of an unhindered carbonyl group (*op. cit.*, p. 74). The generation of a 2 : 3-*cis*-derivative by reduction of the tetramethoxyflavonol (V) is analogous to

the formation of (±)-penta-*O*-methyl*epi*catechin in preference to the catechin ether by hydrogenation of 3 : 5 : 7 : 3′ : 4′-penta-*O*-methylquercetin (Freudenberg and Kammüller, *Annalen*, 1927, **451**, 209).

Melacacidin is so far the only fully characterised flavandiol to have been isolated from natural sources, but in view of the wide distribution of ill-defined phenolic substances which share its anthocyanidin-forming properties, the discovery of analogues can doubtless be expected, and in particular those corresponding to the principal species pelargonidin, cyanidin, and delphinidin. However, neither the anthocyanidin derived from melacacidin nor that related to the only other recognised leucoanthocyanidin, namely, peltogynol (IX) (Robinson and Robinson, *J.*, 1935, 744), has so far been found in Nature.

Following the elucidation of the flavandiol structure for leucoanthocyanidins, in addition to our own synthetic experiments, methods for the preparation of flavan-3 : 4-diols have been described in preliminary notes from other laboratories. Bauer, Birch, and Hillis (*Chem. and Ind.*, 1954, 433) have used the lithium aluminium hydride reduction method of Mirza and Robinson (*Nature*, 1950, **166**, 997) to prepare cyanidin from quercetin penta-acetate, the presumed intermediate flav-2-en-3 : 4-diol being treated with hydrochloric acid. Accompanying the anthocyanidin was a colourless gum which when heated with hydrochloric acid in propan-2-ol yielded a further quantity of cyanidin and which was thus assumed to be the flavan-3 : 4-diol. Swain (*Chem. and Ind.*, 1954, 1144) has reported the sodium borohydride reduction of taxifolin, a natural 2 : 3-dihydroquercetin, to an amorphous, possibly stereoisomeric, flavandiol which gave a crystalline tetramethyl ether, also formed by the similar reduction of taxifolin 5 : 7 : 3′ : 4′-tetramethyl ether. Cyanidin and 5 : 7 : 3′ : 4′-tetra-*O*-methylcyanidin were thereupon obtained by treatment of the respective flavandiols with hot hydrochloric acid. Freudenberg and Roux (*Naturwiss.*, 1954, **41**, 450) prepared a 3 : 4 : 7 : 3′ : 4′ : 5′-hexahydroxyflavan and its crystalline 7 : 3′ : 4′ : 5′-tetramethyl ether by catalytic hydrogenation of natural dihydrorobinetin and subsequent methylation, and state briefly that 3 : 4 : 5 : 7 : 3′ : 4′-hexahydroxyflavan may be similarly obtained from dihydroquercetin. Lithium aluminium hydride was used for the preparation of 4′-methoxy-6-methylflavan-3 : 4-diol (Joshi and Kulkarni, *Chem. and Ind.*, 1954, 1421), and two racemates of the melacacidin structure (III; R = Me), m. p. 131—132° and 179°, have been obtained by catalytic and lithium aluminium hydride reduction, respectively, of synthetic 7 : 8 : 3′ : 4′-tetramethoxydihydroflavonol (Kulkarni and Joshi, *ibid.*, p. 1456).

The employment of dihydroflavonols for the preparation of flavandiols suffers from the disadvantage that they are not readily available by synthesis, and only a limited number of the naturally occurring compounds are known. Moreover, the stereochemistry of the existing dihydroflavonols has not yet been investigated, and consequently the configuration of the resulting flavandiols, which contain three centres of asymmetry and can therefore theoretically exist in four pairs of enantiomers, will be entirely unknown. Mahesh and Seshadri (*Proc. Indian Acad. Sci.*, 1955, **41**, 210) have recently discussed the stereochemistry of 3-hydroxyflavanones.

Kulkarni and Joshi (*loc. cit.*) postulate a 2(e) : 3(e)-arrangement for the aryl and the hydroxyl group in the dihydroflavonol used in their experiments, and a 2(e) : 3(e) : 4(e)-configuration (diol system *trans*) for its lithium aluminium hydride reduction product, m. p. 179° (diacetate, m. p. 122—123°). The product, m. p. 131—132° (diacetate, m. p. 120°), obtained by catalytic hydrogenation is regarded as the 2(e) : 3(e) : 4(a)-isomer, but no experimental evidence has been adduced in support of the *cis*-configuration thus attributed to the diol grouping. Clearly, the higher-melting diol is not (±)-tetra-*O*-methylmelacacidin, and the possible correspondence between the isomer of m. p. 131—132° and our synthetic diol of m. p. 135—136° is not confirmed by a comparison of the respective acetates of m. p. 120° and 157—158°. Moreover, we have been able to verify the non-identity of these products by mixed m. p. determinations with specimens kindly provided by Dr. Kulkarni. It is apparent therefore that three of the four theoretically possible racemates of structure (III; R = Me) are now known, the compound which is the principal subject of this communication being (±)-tetra-*O*-methylmelacacidin.

The transformation of melacacidin into the corresponding anthocyanidin is an oxidation

and it may therefore occur either through the intervention of atmospheric oxygen or by a disproportionation in which an equivalent of the flavandiol is reduced to a lower oxidation state. Rosenheim (*loc. cit.*) and Robinson and Robinson (*Biochem. J.*, 1933, **27**, 206) have shown that air is not essential for the conversion of leucoanthocyanidins into flavylium salts, and assuming that the compounds which were the subject of their observation consisted of flavandiols, this can be construed as support for the disproportionation hypothesis. A possible mechanism leading to the simultaneous formation of a flavan-3-ol (catechin type) has already been propounded (King and Bottomley, *locc. cit.*) and it is significant that catechins have been detected by paper chromatography among the products from the action of acid on cacao " leucocyanidin " (Forsyth, *Nature*, 1953, **172**, 726).

The quantity of anthocyanidin formed in the acid treatment of melacacidin is small and is estimated as approximately 10%. This is in agreement with observations made with crude natural tannins (Bate-Smith and Swain, *Chem. and Ind.*, 1953, 377) which in view of their general similarity to melacacidin can now be regarded as leucoanthocyanidins. The low yield of flavylium salt is due to a competing reaction leading to " phlobaphen," an acid-catalysed polymerisation characteristic of the catechins (flavan-3-ols) and presumably, therefore, also of the analogous flavan-3 : 4-diols. Freudenberg and Weinges have in fact recently demonstrated (*Annalen*, 1954, **590**, 140) that the sensitivity of the flavans towards acid is unaffected by the degree of hydroxylation of the heterocyclic nucleus, and the tendency to condensation is furthermore greatest with those containing hydroxyl substituents at the 7- and the 4'-position, as in melacacidin. However, anthocyanidins have not been detected among the products of acid treatment of the catechins (Bate-Smith and Swain, *loc. cit.*), the 3 : 4-diol grouping therefore being essential to flavylium salt formation (cf. Freudenberg and Weinges, *loc. cit.*).

Experimental

(±)-*Melacacidin Tetramethyl Ether* (7 : 8 : 3' : 4'-*Tetramethoxyflavan*-cis-3 : 4-*diol*) (III; R = Me).—Tetramethoxyflavonol (50—70%), m. p. 222°, was obtained from 2-hydroxy-3 : 4 : 3' : 4'-tetramethoxychalkone, m. p. 126—127° (Crabtree and Robinson, *J.*, 1922, **121**, 1033) by treatment with alkaline peroxide (King and Bottomley, *J.*, 1954, 1399; cf. Kostanecki and Rudse, *Ber.*, 1905, **38**, 935). The 3-*ethoxycarbonyl* derivative (90%) was formed from ethyl chloroformate and crystallised from ethanol in yellow needles, m. p. 143—144° (Found : C, 59·5; H, 5·4; loss, 4·3. $C_{22}H_{22}O_9,H_2O$ requires C, 58·9; H, 5·4; loss, 4·0. Found, in a specimen dried at 110° *in vacuo*: C, 61·9; H, 5·4. $C_{22}H_{22}O_9$ requires C, 61·4; H, 5·2%).

7 : 8 : 3' : 4'-Tetramethoxyflavonol (2 g.) in ethanol (100 c.c.) was hydrogenated for 16 hr. at 100—110°/100 atm. over Raney nickel (*ca.* 4·0 g.). After filtration from catalyst (kieselguhr) the solvent was removed under reduced pressure, and a solution of the residue in benzene (30—40 c.c.) was cautiously diluted with light petroleum (b. p. 40—60°; *ca.* 25 c.c.). Next day, 7 : 8 : 3' : 4'-*tetramethoxyflavan*-cis-3 : 4-*diol* [(±)-*melacacidin tetramethyl ether*] (0·8 g., 40%), m. p. 131—132°, was collected (Found : C, 62·7; H, 6·4. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1%). Recrystallisation from benzene–light petroleum afforded the (±)-diol in prisms, m. p. 135—136° alone and when mixed with (−)-melacacidin tetramethyl ether (needles, m. p. 146°). Light absorption of (±)- and (−)-melacacidin tetramethyl ether in ethanol : $\lambda_{max.}$ 206 (ε 69,400) and 278 mμ (ε 3850). Acetylation of the diol (0·1 g.) with acetic anhydride–pyridine at room temperature overnight afforded the (±)-*diacetate*, needles or small prisms, m. p. 157—158°, from methanol (Found : C, 61·7; H, 5·5; Ac, 21·3. $C_{23}H_{26}O_9$ requires C, 61·9; H, 5·9; Ac, 19·3%). A mixture of the (±)-diacetate with (−)-melacacidin tetramethyl ether diacetate (needles, m. p. 194°; King and Bottomley, *loc. cit.*) melted at 167—168°. The preparation of the (±)-diol was repeated several times, the best results being obtained with small quantities of the flavonol (2—4 g.) and relatively large amounts of catalyst.

The infrared absorption spectra of (±)- and (−)-melacacidin tetramethyl ether were measured in CCl_4 solutions 1 mm. thick, with solvent compensation by double-beam operation. The positions of maxima and minima for each compound coincided exactly throughout the range 2—11·5 μ, although owing to the thickness of solutions (1 mm.), which was necessitated by sparing solubility, the regions of intense solvent absorption (6·2—6·7 and 7·8—8·4 μ) are without significance.

A mixture of the *cis*-diol, m. p. 132° (Kulkarni and Joshi, *Chem. and Ind.*, 1954, 1456) with

(±)-melacacidin tetramethyl ether (m. p. 135—136°) sintered at 111° and melted at 116° to a turbid liquid which cleared at 126°; a similar mixture with (−)-melacacidin tetramethyl ether (m. p. 145—146°) melted at 115—117°. A mixture of the *cis*-diol diacetate (m. p. 120°) (Kulkarni and Joshi, *loc. cit.*) with (±)-melacacidin tetramethyl ether diacetate (m. p. 156—157°) sintered at 109° and melted at 113—115°. We thank Dr. Kulkarni for the provision of the relevant specimens for these mixed m. p. determinations.

(±)-7 : 8 : 3′ : 4′-*Tetra*-O-*methylmelacacidin* 3 : 4-*Carbonate* (VI).—(*a*) A solution of the *cis*-(±)-diol (0·3 g.) in benzene (*ca.* 5 c.c.) was treated dropwise with a solution of carbonyl chloride in toluene (12·5% w/w, 0·75 c.c., 1 equiv.) at room temperature. After 1 hr. triethylamine (1 c.c., *ca.* 9 equiv.) was added and the solution cooled in water during the dropwise addition of further carbonyl chloride solution (2·25 c.c., total 3·0 c.c., 4 equiv.); the mixture was then left at room temperature for 2 hr. before the addition of water and ether. Filtration afforded (±)-7 : 8 : 3′ : 4′-*tetra*-O-*methylmelacacidin carbonate* (0·22 g., 69%), m. p. 205° (decomp.), which crystallised from ethanol (50 c.c.) in aggregates of platelets (0·138 g.), m. p. 205° (decomp.) (Found: C, 61·8; H, 5·2. $C_{20}H_{20}O_8$ requires C, 61·8; H, 5·2%). A mixture of the (±)-carbonate with (−)-melacacidin tetramethyl ether carbonate [needles, m. p. 209° (decomp.)] (King and Bottomley, *loc. cit.*) melted at 201—202° (decomp.). In common with other acyl derivatives of melacacidin tetramethyl ether the carbonate became pale pink when dried in a vacuum-desiccator over sulphuric acid. It was insoluble in exaltone and camphor except at temperatures (180—200°) causing decomposition with evolution of gas.

(*b*) Attempts to prepare the carbonate by the action of ethyl chloroformate on the diol in aqueous alkali (King and Bottomley, *loc. cit.*) failed, but were successful in benzene. Addition of triethylamine (1·0 c.c.) to the diol (0·1 g.) and ethyl chloroformate (1·0 c.c.) in benzene (5 c.c.) containing a little dioxan, caused the solution to boil, and after 2 hr. at room temperature the mixture was filtered and the residue washed with water. Crystallisation of the residue from ethanol (15 c.c.) afforded the carbonate (0·0314 g., 30%), plates, m. p. 204—205° (decomp.), identical with that described under (*a*), and a further 20% (total 50%) was obtained by crystallisation of the residue from evaporation of the benzene solution. The possibility that the formation of the carbonate under these conditions is due to carbonyl chloride present in the ethyl chloroformate was not investigated.

iso*Propylidene Derivative of* (±)-7 : 8 : 3′ : 4′-*Tetramethylmelacacidin* (VII).—(±)-Melacacidin tetramethyl ether (0·093 g.) was dissolved in acetone (6 c.c.) containing hydrochloric acid (1 drop in 100 c.c.), and after 3 days at room temperature triethylamine (2 drops) and water were added to the clear solution. Crystallisation afforded the iso*propylidene derivative* in large elongated leaflets (0·082 g., 80%), m. p. 163°, which crystallised from methanol with almost quantitative recovery in prisms, m. p. 163° (Found: C, 65·6; H, 6·6. $C_{22}H_{26}O_7$ requires C, 65·7; H, 6·5%).

Anthocyanidin Formation.—(±)- and (−)-Melacacidin tetramethyl ether were unaffected by acid in the cold for short periods, but both the synthetic and the natural flavan-3 : 4-diol gave an intense cherry-red colour with hydrochloric acid (10%) in butanol at 100°. On paper chromatograms the two coloured solutions behaved characteristically and identically: with ascending butanol–2N-hydrochloric acid there were observed red spots (attributed to 3-hydroxy-7 : 8 : 3′ : 4′-tetramethoxyflavylium chloride) at R_F 0·94, with a brownish-yellow tail (R_F *ca.* 0·84) due to "phlobaphen," and a further colourless tail (extending to R_F 0·57) which showed a bright greenish-yellow fluorescence in ultraviolet light. In ascending *m*-cresol–2N-hydrochloric acid the flavylium salts moved with the solvent front and these red spots were edged with the brownish-yellow material and by a narrow region which fluoresced in ultraviolet light. (−)- and (±)-Melacacidin tetramethyl ether moved as single substances on paper chromatograms and the colourless spots were revealed by spraying the developed chromatograms with concentrated hydrochloric acid and heating at 100° for 2—3 min., which caused the appearance of the red colour due to 3-hydroxy-7 : 8 : 3′ : 4′-tetramethoxyflavylium chloride. The two materials behaved identically and showed R_F 0·86 in butanol–acetic acid–water (5 : 1 : 4) and R_F 0·96—1·0 in *m*-cresol–acetic acid–water (50 : 2 : 48).

7 : 8 : 3′ : 4′-Tetramethoxyflavanone (40%), m. p. 143—144°, was prepared as described by Geissman and Heaton (*J. Amer. Chem. Soc.*, 1944, **66**, 486—487) except that fractional crystallisation was avoided and isolation simplified by extraction of the concentrated reaction mixture with ether, removal of phenolic material by washing with 0·5N-sodium hydroxide, and evaporation of the ether to yield the crystalline flavanone.

THE UNIVERSITY, NOTTINGHAM. [*Received, April 22nd,* 1955.]