803. *Flavan Derivatives. Part III.*[1] *Melacacidin and Isomelacacidin from* Acacia *Species.*

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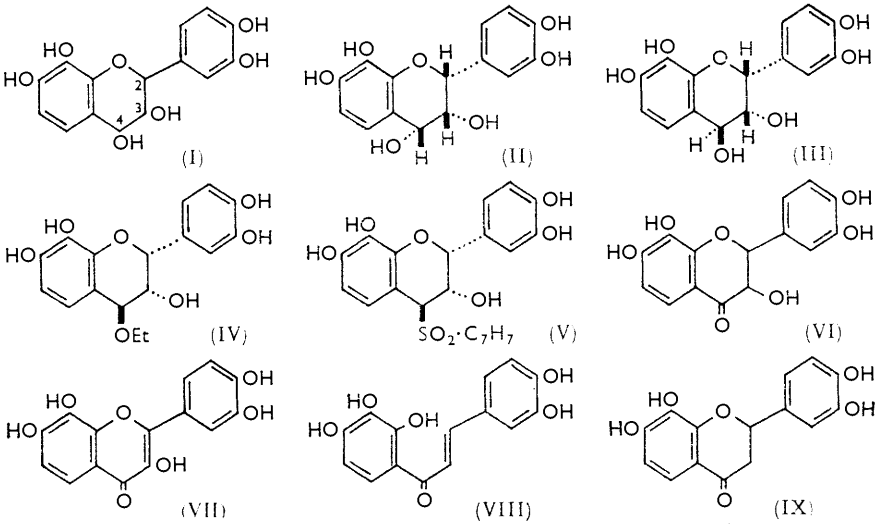
A new leucoanthocyanidin, isomelacacidin, has been found with its 4-epimer, melacacidin, in the heartwood of three species of *Acacia*. Extraction and separation of the epimers is described, and melacacidin obtained pure and crystalline. Isomelacacidin behaves as a reactive *p*-hydroxybenzyl alcohol and readily forms an ethyl ether by reaction with ethanol, and this distinction from melacacidin facilitates its separation therefrom. The comparative inertness of melacacidin is attributed to an unexpected conformational stability in its 2(*eq*),3(*ax*),4(*eq*)-half-chair conformation, which inhibits resonance stabilisation of the 4-carbonium ion and so reduces the benzylic character of the 4-hydroxyl group.

Heartwoods of three Australian *Acacia* species (*A. excelsa*, *A. harpophylla*, and *A. melanoxylon*) have each been found to contain melacacidin (0·2—1·0%) and a new leucoanthocyanidin, isomelacacidin (>0·2%). Melacacidin was first isolated, in amorphous form, from *A. melanoxylon* by King and Bottomley,[2] who proved its structure (I). It was later shown [3] to have the geometrical configuration (II); from comparison of the specific rotations of the free phenol, its 7,8,3′,4′-tetramethyl ether, and the methyl ether diacetate



with corresponding values in the (−)-epicatechin series, (−)-melacacidin is more probably represented by (II) than by its mirror image.* Melacacidin has now been extracted by a different method, purified by counter-current distribution, and obtained crystalline from both *A. excelsa* and *A. harpophylla*; the latter appears to be the best source. Crude extractives from *Acacia* heartwoods were shown by paper chromatography to contain melacacidin and two other monomeric leucoanthocyanidins, isomelacacidin and *O*-ethylisomelacacidin. The three compounds were separated by counter-current distribution, but their isolation was simplified after recognition that *O*-ethylisomelacacidin was an artefact

* *Note added in proof.* The absolute configuration (II) of (−)-melacacidin has now been established (unpublished work with E. F. Katekar).

[1] Part II, *J.*, 1960, 2433.
[2] King and Bottomley, *Chem. and Ind.*, 1953, 1368; *J.*, 1954, 1399.
[3] King and Clark-Lewis, *J.*, 1955, 3384.

very readily formed from isomelacacidin and the ethanol which had been used in manipulating the extractives. The crude leucoanthocyanidin mixture was boiled with ethanolic 1% acetic acid, to convert isomelacacidin into *O*-ethylisomelacacidin, and this was easily separated from melacacidin by counter-current procedures because of its enhanced distribution ratio; the separatory-funnel procedure described by Bush and Densen [4] proved useful for preparative separations. Hydrolysis of crystalline *O*-ethylisomelacacidin gave isomelacacidin, which has not yet been induced to crystallise and also hinders crystallisation of melacacidin.

The structure of isomelacacidin (III) was inferred largely from the properties of its ethyl derivative, *O*-ethylisomelacacidin (IV), which crystallised as a hydrate but yielded the anhydrous compound $C_{17}H_{18}O_7$ when dried, and gave the expected values in the Zeisel and Kuhn–Roth determinations. *O*-Ethylisomelacacidin crystallised unchanged from aqueous methanol, and on paper chromatograms it was separable from isomelacacidin and *O*-methylisomelacacidin; moreover, *O*-ethylisomelacacidin was separated from isomelacacidin by counter-current distribution between solvents, so that it is clearly an ether and not merely a solvate. *O*-Ethylisomelacacidin gave an alkali-insoluble tetramethyl ether which yielded a toluene-*p*-sulphonate, thus disclosing the presence of four phenolic groups and an alcoholic hydroxyl group. The anthocyanidin formed from *O*-ethylisomelacacidin and hot 3N-hydrochloric acid was chromatographically indistinguishable from 3,7,8,3′,4′-pentahydroxyflavylium chloride similarly derived from melacacidin, so that *O*-ethylisomelacacidin is either (IV) or the analogous 3-ethoxy-4-hydroxy-compound. The extreme lability of the ethyl group in *O*-ethylisomelacacidin excludes this alternative and requires the benzylic ether structure (IV). The close relation between *O*-ethylisomelacacidin, isomelacacidin, and melacacidin was confirmed by acid-catalysed hydrolysis and epimerisation. Thus *O*-ethylisomelacacidin was rapidly hydrolysed to isomelacacidin by hot water or dilute acetic acid (see p. 4111). Conversion of isomelacacidin into melacacidin was very slow but melacacidin was epimerised rapidly to isomelacacidin (*ca.* 90%) by hot dilute hydrochloride acid. The equilibrium between the two leucoanthocyanidins in aqueous acids was therefore greatly in favour of isomelacacidin, but conversion of melacacidin into *O*-ethylisomelacacidin was negligible in ethanol containing acetic acid.

Isomelacacidin was further characterised as the sulphone (V), a derivative suggested by the investigations of reactive benzyl alcohols by Kenyon and his co-workers [5] who found them to undergo reversible formation of sulphones by reaction with sulphinic acids. This sulphone (V) crystallised readily and was easily purified; it gave a penta-acetate, a tetramethyl ether, and a tetramethyl ether acetate. *O*-Ethylisomelacacidin and toluene-*p*-sulphinic acid gave the sulphone (V) when heated in weakly acidic solution, but melacacidin did not react until the acidity was increased to that already known to cause isomerisation. The product from melacacidin was the same sulphone (V), and this establishes the steric identity in melacacidin and isomelacacidin of those positions not affected by acid. The properties of isomelacacidin accord well with those of other benzyl alcohols activated by *o*- and *p*-hydroxyl groups,[6] and the reactivity of these alcohols is attributed to resonance stabilisation of the related benzyl carbonium ions. This indicates that the 4-hydroxyl group in isomelacacidin is axial (or can easily become axial) and that the 2(*eq*),3(*ax*),4(*ax*)-conformation is preferred—it permits maximum resonance stabilisation of the 4-carbonium ion through coplanarity of the attached groups. The preferred conformation of melacacidin [3] is, however, 2(*eq*),3(*ax*),4(*eq*) and it is probable that intramolecular hydrogen bonding between the heterocyclic oxygen atom and the 3(*ax*)-hydroxyl group stabilises both epimers

[4] Bush and Densen, *Analyt. Chem.*, 1948, **20**, 121.
[5] Balfe, Downer, Evans, Kenyon, Poplett, Searle, and Tárnoky, *J.*, 1946, 797; Balfe, Kenyon, and Wicks, *J.*, 1946, 807; Kenyon and Mason, *J.*, 1952, 4964.
[6] Mikawa, *Bull. Chem. Soc. Japan*, 1954, **27**, 53; de Jonge and Bibo, *Rec. Trav. chim.*, 1955, **74**, 1448.

in the conformations mentioned. The comparatively unreactive nature of melacacidin is a consequence of its conformational stability in the 2(*eq*),3(*ax*),4(*eq*)-conformation which is unfavourable for resonance stabilisation of the 4-carbonium ion (attached groups not coplanar with the benzene ring).

The rapid conversion of melacacidin into isomelacacidin indicates that epimerisation is probably the first step in formation of the related anthocyanidin from melacacidin and aqueous acid. Formation of the 4-carbonium ion from isomelacacidin occurs very readily and would undoubtedly account for one process of the polymerisation to phlobaphenes which accompanies formation of anthocyanidins from leucoanthocyanidins.[2] Analogous polymerisations through the 2-carbonium ions formed by ring fission of flavans have been discussed by others.[7]

Peltogynol and peltogynol B occur in *Peltogyne porphyrocardia*,[8] and teracacidin and isoteracacidin are found together in *Acacia intertexta*,[9] so that flavan-3,4-diols may frequently, or even generally, occur as mixtures of 4-epimers. Such epimers clearly could arise in the plant through non-specific reduction of a dihydroflavonol although, from the ease of epimerisation, isomelacacidin might also be formed from melacacidin. A dihydrotetrahydroxyflavonol (probably VI) was obtained from *A. excelsa* but not examined more closely, and 7,8,3′,4′-tetrahydroxyflavonol (VII) was isolated from *A. harpophylla*. Okanin (VIII) was also obtained from this source but appeared to be formed during isolation so that we infer the presence in the wood of the corresponding flavanone (IX).

Experimental

Paper chromatograms were run with butan-1-ol–acetic acid–water [10] (4 : 1 : 5) except where otherwise indicated. Water–acetic acid–concentrated hydrochloric acid (10 : 30 : 3) (Forestal solvent [11]) was used for chromatography of anthocyanidins.

Wood specimens were obtained by courtesy of the Chemical Research Laboratories, C.S.I.R.O., and collected from the following botanically identified *Acacia* species (herbarium specimen numbers in brackets): *A. harpophylla* (54 and 500), *A. excelsa* (55 and 890), and *A. melanoxylon* (481). Heartwood and sapwood were separately reduced to fine shavings; a total of 53 kg. was extracted.

Extraction of the Wood.—Milled heartwood (2335 g.) of *Acacia excelsa* was extracted with acetone (24 hr.), ethanol (8 hr.), and water (8 hr.) by continuous hot percolation in a stainless-steel extractor. The viscous residue (376 g.) obtained by concentrating the acetone extract was stirred with water (4 l.), and next day the filtrate was concentrated under reduced pressure to 600 c.c. Continuous extraction of this solution with ethyl acetate then gave in successive 8 hr. periods 116 g., 8 g., and 4 g. of extractive. The ethanol extract was similarly treated and gave 14 g. of material soluble in ethyl acetate. The ethyl acetate-soluble extractive (142 g.) was boiled with ethanol (1 l.) and acetic acid (10 c.c.) for 2 hr., to convert isomelacacidin into *O*-ethylisomelacacidin. Evaporation to dryness under reduced pressure then left a residue which was dissolved in water (400 c.c.) and distributed between ethyl acetate (240 c.c.) and water (400 c.c.) on each occasion in a counter-current procedure with five separatory funnels essentially as described by Bush and Densen.[4] The ethyl acetate fractions from the distribution were evaporated and left fraction A (77·7 g.). The water-soluble fractions were evaporated under reduced pressure at 55° to 400 c.c. and then continuously extracted with ether, which gave in 32 hr. a total of 10·6 g. (0·45%) of crystalline melacacidin and 26·6 g. of non-crystalline ether-soluble material. Fraction A was heated on a steam-bath with water (400 c.c.) and filtered when cold from a dihydroflavonol (5·2 g.; see below). The filtrate therefrom was distributed between ethyl acetate and water in separatory funnels as indicated above. Evaporation of these combined aqueous phases gave a residue which, after being boiled with ethanol (300 c.c.) and

[7] Brown, Cummings, and Somerfield, *J.*, 1957, 3757; Brown and Cummings, *J.*, 1958, 4302; Freudenberg and Weinges, *Fortschritte der Chemie organischer Naturstoffe*, 1958, **16**, p. 1.
[8] Chan, Forsyth, and Hassall, *J.*, 1958, 3174.
[9] Clark-Lewis, Katekar, and Mortimer, unpublished work.
[10] Partridge, *Biochem. J.*, 1948, **42**, 238.
[11] Bate-Smith, *Biochem. J.*, 1954, **58**, 122.

acetic acid (3 c.c.) for 2½ hr., evaporated to dryness, and continuously extracted with ether from an aqueous solution neutralised with sodium hydrogen carbonate, gave crystalline *O*-ethylisomelacacidin (7·6 g., 0·3%). The water-soluble portion from the ethanol extract of the wood contained pipecolic acid and 4-hydroxypipecolic acid.[12]

Melacacidin and isomelacacidin were also obtained from *A. melanoxylon* and *A. harpophylla* heartwoods; the latter appeared to be the best source of melacacidin and one specimen gave 0·6% and 1·0%, and another, 0·2% yield. Melacacidin and isomelacacidin (as *O*-ethyl derivative) were conveniently separated in a 50-tube (50 c.c.) counter-current apparatus by distribution between ethyl acetate and 0·067M-phosphate buffer (pH 7·0) when dealing with quantities up to about 30 g. Peak concentrations were as follows: *O*-ethylisomelacacidin (tube 44), melacacidin (tube 18), and isomelacacidin (tube 14).

The crude dihydroflavonol (5·2 g.) mentioned above crystallised from boiling water in pale yellow flakes (*ca.* 0·05 g. per 100 c.c. of water), and two recrystallisations from aqueous ethanol (covered with light petroleum to retard oxidation) gave *dihydro*-(?7,8,3′,4′)-*tetrahydroxyflavonol*, m. p. 284—285° after sintering at 170—180° (Found, on material dried for 24 hr. *in vacuo* over P_2O_5: C, 57·6; H, 4·4. $C_{15}H_{12}O_7,\frac{1}{2}H_2O$ requires C, 57·6; H, 4·2%). This gave red colours stable for several hours when treated in aqueous-ethanolic hydrochloric acid with magnesium or zinc.[13] On paper chromatograms it gave a streak (R_F 0·57—0·79) with faint blue fluorescence under untraviolet light, invisible in daylight, but becoming yellow after 3—4 hr. Hydrochloric acid or ethanolic 3% toluene-*p*-sulphonic acid gave a deep yellow colour immediately.

Isolation of Okanin (VIII) *and* 7,8,3′,4′-*Tetrahydroxyflavonol* (VII).—Milled heartwood of *A. harpophylla* (2756 g.) was extracted with light petroleum (10 hr.), ether (67 hr.), and acetone (7 hr.), and the acetone solution was concentrated on a steam-bath to *ca.* 220 c.c. and filtered from a residue (5·9 g.) consisting mainly of 7,8,3′,4′-tetrahydroxyflavonol. The filtrate was diluted with water to 2 l. and after several days the solution was filtered and concentrated to 400 c.c. under reduced pressure before continuous extraction with ether for 24 hr. gave 36·2 g. of a yellow mixture of polyphenols. This was dissolved in water (25 c.c.) and was continuously extracted with light petroleum (b. p. 60—80°), which dissolved amorphous material (0·06 g.) in 24 hr. and caused separation of orange-red crystals (1·47 g.) of okanin (3,4,2′,3′,4′-pentahydroxychalcone) in the aqueous layer. Recrystallisation from an ethanol (30 c.c.) solution diluted with water (100 c.c.) gave okanin [14] in orange needles, m. p. 238° raised to 240° on admixture with authentic okanin, m. p. 245° (penta-acetate, m. p. 136°; lit., 141°). On paper chromatograms both samples of okanin gave tailing spots with R_F 0·52 (leading edge).

The 7,8,3′,4′-tetrahydroxyflavonol fraction was identified by acetylation to 3,7,8,3′,4′-penta-acetoxyflavone, m. p. 176° (lit.,[15] m. p. 173°) (Found: C, 58·6; H, 4·0; Ac, 43·4. Calc. for $C_{25}H_{20}O_{12}$: C, 58·6; H, 3·9; Ac, 42·0%), and by conversion in acetone with methyl sulphate and potassium carbonate into 3,7,8,3′,4′-pentamethoxyflavone, m. p. 151° (lit.,[2] m. p. 151°) alone and when mixed with a specimen similarly prepared from synthetic 7,8,3′,4′-tetramethoxyflavonol (Found: C, 64·5; H, 5·5; OMe, 40·9. Calc. for $C_{20}H_{20}O_7$: C, 64·7; H, 5·4; OMe, 41·7%).

Melacacidin (II).—Crystalline melacacidin (0·5 g.) was dissolved in ethanol (30 c.c.), and the filtrate was concentrated to 4 c.c. *Melacacidin* (0·36 g.) crystallised rapidly from the seeded solution, and recrystallisation from ethanol containing a trace of acetic acid (charcoal) gave colourless prisms, m. p. 229° (decomp.) after becoming brown and sintering at 200—225° (Found: C, 58·9; H, 4·9; loss on drying, <0·1. $C_{15}H_{14}O_7$ requires C, 58·8; H, 4·6%). Melacacidin had $[\alpha]_D^{16}$ −75° (0·2% in EtOH, 4 dm. tube), $[\alpha]_D^{24}$ −85° (1% in 1 : 1 acetone–water), and $[\alpha]_D^{22}$ −75° (1% in 1 : 1 dioxan–water) unchanged by the addition of 10N-hydrochloric acid (1%). In 95% ethanol it had $\lambda_{max.}$ 280 mμ (log ε 3·5) and $\lambda_{min.}$ at 254 mμ (log ε 2·77). Pure melacacidin, R_F 0·25—0·32 in butanol–acetic acid–water and R_F 0·30—0·42 in 2% acetic acid, was almost insoluble in water and its solubility in ethanol was less than 0·5% at room temperature and *ca.* 2% at the b. p. A solution of melacacidin in ethanol was kept for 20 months without exclusion of light or air; it did not darken appreciably and was then found by chromatography to contain only melacacidin.

[12] Clark-Lewis and Mortimer, *J.*, 1960, in the press.
[13] Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031; Shimizu, *J. Pharm. Soc. Japan*, 1952, **72**, 338; *Chem. Abs.*, 1953, **47**, 2758.
[14] King and King, *J.*, 1951, 569.
[15] Kostanecki and Rudse, *Ber.*, 1905, **38**, 935.

Melacacidin tetramethyl ether crystallised from ethanol–ether in needles, m. p. 144—145° alone and when mixed with authentic material, $[\alpha]_D^{25}$ −83·5° (1% in EtOH) (lit.,[2] m. p. 145—146°, α_D −84·4°) (Found: C, 63·0; H, 6·1; OMe, 34·3. Calc. for $C_{19}H_{22}O_7$: C, 62·9; H, 6·1; OMe, 34·2%). The tetramethyl ether diacetate had m. p. 191—192°, $[\alpha]_D$ −39·5° (0·2% in EtOH; 4 dm. tube) (lit.,[2] m. p. 193—194°, α_D −39·2°).

Hot solvent (as below) (1 c.c.) was added to crystalline melacacidin (0·02 g.), and the mixture was heated in a boiling-water bath; it was examined by paper chromatography of samples withdrawn within ½—1 min. (when dissolution was complete) and at intervals of 10, 20, 30, 60, and 90 min. Melacacidin was unchanged by water at 100° for 1½ hr. but in 0·1N-acetic acid melacacidin was detected after 1 and 1½ hr. Melacacidin was progressively converted by 0·5N-acetic acid into isomelacacidin (*ca.* 50% after 1 hr., *ca.* 67% after 1½ hr.) at 100°, and in 0·01N-hydrochloric acid conversion into melacacidin reached 90% (apparently equilibrium proportion) after 10 min. at 100°; polymeric material was detected after 1 hr. Results with 0·05N-hydrochloric acid at 100° were similar except that polymeric material appeared after 20 min. Less than 1% of *O*-ethylisomelacacidin was formed from melacacidin (0·05 g.), ethanol (10 c.c.), and acetic acid (0·2 c.c.) at the b. p. in 1½ hr.

O-*Ethylisomelacacidin* (IV).—The ethyl ether was prepared from isomelacacidin extracted from *A. excelsa* as already described, and also from *A. melanoxylon* heartwood (8·8 kg.) extractive after removal of melacacidin and ketonic flavonoids by distribution between solvents: crude isomelacacidin (106 g.) was boiled for 2 hr. with ethanol (1 l.) and acetic acid (10 c.c.), and evaporation left a residue which was distributed between ethyl acetate and water in separatory funnels.[4] Crystallisation of the ethyl acetate-soluble material (56 g.), after dilution of its ethanolic solution (112 c.c.) with water (450 c.c.) and storage at 0°, gave O-*ethylisomelacacidin hydrate* (20·4 g.) (Found: C, 51·5; H, 6·0; *C*-Me, 3·7; OEt, 10·0; loss on drying, 15·9. $C_{17}H_{18}O_7,3\frac{1}{2}H_2O$ requires C, 51·4; H, 6·3; *C*-Me, 3·8; OEt, 11·4; H_2O, 15·8%). The anhydrous *compound* was obtained at 90° *in vacuo* over phosphoric oxide (Found: C, 60·8; H, 5·5; OEt, 11·7. $C_{17}H_{18}O_7$ requires C, 61·1; H, 5·4; OEt, 13·3%) and remained chromatographically homogeneous (R_F 0·58—0·67; R_F 0·58—0·70 in 2% acetic acid), and indistinguishable from the undried material which had $[\alpha]_D^{22}$ −31° (0·9% in EtOH), $[\alpha]_D^{23}$ −40° (2·4% in 1 : 1 acetone–water) increased to $[\alpha]_D^{17}$ −70° during 6 hr. after addition of 10N-hydrochloric acid (1%). The anthocyanidin formed by heating *O*-ethylisomelacacidin with 3N-hydrochloric acid for 15 min. was extracted with pentyl alcohol and chromatographed with Forestal solvent; it possessed the same R_F as the anthocyanidin from melacacidin and behaved similarly when sprayed with alcoholic aluminium chloride.

Hot solvent (1 c.c.) was added to *O*-ethylisomelacacidin (0·02 g.), and the mixture was heated in a boiling-water bath and examined by paper chromatography as for melacacidin. *O*-Ethylisomelacacidin was slowly hydrolysed by water to isomelacacidin (>50% in 1 hr.), and in 0·1N-acetic acid conversion was almost complete in 10 min., and no other polyphenol was formed. In 0·5N-acetic acid 50% conversion into isomelacacidin was reached in ½ min. and melacacidin was barely detectable after 10 min. and thereafter increased in concentration which however remained significantly lower than that of isomelacacidin. Considerable conversion into isomelacacidin occurred in 1 min. in 0·01N-hydrochloric acid, and melacacidin was detected after 10 min. but was obscured by polymeric material in later samples.

O-*Ethylisomelacacidin* 7,8,3′,4′-*Tetramethyl Ether* 3-*Toluene*-p-*sulphonate*.—The *tetramethyl ether* prepared by the action of diazomethane on *O*-ethylisomelacacidin was obtained as a viscous oil, b. p. 245°/1 mm. (Found: C, 65·3; H, 7·2; OAlk, 35·3. $C_{21}H_{26}O_7$ requires C, 64·6; H, 6·7; OAlk, calc. as OMe, 38·7%). Crystallisation of this and subsequent preparations could not be induced, nor that of the acetate or *p*-nitrobenzoate. The *toluene*-p-*sulphonate*, prepared in pyridine at room temperature, crystallised from ethanol in prisms (*ca.* 70%), m. p. 125° unchanged by two recrystallisations from ethanol (5 c.c. per g.), $[\alpha]_D^{23}$ −19° (0·04% in EtOH). The crystals became opaque at 110—120°, and were dried at room temperature over phosphoric oxide for analysis (Found: C, 61·5; H, 6·1; S, 6·3; OMe, 27·6. $C_{28}H_{32}O_9S$ requires C, 61·8; H, 5·9; S, 5·9; OMe, 28·5%).

O-*Methylisomelacacidin*.—The isomelacacidin fraction (33·5 g.) from *A. harpophylla* heartwood (2165 g.) was boiled with methanol (300 c.c.) and acetic acid (10 c.c.) for 2 hr. before evaporation under reduced pressure. Slow crystallisation of the residue from aqueous methanol at 0° gave O-*methylisomelacacidin* (5·8 g.) initially as needles and then plates, decomp. when heated, $[\alpha]_D^{12}$ −56° (0·9% in MeOH), −68° (1% in 1 : 1 acetone–water). When stored over

anhydrous calcium chloride it gave a pink powder (Found: C, 58·4; H, 5·4; OMe, 9·0. $C_{16}H_{16}O_7,\frac{1}{2}H_2O$ requires C, 58·4; H, 5·2; OMe, 9·4%). Crystalline material lost 10·4% of its weight when dried at 90° for 9 hr. On paper chromatograms it separated (R_F 0·63—0·73) from melacacidin (R_F 0·42—0·48) and *O*-ethylisomelacacidin (R_F 0·73—0·82); separation also occurred with 2% acetic acid (R_F 0·53—0·66, 0·35—0·47, and 0·63—0·74 respectively). The anthocyanidin formed from *O*-methylisomelacacidin and hot 3N-hydrochloric acid was chromatographically indistinguishable in Forestal solvent from the anthocyanidin similarly obtained from melacacidin and gave the same colour change with aluminium chloride. Methylation of *O*-methylisomelacacidin with diazomethane gave a product which did not crystallise or yield a crystalline acetate or toluene-*p*-sulphonate.

p-*Tolyl Sulphone* (V).—(*a*) Crystalline *O*-ethylisomelacacidin (0·371 g.) dissolved in 0·01N-hydrochloric acid (5 c.c.) on a steam-bath in 1 min.; acetic acid (0·35 c.c.) and sodium toluene-*p*-sulphinate dihydrate (0·643 g.) were added, and the mixture was heated for 30 min. on a steam-bath. Crystallisation at 0° gave the *sulphone* (0·39 g., 81%), m. p. 103—111°, $[\alpha]_D^{26}$ −25° (1% in acetone) (Found, after drying at room temperature and pressure over P_2O_5: C, 54·8; H, 5·0; S, 6·6. $C_{22}H_{20}O_8S,2H_2O$ requires C, 55·0; H, 5·0; S, 6·7%). The *penta-acetate* was prepared by acetylation with acetic anhydride and pyridine at room temperature overnight, and crystallised from ethanol in needles, m. p. 193°, $[\alpha]_D^{23}$ −13·5° (1% in acetone) (Found: C, 59·0; H, 5·0; S, 5·3; Ac, 30·3. $C_{32}H_{30}O_{13}S$ requires C, 58·7; H, 4·6; S, 4·9; Ac, 32·9%). With 3N-hydrochloric acid on a steam-bath for 15 min. the phenolic sulphone gave 3,7,8,3′,4′-pentahydroxyflavylium chloride, chromatographically homogeneous and inseparable from the flavylium salt derived from melacacidin (R_F 0·58; cyanidin R_F 0·55; in Forestal solvent).

(*b*) Crystalline melacacidin (0·612 g.) in 0·01N-hydrochloric acid (10 c.c.) was heated on a steam-bath for 20 min. and acetic acid (0·7 c.c.) and sodium toluene-*p*-sulphinate dihydrate (1·27 g.) were then added, and heating was continued for 30 min. The sulphone (0·347 g., 36%) crystallised from the cooled solution, and recrystallisation (charcoal) from 5% acetic acid (20 c.c.) gave the sulphone in pale pink prisms (0·273 g.), $[\alpha]_D^{23}$ −24° (1% in acetone), m. p. 102—110° alone and when mixed with that prepared by method (*a*). The acetate had m. p. 192—193° alone and when mixed with that described under (*a*).

(*c*) The sulphone (1 g.) was kept in ethereal diazomethane at room temperature for 45 hr., and the solution was concentrated to 5 c.c.; slow evaporation left a clear gum which crystallised very slowly. The product was triturated with ether, and the insoluble residue (0·85 g.) crystallised from a very concentrated solution in acetone in plates, m. p. 153—154°, consisting of the p-*tolyl sulphone* 7,8,3′,4′-*tetramethyl ether*, $[\alpha]_D^{25}$ −44° (1% in acetone) (Found: C, 62·5; H, 5·9; S, 6·4; OMe, 24·4. $C_{26}H_{28}O_8S$ requires C, 62·4; H, 5·6; S, 6·4; OMe, 24·8%). The *sulphone tetramethyl ether acetate* (0·63 g., 47%), m. p. 117—118° raised to m. p. 119—122° by crystallisation (needles, 0·28 g.) from methanol, $[\alpha]_D^{23}$ −34° (1% in acetone), was prepared by methylation of the sulphone (1·5 g.) with ethereal diazomethane, evaporation of the ether, and acetylation of the viscous residue with acetic anhydride (2 c.c.) and pyridine (10 c.c.) at room temperature for 14 hr. (Found: C, 60·8, 60·8; H, 5·6, 5·9; S, 5·7; OMe, 22·5; Ac, 8·7. $C_{28}H_{30}O_9S,\frac{1}{2}H_2O$ requires C, 61·0; H, 5·7; S, 5·8; OMe, 22·3; Ac, 7·8%).

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