The Chemistry of Extractives from Hardwoods. Part XVII. The Occurrence of a Flavan-3 : 4-diol (Melacacidin) in* Acacia melanoxylon.

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The amorphous material extracted by ether from Australian Blackwood (*Acacia melanoxylon*) consists largely of melacacidin, 3 : 4 : 7 : 8 : 3′ : 4′-hexahydroxyflavan, a compound of novel type characterised by crystalline derivatives. Its constitution has been demonstrated by oxidation of the tetramethyl ether, with potassium permanganate to 2-hydroxy-3 : 4-dimethoxybenzoic acid and veratric acid, and by the Oppenauer method to 7 : 8 : 3′ : 4′-tetramethoxyflavonol

As already briefly reported (King and Bottomley, *Chem. and Ind.*, 1953, 1368), melacacidin combines the properties of both leucoanthocyanidin and phlobatannin, thereby explaining the observations of Bate-Smith and Swain (*ibid.*, 1953, 377) and indicating a flavan-3 : 4-diol structure for this widely distributed group of natural products.

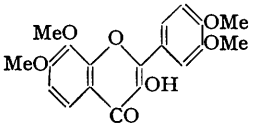
Acacia melanoxylon is a tree of medium size found in many parts of Australia and Tasmania, where it is of value as the source of a cabinet timber. A quantity of the heartwood procured through the kindness of the Conservator of Forests, Western Australia, has been investigated with respect to the nature of its extractable constituents. The only noteworthy product was a light amorphous powdery solid, amounting to approximately 1—1½% of the wood, phenolic in nature and largely soluble in cold water. No homogeneous compound was isolated from the extract nor were its various acylation products obtained crystalline. Nevertheless it is substantially a single compound and when treated with diazomethane it gave a crystalline optically active derivative, $C_{19}H_{22}O_7$, containing four methoxyl groups. Crude melacacidin was shown by Zeisel analysis to be methoxyl-free; hence the compound $C_{19}H_{22}O_7$ is melacacidin tetramethyl

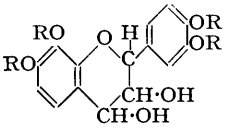
* Part XVI, preceding paper.

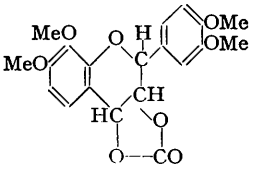
ether. The derivative exhibited no colour with ferric chloride and was insoluble in alkalis, but its formation of a diacetate indicated the presence of two alcoholic groups. Oxidation of the tetramethyl ether with potassium permanganate afforded a mixture of acids which were separated by conversion into methyl esters, one of them being phenolic and therefore alkali-soluble. The phenolic ester was identified as methyl 2-hydroxy-3 : 4-dimethoxybenzoate and the accompanying neutral ester as methyl veratrate.

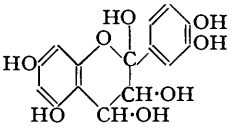
From the formation of these two oxidation products, one of them an *o*-hydroxy-acid, it seemed probable that melacacidin contained a dihydrodihydroxypyran nucleus. The ready oxidation of melacacidin tetramethyl ether with periodic acid proved that the two alcoholic substituents were present as an α-glycol unit; 2-hydroxy-3 : 4-dimethoxybenzaldehyde and an unidentified transformation product were isolated from this reaction, the former disclosing the position of the diol system with respect to the principal aromatic ring. Conclusive evidence as to the total structure of melacacidin was then obtained from an Oppenauer oxidation of the tetramethyl ether which yielded 7 : 8 : 3′ : 4′-tetramethoxyflavonol (I) (Kostanecki and Rudse, *Ber.*, 1905, **38**, 935), a sample of which was synthesised from 2-hydroxy-3 : 4 : 3′ : 4′-tetramethoxychalkone by oxidation with alkaline hydrogen peroxide. Melacacidin is therefore one of the stereoisomeric 7 : 8 : 3′ : 4′-flavan-3 : 4-diols represented as (II; R = H).

Methylation of the crude flavandiol with methyl sulphate–potassium carbonate in acetone for a limited time also resulted in a good yield of the tetramethyl ether (II; R = Me). Prolonged treatment under these conditions, on the other hand, gave a mixture of (II; R = Me) with two other crystalline products, one of them a pentamethyl compound [melacacidin 3(or 4) : 7 : 8 : 3′ : 4′-pentamethyl ether]. The identity of the remaining derivative, $C_{20}H_{20}O_8$, became apparent after it had been subjected to mild hydrolysis with

(I) 

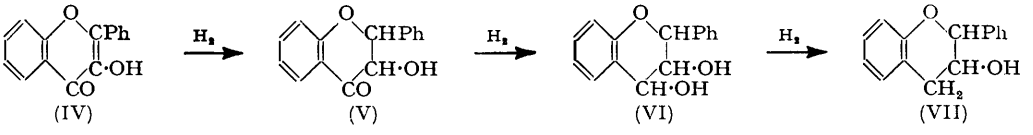
 (II)

(III) 

 (VIII)

alkali, the formation of the tetramethyl ether $C_{19}H_{22}O_7$ (II; R = Me) showing it to be a cyclic carbonate (III). This was afterwards confirmed by a synthesis of (III) from tetramethylmelacacidin and ethyl chloroformate in an aqueous alkaline medium. From the ready production of the cyclic carbonate (III) under the Robertson–Robinson methylation conditions it may be concluded that the diol system has the *cis*-configuration. The nature of this unusual reaction and also its possible application to other polyhydric compounds, *e.g.*, carbohydrates, appears to warrant further investigation.

Flavan-3 : 4-diols seem not to have been recognised hitherto as natural products, though closely related to the widely distributed series of flavonols. The discovery of melacacidin therefore completes the reduction sequence illustrated in prototype by the



(IV) (V) (VI) (VII)

structures (IV)—(VII) beginning with flavonols (type IV) and ending with the catechins (type VII), several flavanolones (type V) having in recent years been discovered in Nature (cf. Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031; Erdtman, "Progress in Organic Chemistry," Butterworth, ed. J. W. Cook, Vol. I, pp. 31, 37; Hillis, *Austral. J. Sci. Res.*, 1952, *A*, **5**, 379).

A structure resembling that of melacacidin, namely (VIII), was proposed by Robinson and Robinson (*Biochem. J.*, 1933, **27**, 206) for a leucoanthocyanidin, and the essential validity of their suggestion is clear from the properties of the amorphous extractive of *A. melanoxylon*, to which reference has been made in a preliminary publication (King and Bottomley, *Chem. and Ind.*, 1953, 1368). Thus heating it with mineral acid produces a deep scarlet solution similar to that of the anthocyanidins, although the amount of colouring matter generated is relatively small, the principal product being a red-brown amorphous material. Comparison with a synthetic specimen by means of paper chromatography, using acetic acid–butanol–water, has shown that the resulting anthocyanidin is the expected 3 : 7 : 8 : 3′ : 4′-pentahydroxyflavylium salt (Dr. J. W. Clark-Lewis, unpublished work). Although no natural anthocyanidin with the phenolic hydroxyl pattern of melacacidin has so far been encountered, in the light of observations by Bate-Smith and Swain (*Chem. and Ind.*, 1953, 377), who have concluded that the properties of so-called leucoanthocyanidins occurring in many plant products are indistinguishable from those of condensed tannins, the behaviour of melacacidin with acids is of considerable significance since it implies that the hitherto imperfectly defined " phlobatannins " also are derivatives of flavan-3 : 4-diol. Certain resemblances, *e.g.*, in ultra-violet light absorption, between the leucoanthocyanidins and catechins noted by Bate-Smith and Swain (*loc. cit.*) are therefore readily explained by the similarity of their structures, or may even be due to the actual conversion of the leucoanthocyanidins (in part) into catechins, as was recently demonstrated chromatographically by Forsyth (*Nature*, 1953, **172**, 726) in the case of cacao bean " leucoanthocyanin." The formation of a flavylium salt from the corresponding leucoanthocyanidin is an oxidation process and if it were to take place through a compensating reduction of the flavandiol to catechin the reason for the tannin reactions of the leucoanthocyanidins becomes obvious. On the other hand, the relatively small yield of anthocyanidin produced by the action of acids compared with that of the amorphous (presumed) polymer indicates that the mechanism of polymerisation is to a large degree independent of flavylium salt formation. Further investigation will no doubt show whether this new hypothesis as to the structure of phlobatannins or their precursors provides a satisfactory alternative to the flavpinacol theory due to Russell (*Chem. Reviews*, 1935, **17**, 155).

Evaporation of the ethereal solution remaining from the extraction of melacacidin gave an orange powder from which a small amount (0·01%) of 7 : 8 : 3′ : 4′-tetrahydroxyflavonol was obtained—the first indication of the natural occurrence of this compound.

Experimental

Melacacidin (II; R = H).—The ground heartwood of *A. melanoxylon* (2·8 kg.) was first extracted with boiling light petroleum and then for 3—4 days with ether, the metal extractor being heated on a steam-bath to avoid decomposition of the crude melacacidin which was deposited as a buff-coloured layer on the base of the vessel (yield, 28—45 g., 1—1·6%). The product was largely soluble in water, the simple alcohols, acetone, and acetic acid. Its aqueous solution gave a dark brown ferric reaction, and in boiling 10% hydrochloric acid a deep crimson solution from which gradually a brown precipitate separated. Both soluble and precipitated products were extracted into *iso*amyl alcohol to give deep red solutions. Melacacidin failed to give a crystalline acetate, benzoate, or *p*-nitrobenzoate; it was characterised by its methyl ethers (see below).

Melacacidin Tetramethyl Ether (3 : 4-*Dihydroxy*-7 : 8 : 3′ : 4′-*tetramethoxyflavan*) (II; R = Me).—(*a*) The crude extractive (1 g.) in methanol (10 c.c.) was treated with an ethereal solution of diazomethane and set aside overnight. Evaporation of the solvent under diminished pressure and crystallisation of the residue from ethanol yielded *tetramethylmelacacidin* (0·8 g.) as colourless needles, m. p. 145—146°, $[\alpha]_D$ −84·4° in EtOH (Found in a sample dried *in vacuo* at 100°: C, 63·1; H, 6·2; OMe, 33·6. $C_{19}H_{22}O_7$ requires C, 63·0; H, 6·1; 4OMe, 34·2%); light absorption: max., 217, 278 mμ; log ε 4·36, 3·39. Dissolved in hot alcoholic hydrogen chloride it gave a pale pink solution. *Tetramethylmelacacidin diacetate* crystallised from ethanol in needles, m. p. 193—194°, $[\alpha]_D$ −39·2° in EtOH (Found: C, 61·8; H, 5·9; OMe, 27·8; OAc, 20·2%; *M*, 456. $C_{23}H_{26}O_9$ requires C, 61·9; H, 5·9; 4OMe, 27·7; 2OAc, 19·3%; *M*, 446).

(*b*) A mixture of melacacidin (5 g.), potassium carbonate (35 g.), methyl sulphate (10 g.), and acetone (150 c.c.) was heated under reflux for 45 min. The filtered solution was then evaporated and the residue mixed with aqueous ammonia and set aside for several hours. The mixture was then acidified and the solid collected and crystallised from ethanol, melacacidin tetramethyl ether being obtained as needles (3 g.), m. p. 145—146°.

Tetramethylmelacacidin Carbonate (III).—(*a*) After the methylation of melacacidin (5 g.) with excess of methyl sulphate–potassium carbonate in acetone for 18 hr., the filtered liquid was evaporated to dryness and the residue mixed with benzene (20 c.c.). The insoluble solid, consisting of tetramethylmelacacidin, was removed, the solution evaporated, and the residue dissolved in boiling ethanol; *tetramethylmelacacidin carbonate* separated in needles, m. p. after recrystallisation, 209° (Found: C, 61·7; H, 5·4; OMe, 32·7%; *M*, 400. $C_{20}H_{20}O_8$ requires C, 61·85; H, 5·2; 4OMe, 31·9%; *M*, 388). A solution of the carbonate (0·1 g.) in acetone (5 c.c.) and N-aqueous sodium hydroxide (5 c.c.) was heated on a steam-bath until the acetone had evaporated. The solid collected from the cold solution crystallised from ethanol as needles, m. p. 145—146° alone or mixed with the compound (II; R = Me); it had $[\alpha]_D$ −84°.

(*b*) A mixture of tetramethylmelacacidin (0·1 g.) in acetone (4 c.c.) and water (4 c.c.) was treated with ethyl chloroformate (0·4 g.), and the solution made faintly alkaline with N-sodium hydroxide. After 10 min., alcohol (15 c.c.) was added, and when filtered from sodium chloride the solution was concentrated to *ca.* 10 c.c. The solid which was then collected crystallised from ethanol in needles, m. p. 209° alone or mixed with tetramethylmelacacidin carbonate.

Pentamethylmelacacidin [3(or 4)-*hydroxy*-4(or 3) : 7 : 8 : 3′ : 4′-*pentamethoxyflavan*] was isolated from the alcoholic solution remaining from the crystallisation of the carbonate (III) obtained by methylating melacacidin with methyl sulphate–potassium carbonate; it formed needles, m. p. 149°, from ethanol (Found: C, 63·7; H, 6·4; OMe, 39·8. $C_{20}H_{24}O_7$ requires C, 63·8; H, 6·4; 5OMe, 41·2%). Only the carbonate (III) and melacacidin pentamethyl ether were obtained after 48 hours' methylation of melacacidin.

Oxidation of Tetramethylmelacacidin with Potassium Permanganate.—A mixture of tetramethylmelacacidin (2 g.), potassium permanganate (3 g.), and acetone (100 c.c.) was heated under reflux for 4 hr. After the addition of water, the acetone was evaporated and the solution saturated with sulphur dioxide. Extraction with ether and shaking the ethereal solution with aqueous sodium hydrogen carbonate afforded a mixture of acids which was liberated from the carbonate solution, dissolved in ether, dried, and treated with excess of diazomethane. After 10 min. the solution was evaporated and the residue shaken with 2N-sodium hydroxide. The insoluble material consisted of methyl veratrate which when crystallised from alcohol had m. p. and mixed m. p. 59°.

The alkali-soluble ester was precipitated with hydrochloric acid and crystallised from aqueous methanol in prisms, m. p. 74—75° alone or mixed with synthetic methyl 2-hydroxy-3 : 4-dimethoxybenzoate. Hydrolysis of the ester with 2N-sodium hydroxide gave 2-hydroxy-3 : 4-dimethoxybenzoic acid, m. p. and mixed m. p. 170—171°.

Oxidation of Tetramethylmelacacidin with Periodic Acid.—The tetramethyl derivative (II; R = Me) (1 g.), dissolved in ethanol (20 c.c.), was treated with a solution of periodic acid (1·5 g.) in water (20 c.c.). After 4 hr., most of the alcohol was evaporated under diminished pressure and the residual liquid extracted with ether, which was washed in turn with aqueous sodium hydrogen carbonate and sodium hydroxide. Evaporation of the washed ethereal solution then yielded a semi-solid product from which was prepared a *semicarbazone*, m. p. 212°, alone or mixed with 2-hydroxy-3 : 4-dimethoxybenzaldehyde semicarbazone (Found: C, 50·2; H, 5·7; N, 17·6; OMe, 25·2. $C_{10}H_{13}O_4N_3$ requires C, 50·2; H, 5·5; N, 17·6; 2OMe, 25·9%).

The material isolated from the sodium hydroxide extract was redissolved in ether and again shaken with alkali. Acidification of the alkaline solution and ether-extraction yielded a substance crystallising from aqueous methanol in prisms, m. p. 103°, no longer soluble in sodium hydroxide and showing no reaction with ferric chloride (Found: C, 67·9; H, 5·7; OMe, 41·3%; *M*, 306).

Oppenauer Oxidation of Tetramethylmelacacidin.—To a solution of melacacidin (2 g.) in toluene (45 c.c.), prepared by concentrating a more dilute solution, *cyclo*hexanone (12 c.c.) and a saturated solution of aluminium *tert.*-butoxide in toluene (20 c.c.) were added and the mixture was heated under reflux for 1½ hr. It was then treated with water, acidified, and steam-distilled to remove solvent, etc., after which the residual solid was collected and crystallised from acetic acid. 7 : 8 : 3′ : 4′-Tetramethoxyflavonol (I) was thus obtained as small yellow prisms (0·4 g.), m. p. 220—221° alone or mixed with the synthetic specimen (Found: C, 63·4; H, 5·2. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·1%). The acetate, needles from methanol,

had m. p. and mixed m. p. 174—175° (Found : C, 63·1; H, 4·8. Calc. for $C_{21}H_{20}O_8$: C, 63·0; H, 5·0%).

Synthesis of 7 : 8 : 3′ : 4′-*Tetramethoxyflavonol* (cf. Kostanecki and Rudse, *loc. cit.*).—2-Hydroxy-3 : 4 : 3′ : 4′-tetramethoxychalkone (Crabtree and Robinson, *J.*, 1922, **121**, 1033) (5 g.) was dissolved in 50% aqueous ethanol (100 c.c.) containing sodium hydroxide (10 g.) to which 30% hydrogen peroxide (10 c.c.) was then added. During the first exothermic reaction the mixture was cooled and then set aside at room temperature for 1 hr. When the crystalline slurry had been acidified, 7 : 8 : 3′ : 4′-tetramethoxyflavonol was collected and crystallised from acetic acid, to give yellow prisms, m. p. 220—221° (Found : C, 63·5; H, 5·0; OMe, 34·7%); the acetate formed needles, m. p. 174—175° (Found : C, 63·1; H, 4·8; OMe, 30·5%).

Isolation of 7 : 8 : 3′ : 4′-*Tetrahydroxyflavonol.*—After being decanted from the deposit of melacacidin, the ethereal solution from the extraction of 15 kg. of the wood was evaporated and the residue heated in boiling water (200 c.c.). The boiling liquid was filtered and the insoluble residue (1·7 g.) washed with a little hot acetic acid and crystallised from ethanol. 7 : 8 : 3′ : 4′-Tetrahydroxyflavonol was isolated as yellow blades, m. p. (decomp.) *ca.* 312—320° (Found : C, 59·2; H, 3·6. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%).

Methylation with methyl sulphate–potassium carbonate in acetone for 20 min. gave 7 : 8 : 3′ : 4′-tetramethoxyflavonol, m. p. and mixed m. p. 220—221° (acetate, m. p. and mixed m. p. 174—175°). Continued methylation under these conditions yielded 3 : 7 : 8 : 3′ : 4′-pentamethoxyflavone as needles, m. p. 151° alone or with a synthetic specimen. Row, Seshadri, and Thiruvengadam (*Proc. Indian Acad. Sci.*, 1948, **28**, A, 98) give m. p. 153—154° (Found : C, 64·5; H, 5·1; OMe, 40·8. Calc. for $C_{20}H_{20}O_7$: C, 64·5; H, 5·4; 5OMe, 41·6%).

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