543. *Leucoanthocyanidins of Plants. Part III.* Leucopelargonidin from* Eucalyptus calophylla *Kino.*

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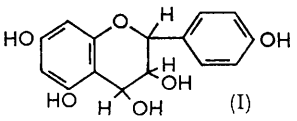
Eucalyptus calophylla kino yields, besides aromadendrin and sakuranetin, lævorotatory leucopelargonidin (3,4,5,7,4′-pentahydroxyflavan). Reduction of aromadendrin trimethyl ether yields two isomeric flavandiols; the lower-melting racemate agrees closely in properties with the methyl ether of the natural leucopelargonidin.

The gums of Eucalyptus species, called eucalyptus kinos, have been used in medicine and a few have been chemically examined recently. Hillis [1] reported the presence, in *E. calophylla* kino of Australian origin, of aromadendrin, ellagic acid, and a small quantity of kaempferol. Satwalekar, Gupta, and Rao [2] found that *E. citriodora*, *E. robusta*, *E. globulus* and *E. pilularis* contained ellagic acid, and that *E. citriodora* yielded also aromadendrin 7-methyl ether and kaempferol 7-methyl ether. More recently work on *E. pilularis* kino [3] in this laboratory showed that is was quite rich in leucodelphinidin. Hence a further study of the kino of *E. calophylla* obtained from W. Australia has been carried out, fractional extraction being used. Light petroleum yielded a very small amount of material giving positive tests for triterpenoids. An ether extract was separated into fractions by using in succession aqueous sodium carbonate and sodium hydroxide, and the products were identified as aromadendrin and sakuranetin, respectively. Subsequent extraction of the gum with acetone yielded a phenolic substance $C_{15}H_{14}O_6$ that gave the reactions of leucoanthocyanidins and formed pelargonidin chloride when boiled with alcoholic hydrochloric acid. It was lævorotatory, formed a penta-acetate, and with

* Part II, *Tetrahedron*, 1959, **6**, 21.

[1] Hillis, *Austral. J. Sci.*, 1952, **5**, 379.
[2] Satwalekar, Gupta, and Rao, *J. Indian Inst. Sci.*, 1957, **39**, 195.
[3] Ganguly, Seshadri, and Subramanian, *Tetrahedron*, 1958, **3**, 225.

diazomethane yielded a trimethyl ether from which a diacetate was obtained. These results indicated three phenolic and two alcoholic hydroxyl groups. The methyl ether was strongly lævorotatory and its infrared spectrum had no carbonyl band. The ultraviolet spectrum was similar to those of leucocyanidin [4] and leucodelphinidin.[3]



(I)

Permanganate oxidised the methyl ether to *p*-anisic acid. It was, therefore, concluded that the compound was leucopelargonidin (3,4,5,7,4′-pentahydroxyflavan) (I).

In efforts to prepare a synthetic leucopelargonidin derivative aromadendrin was reduced by sodium borohydride, but the product did not crystallise; after acetylation, however, a crystalline acetate was isolated; this differed in melting point from the acetate of the natural sample but agreed with it closely in the infrared spectrum. Its composition indicated that it was probably the corresponding flaven-3-yl acetate resulting from the diol by the loss of the elements of water during acetylation. In the hope of better success aromadendrin trimethyl ether was then reduced by the same reagent: two isomeric trimethoxyflavan-3,4-diols were obtained. Repeated fractional crystallisation yielded isomers of m. p. 197—200° and 150—153°. The latter agreed in melting point with the trimethyl ether obtained from natural leucopelargonidin. Janes and Morgan,[5] using different conditions, obtained only one product, melting at 161—162°. As Robertson [6] remarked, the nature of the reduction product and the proportion and ease of separation of the isomers seem to depend on the optical purity of the starting material as well as on the experimental conditions.

Though the presence of leucopelargonidin was noted earlier by qualitative methods, the isolation and description of leucopelargonidin is given here for the first time. The presence of sakuranetin in this kino is also new; it is a 7-methyl ether, in this respect similar to the 7-methyl ethers of aromadendrin and kaempferol found in *E. citriodora*.[2]

Experimental

Extraction.—*Eucalyptus calophylla* kino was procured through the help of the Council of Scientific and Industrial Research Organisation, Western Australia. The powdered gum (600 g.) was extracted repeatedly with low-boiling light petroleum (b. p. 40—60°): concentration of the extracts yielded a small amount of a substance which answered the Liebermann–Burchard reaction. The residual gum was extracted repeatedly with cold ether, and the ether extract was concentrated to about 800 c.c. and shaken with aqueous saturated sodium hydrogen carbonate (no extraction), 20% aqueous sodium carbonate (fraction A), and 3% aqueous sodium hydroxide (fraction B). Complete evaporation of the remaining ether solution left no residue. The gum left after the ether-extraction was subsequently extracted repeatedly with cold acetone, and the combined acetone extracts were evaporated under reduced pressure. The red sticky solid thus obtained was extracted repeatedly with ethyl acetate in the cold and these extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The concentrate was subjected to fractional precipitation by light petroleum (b. p. 40—60°) whereby coloured impurities were precipitated first and the leucoanthocyanidin (fraction C) was eventually obtained crystalline.

Fraction A (*Aromadendrin*).—When acidified with hydrochloric acid fraction A yielded a light brown solid (12 g.) that was filtered off, dried, and crystallised from methanol, yielding colourless needles, m. p. 246—247°. In alcoholic solution it gave a red colour with magnesium or zinc and hydrochloric acid. It gave a purple (changing to brown) ferric reaction. When methylated by dimethyl sulphate (3 mol.) and potassium carbonate (excess) in acetone for 10 hr. by the procedure of Goel *et al.*,[7] it gave a colourless *trimethyl ether* that crystallised from dilute methanol as rectangular tablets, m. p. 144—146°, and gave no colour with alcoholic

[4] Ganguly and Seshadri, *Tetrahedron*, 1959, **6**, 21.
[5] Janes and Morgan, *J.*, 1960, 2562.
[6] Robertson, *Canad. J. Chem.*, 1959, **37**, 1946.
[7] Goel, Narasimhachari, and Seshadri, *Proc. Indian Acad. Sci.*, 1954, **39**, *A*, 254.

ferric chloride. With acetic anhydride and pyridine the methyl ether yielded an *acetate*, needles (from methanol), m. p. 126—128° (Found: C, 64·2; H, 5·7. $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%). With iodine and fused potassium acetate in acetic acid (cf. Mahesh and Seshadri [8]) the aromadendrin gave a good yield of kaempferol.

Fraction B (*Sakuranetin*).—On acidification fraction B yielded a yellow solid (5 g.) which was filtered off and dried. The compound could not be satisfactorily purified by crystallisation and hence its benzene solution was passed through a column of cellulose powder and the column washed repeatedly with benzene. The solid recovered by evaporation of the combined percolates, crystallised from dilute methanol as colourless needles, m. p. 152—153°, alone or mixed with authentic sakuranetin. Its alcoholic solution gave a wine-red colour with ferric chloride and a red colour with magnesium and hydrochloric acid. It gave no colour with zinc and hydrochloric acid. Its acetate melted at 97—98° alone or mixed with sakuranetin diacetate.

Fraction C (*Leucopelargonidin*) (I).—The leucoanthocyanidin was passed in aqueous alcohol through a long cellulose column, and the column washed repeatedly with water. The combined percolates were saturated with salt and extracted repeatedly with ethyl acetate; the extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Fractional precipitation by light petroleum then first removed impurities, and *leucopelargonidin* was obtained as colourless crystals (20 g.) (Found: C, 55·0, 54·7; H, 5·6, 5·8. $C_{15}H_{14}O_6,2H_2O$ requires C, 55·2; H, 5·5%). Its alcoholic solution was lævorotatory. It was soluble in water, alcohol, and acetone but insoluble in benzene and chloroform. The alcoholic solution gave a bluish-violet ferric reaction. When boiled with alcoholic hydrochloric acid it formed a red solution. Conversion into pelargonidin chloride was effected by the procedure described for leucodelphinidin [3] [yield 10%; R_F (circular), phenol–water lower layer at 30°, 0·9]. An ethanolic hydrochloric acid solution of the anthocyanidin chloride had $\lambda_{max.}$ 530 mμ.

Leucopelargonidin penta-acetate was prepared by acetic anhydride and pyridine in the cold; it crystallised from ethyl acetate–light petroleum as prisms, m. p. 170° (decomp.; sintering at 163°) (Found: C, 59·8; H, 5·0. $C_{25}H_{24}O_{11}$ requires C, 60·0; H, 4·8%). Its infrared spectrum had main bands at 5·66s, 6·20m, 6·66s, 7·30s, and 8·31w.

Methylation of the leucopelargonidin was by ethereal diazomethane. The colourless *trimethyl ether* crystallised from ethyl acetate–light petroleum (b. p. 40—60°) as prisms, m. p. 148—150°, $[\alpha]_D$ −122·8° (Found: C, 64·8; H, 6·6. $C_{18}H_{20}O_6$ requires C, 65·0; H, 6·1%). An alcoholic solution gave no ferric reaction and with hydrochloric acid the ether gave a red colour. The infrared spectrum had main bands at 6·20s, 6·28s, 6·88s, 7·45m, 8·71s, 8·95s, 9·32s, 9·50s, 9·65s, and 10·30w. The ether with acetic anhydride and pyridine gave its *diacetate*, m. p. 182—184° [from ethyl acetate–light petroleum (b. p. 40—60°)] (Found: C, 63·7; H, 5·8. $C_{22}H_{24}O_8$ requires C, 63·4; H, 5·8%).

Potassium permanganate was added to a boiling acetone solution of the methyl ether during 6 hr. and the product worked up as described in the case of leucodelphinidin.[3] The purified acid product melted at 182—183°, alone or mixed with *p*-anisic acid.

Synthesis of Leucopelargonidin Methyl Ethers.—To a methanolic solution (50 c.c.) of aromadendrin trimethyl ether (0·1 g.) at 0° sodium borohydride (30 mg., excess) was added in one lot. After 48 hr. at room temperature (30°) the mixture was acidified with acetic acid, filtered, and evaporated almost completely under reduced pressure, and the residue was kept in the vacuum-desiccator over potassium hydroxide. The resulting colourless solid was treated with boiling water to decompose the boron complex and filtered hot. The residue was dried and crystallised fractionally from methanol; two fractions were obtained. After repeated recrystallisation the first fraction (50 mg.) formed colourless needles, m. p. 150—153° (Found: C, 64·5; H, 6·3. Calc. for $C_{18}H_{20}O_6$: C, 65·0; H, 6·1%). The second fraction (30 mg.) crystallised from methanol as colourless cubes, m. p. 197—200° (Found: C, 65·0; H, 5·9%). A mixed m. p. of each fraction with aromadendrin methyl ether was depressed. The first fraction did not depress the m. p. of the methyl ether of leucopelargonidin (natural) and its infrared spectrum had the same main bands.

Reduction of Aromadendrin.—To an ice-cold solution of aromadendrin (0·1 g.) in methanol (50 c.c.) sodium borohydride (30 mg., excess) was added in one lot. It was worked up as above and the crude product was treated with acetic anhydride and pyridine at room temperature. The colourless *acetate* crystallised from ethyl acetate–light petroleum (b. p. 40—60°) as cubes,

[8] Mahesh and Seshadri, *Proc. Indian Acad. Sci.*, 1955, **41**, *A*, 210.

m. p. 275° (decomp.; after sintering at 262°) [Found: C, 62·9; H, 4·9. $C_{23}H_{20}O_9$ (enol acetate) requires C, 62·7; H, 4·6%]. The infrared spectrum had main bands at 5·67s, 6·21s, 6·63s, 7·28s, and 8·30w and agreed with that of the leucoanthocyanidin acetate though the m. p. did not.

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