418. *Flavanones in* Angophora lanceolata.

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The kino of *Angophora lanceolata* contains a small proportion of flavanones, among which have been recognised (−)-farrerol (I; R = R′ = H) and (±)-angophorol (I; R = H, R′ = Me).

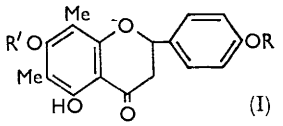
The kinos of some Australian *Myrtaceae* (*Angophora* and *Eucalyptus*) have been briefly examined [1] and on the basis chiefly of colour reactions were thought to contain aromadendrin (3′,4′,5,7-tetrahydroxyflavanone). However, the kino of *E. maculata* has been shown [2] to contain naringenin (4′,5,7-trihydroxyflavanone) and the 7-methyl ether of aromadendrin. In view of our interest in the biosynthesis of chromone derivatives we have examined other kinos for compounds whose structures might illuminate this subject.

Angophora lanceolata ("Sydney red-gum") produces large quantities of kino consisting chiefly of tannins. From it was isolated a small proportion of material soluble in ether. Fractionation of this into material soluble in sodium hydrogen carbonate, sodium carbonate, and sodium hydroxide solution gave respectively a small amount of lower fatty acid, a crystalline flavanone, m. p. from ~212° to 220° (different specimens), later identified as (−)-farrerol, and a flavanone, m. p. 150° (angophorol). The proportions of these flavanones varied with the geographical source of the kino: one sample from Lane Cove, N.S.W., contained mainly farrerol, another from National Park, Sydney, mainly angophorol.

(−)-*Farrerol.*—After the structure of the substance, m. p. 212°, had been shown [3] to be (I; R = R′ = H) Arthur [4] recorded the isolation and structural determination of farrerol (I; R = R′ = H) from a *Rhododendron*. Comparisons of our substance with (−)-farrerol kindly supplied by him and also of the dimethyl ethers of substance from the two sources

[1] Summarised by Hillis, *Austral. J. Sci. Res.*, *A*, 1952, **5**, 379.
[2] Gell, Pinhey, and Ritchie, *Austral. J. Chem.*, 1958, **11**, 373.
[3] Pettit, B.Sc. Thesis, Sydney, 1955.
[4] Arthur, *J.*, 1955, 3740.

(methylmatteucinol, I; R = R′ = Me) showed them to be identical. Our structural evidence can be summarised very briefly. Farrerol, m. p. 212°, $C_{17}H_{16}O_5$, $[\alpha]_D$ −20° (in ethanol), gave colour tests for a flavanone rather than a flavanon-3-ol,[5] contained no methoxyl but 2*C*-Me groups, gave a green ferric test, and was soluble in potassium carbonate solution. Diazomethane gave methylmatteucinol (I; R = R′ = Me), m. p. 100—102°, undepressed by an authentic specimen. Alkali-fusion produced 4-hydroxycinnamic acid, and 2,4-dimethylphloroglucinol identified by paper chromatography. This evidence can only be rationalised on the basis of formula (I; R = R′ = H).



(I)

(±)-*Angophorol*.—This substance, $C_{18}H_{18}O_5$, m. p. 150° as first isolated, contained one methoxy- and two *C*-Me groups and gave colour reactions for a flavanone rather than a flavanon-3-ol.[5] The ultraviolet spectrum was similar to those of known flavanones and the infrared spectrum demonstrated the presence of conjugated and probably hydrogen-bonded carbonyl and hydrogen-bonded hydroxyl groups. The substance gave a green ferric test and was insoluble in carbonate solution, but soluble in sodium hydroxide solution. Alkali-fusion gave *p*-hydroxybenzoic acid and 4-hydroxycinnamic acid, recognised by paper chromatography. The phloroglucinol derivative also formed was not identical (paper chromatography) with phloroglucinol or monomethyl- or 2,4-dimethyl-phloroglucinol. After the action of boiling hydrobromic acid, however, alkali fusion did produce 2,4-dimethylphloroglucinol. The action of diazomethane on angophorol produced methylmatteucinol, m. p. 100°, undepressed by an authentic specimen, m. p. 102°, the two compounds showing the same behaviour on paper chromatography.

The methylated product was unexpectedly difficult to purify, so angophorol was re-examined by paper chromatography for purity. It was thus revealed as a mixture, but the major component, now named angophorol, was separated by chromatography on paper strips and had m. p. 166—168°. The chemical properties were identical with those observed for the mixture. When the insolubility in carbonate solution and non-identity with matteucinol are taken into account, angophorol must have structure (I; R = H, R′ = Me). This was supported by partial synthesis by the action of diazomethane on (−)-farrerol. The mixture of products was separated by chromatography on paper strips to give a substance, m. p. 166° undepressed by angophorol, and identical with this in ultraviolet spectrum. The infrared spectra were almost identical but the optical rotation could not be examined because of the small amount available. It is likely that angophorol is racemised during extraction and purification.

The presence of two methyl groups on the phloroglucinol ring, itself derived from acetic acid,[6] reinforces the suggestion [7] that methyl groups may be introduced biosynthetically into such substances in a stage distinct from the formation of the ring.

Experimental

Isolation of Flavanones.—The kino (150 g.) collected in Lane Cove, N.S.W., was dissolved in acetone and filtered. Ether (200 c.c.) precipitated most of the material as a red gum giving tannin reactions. The solution was decanted and was extracted with water (400 c.c.), and then with saturated sodium hydrogen carbonate solution (50 c.c.) (extract A). Ice-cold 3% sodium hydroxide solution (3 × 40 c.c.) gave extract B. Evaporation of the ether left only a small amount of gum. Acidification of extract A, and ether-extraction, gave a small amount of oily acid with an odour of isobutyric acid. Acidification of extract B gave a gum which crystallised from ether–benzene to yield colourless (−)-farrerol, m. p. 211·5—212·5°. In some

[5] Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031.
[6] Underhill, Watkins, and Neish, *Canad. J. Biochem. Physiol.*, 1957, **35**, 219; Geissman and Swain, *Chem. and Ind.*, 1957, 984; Shibata and Yamazaki, *Pharm. Bull.* (*Japan*), 1958, **6**, 42.
[7] Linstedt, *Acta Chem. Scand.*, 1951, **5**, 129; Birch, Elliott, and Penfold, *Austral. J. Chem.*, 1954, **7**, 169.

experiments the m. p. was as high as 218—220°, possibly owing to varying degrees of racemisation.

The mother-liquor was taken up in ether and extracted exhaustively with 5% potassium carbonate solution to yield more farrerol. The residual gum was crystallised repeatedly from benzene as colourless prisms, having m. p. 150° which could not be raised further by this procedure. The m. p. was depressed on admixture with matteucinol, m. p. 175°. The total yield of flavanones was of the order 0·5%; the ratio of the two substances varied considerably according to the source; one specimen of kino from National Park, Sydney, contained only angophorol.

(−)-*Farrerol.*—The substance formed colourless prisms, m. p. between 212° and 220° according to the specimen (Found: C, 67·8; H, 5·4; *C*-Me, 8·4. Calc. for $C_{17}H_{16}O_5$: C, 68·0; H, 5·4; 2*C*-Me, 9·9%), $[\alpha]_D$ −20° (EtOH), $\lambda_{max.}$ 298 mμ (log ε 4·25), $\lambda_{min.}$ 254 mμ (log ε 3·28). It gave a green ferric test, dissolved in dilute potassium carbonate to a yellow solution, and gave a red colour on reduction with magnesium but not with zinc and hydrochloric acid in methanol,[5] indicating a flavanone and not a flavanon-3-ol structure. Alkali-fusion by the method of Lindstedt and Misiorny [8] and paper chromatography gave a spot R_F 0·94, visible as a blue spot under ultraviolet light with ammonia vapour. This corresponds to authentic 2,4-dimethylphloroglucinol (phloroglucinol R_F 0·75 and 2-methylphloroglucinol R_F 0·79). Hydrolysis with 10% aqueous sodium hydroxide at 100° for 90 min. produced 4-hydroxycinnamic acid, m. p. 206—208°, undepressed on admixture with an authentic specimen, m. p. 210°, and identical in chromatographic behaviour.

(−)-Farrerol (20 mg.) in methanol (2 c.c.) with diazomethane in ether (contact time 30 min.) gave an alkali-insoluble compound, m. p. 100—102° (Found: C, 69·7; H, 6·5. Calc. for $C_{19}H_{20}O_5$: C, 69·5; H, 6·1%). This still gave a green ferric test, although insoluble in dilute aqueous sodium hydroxide. It was identified as methylmatteucinol (authentic specimen m. p. 102°) by mixed m. p. and identity of infrared spectrum.

Angophorol.—The substance formed colourless prisms, m. p. 150° as first isolated (Found: C, 68·5; H, 5·8; OMe, 10·0; *C*-Me, 7·1. $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·8; 1OMe, 9·9; 2*C*-Me, 9·55%). It gave a green ferric test and a colour reaction as above for a flavanone, and possessed absorption bands in accord with such a structure [$\lambda_{max.}$ 216, 283, 353 mμ (log ε 4·72, 4·49, 3·96)].

Angophorol (1 mg.) was refluxed in 50% aqueous potassium hydroxide (1 c.c.) for 10 min. The solution was acidified with 10N-hydrochloric acid and extracted with ether, and the extract applied to Whatman No. 1 paper. Development with butanol–acetic acid–water and coupling with bisdiazotised benzidine gave only one visible spot (R_F 0·93), not identical with phloroglucinol or its 2-methyl or 2,4-dimethyl derivative. In a similar process the chromatogram was developed with butanol–water and sprayed with diazotised *p*-nitroaniline, then oversprayed with aqueous sodium carbonate solution, to reveal the presence of *p*-hydroxybenzoic acid, R_F 0·15 (red spot), and 4-hydroxycinnamic acid, R_F 0·22 (indigo-blue spot). Angophorol was refluxed with hydrobromic acid (*d* 1·5) for 1 hr., and the product was extracted with ether and submitted to alkaline-hydrolysis and paper chromatography by the method of Lindstedt and Misiorny,[8] to give a spot of R_F 0·94, rendered visible in ultraviolet light by ammonia vapour and identical in position and general behaviour with that due to authentic 2,4-dimethylphloroglucinol.

An excess of diazomethane in ether was added to angophorol (50 mg.) in methanol (2 c.c.), and the mixture was left for 1 hr. and then washed with sodium hydroxide solution. Evaporation of the organic solution left a gum which crystallised in contact with light petroleum and was recrystallised several times from methanol, giving prisms of methylmatteucinol, m. p. 100°, undepressed by authentic substance, m. p. 102°, and identical in infrared spectrum and chromatographic behaviour (Found: C, 69·7; H, 6·3. Calc. for $C_{19}H_{20}O_5$: C, 69·5; H, 6·1%). The yield was poor (about 10 mg.) and the original angophorol was therefore chromatographed in Lindstedt's benzene–ligroin–methanol–water mixture, and compared with available related compounds, spots being detected by spraying with bisdiazotised benzidine. The results were: methylmatteucinol (authentic and product above), R_F 0·95 (brick-red); farrerol, R_F 0·3—0·4 (pale cream); matteucinol, R_F 0·85 (cream); angophorol, m. p. 150°, R_F 0·85 (yellow) and 0·75 (pink). The angophorol was clearly a mixture, but did not contain the other substances listed. Separation on paper strips in the above solvent showed that the substance of R_F 0·85 was the

[8] Lindstedt and Misiorny, *Acta Chem. Scand.*, 1951, **5**, 1.

main constituent and was obtained pure, with m. p. 167°, $[\alpha]_D$ 0° (Found: C, 68·7; H, 5·8. Calc. for $C_{18}H_{18}O_5$: C, 68·8; H, 5·8%); the constituent of R_F 0·75 could not be isolated pure. From the virtual identity of analyses of the substance, m. p. 150°, with that of m. p. 167°, the second component must be isomeric with, or very closely related to, pure angophorol.

(−)-Farrerol (100 mg.) in methanol (2 c.c.) was treated with about 1·2 equivalents of diazomethane in ether. The product was a mixture, but several chromatographic separations on paper, as above, with isolation of material of R_F 0·85, yielded angophorol, m. p. 166—168° identical (mixed m. p.; infrared spectrum) with the natural material.

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