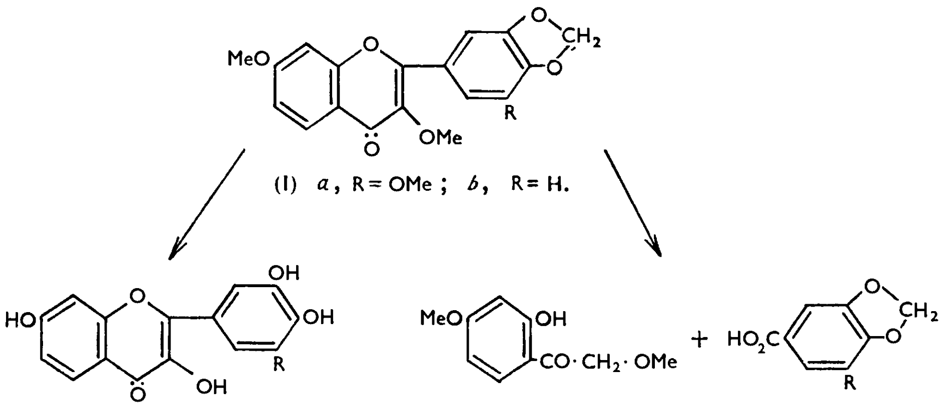
426. *Demethoxykanugin: a New Crystalline Compound from* Pongamia glabra.

By O. P. Mittal and T. R. Seshadri.

Besides kanugin a second crystalline compound has now been isolated from the root as well as the stem bark of *Pongamia glabra*. By degradation and synthesis it has been shown to be 3 : 7-dimethoxy-3′ : 4′-methylenedioxyflavone and so is demethoxykanugin.

The roots of *Pongamia glabra* were examined earlier by Rangaswami, Rao, and Seshadri,[1] who found therein kanugin (I*a*) and a closely related substance, the latter occurring especially in the thicker roots. Though they obtained kanugin free from the second compound, they were not able to isolate or characterise the latter. Rangaswami[2] isolated from the stem bark only a small quantity of kanugin, in a yield one-tenth of that from the root bark. The fresh root bark and the stem bark have now been re-examined and the second component has been isolated and characterised.



(I) *a*, R = OMe; *b*, R = H.

(IV) *a*, R = OH; *b*, R = H. (III) (II) *a*, R = OMe; *b*, R = H.

A crude mixture obtained by alcoholic extraction of the root bark gave colour reactions characteristic of flavonoid ethers and of methylenedioxy-groups. With alcoholic potassium

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[1] Rangaswami, Rao, and Seshadri, *Proc. Indian Acad. Sci.*, 1942, **16**, *A*, 319; cf. Rangaswami and Seshadri, *ibid.*, 1943, **17**, *A*, 20; Rajagopalan, Rangaswami, Rao, and Seshadri, *ibid.*, 1946, **23**, *A*, 60.

[2] Rangaswami, *Current Sci.*, 1946, **15**, *A*, 127.

hydroxide it yielded myristicic acid (II*a*), piperonylic acid (II*b*), and 2-hydroxy-4 : ω-dimethoxyacetophenone (III); the first and the third were obviously derived from kanugin. The formation of piperonylic acid indicated the nature of the second component which was supported by the demethylation of the mixture and examination of the product by circular paper chromatography: this indicated the presence of robinetin (IV*a*) and fisetin (IV*b*). Kanugin, being a derivative of robinetin, the second component should be a fisetin derivative.

Chromatography on an alumina column, but not fractional crystallisation, was effective for separation of the mixture, yielding kanugin and a second colourless substance. The latter gave the same colour reactions as the mixture, had the formula $C_{18}H_{14}O_6$, contained two methoxyl groups, and on fission yielded 2-hydroxy-4 : ω-dimethoxyacetophenone and piperonylic acid, showing that it was a fisetin derivative having a 3′ : 4′-methylenedioxy-group and 3- and 7-methoxyl groups (I*b*). This constitution was supported by demethylation, whereby fisetin (IV*b*) was obtained and identified by its colour reactions and as acetate. Since the new substance contains one methoxyl group less than kanugin, it is named demethoxykanugin.

This constitution has been confirmed by synthesis; 7-hydroxy-3-methoxy-3′ : 4′-methylenedioxyflavone [3] was methylated and the product found to be identical with the natural sample.

Both kanugin and demethoxykanugin were found in the stem bark also, the yield of the crude mixture being about half of that obtained from the root bark with a lower proportion of demethoxykanugin.

EXPERIMENTAL

Extraction of the Root Bark.—The bark (5 lb.) was collected in Delhi from fresh mature roots (diameter 1—3 in.), chopped, and extracted with boiling alcohol (3 × 10 hr.). The total extract (20 l.) was concentrated under reduced pressure till a dark brown emulsion (600 c.c.) was left. This was repeatedly extracted with ether, and the yellow ether solution (1·5 l.) concentrated to one-third of its volume and then washed repeatedly with 5% aqueous sodium hydroxide, which removed dark saponin-like material. The ether solution, on evaporation, left a brownish-yellow mass which, after drying in the vacuum-desiccator, was dissolved in the minimum quantity of anhydrous acetone. Treatment with light petroleum precipitated a small quantity of a yellow oil which was removed by filtration through charcoal. The filtrate, on slow evaporation, deposited a light yellow granular solid melting indefinitely between 110° and 150°. Washing it with a small quantity of ether removed most of the colour and the adhering oil. The crude product (mixture M) melted at 150—151° after sintering at 122°. The yield was 0·2% of the weight of the fresh bark. The mixture was insoluble in dilute sodium hydroxide, even when boiled for a few minutes. Its alcoholic solution did not give a colour with ferric chloride but gave a deep red colour with magnesium and hydrochloric acid. When warmed with sulphuric acid and a little gallic acid, it produced a deep emerald-green colour.

Alkali Fission of Mixture M.—The mixture (2 g.) was refluxed with 8% absolute-alcoholic potassium hydroxide (100 c.c.) for 8 hr., then evaporated under reduced pressure, water (100 c.c.) was added, and the reddish-brown solution filtered through cotton wool. The filtrate was acidified and extracted with ether, and the ethereal solution shaken with 5% aqueous sodium hydrogen carbonate (2 × 50 c.c.). The aqueous solution, on acidification, yielded a colourless acid mixture. The ether layer was finally washed with water, dried, and evaporated. The residue solidified and crystallised from aqueous alcohol as colourless rectangular plates, m. p. 68—69° alone or in admixture with 2-hydroxy-4 : ω-dimethoxyacetophenone. The 2 : 4-dinitrophenylhydrazone crystallised from acetic acid as bright red rectangular plates, m. p. 222—223°.

The colourless acid mixture when examined by circular paper chromatography (Whatman No. 1), with ammonia-saturated butanol as solvent and *p*-bromophenol-blue as indicator,[4] gave two rings with R_F 0·50 and 0·32. On crystallisation from aqueous methanol, the first

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[3] Rao and Seshadri, *Proc. Indian Acad. Sci.*, 1946, **23**, *A*, 149.

[4] Fewster and Hall, *Nature*, 1951, **168**, 78.

crop consisted of colourless needles, m. p. 229—230°, giving no ferric reaction but a positive methylenedioxy-test. It was identified as piperonylic acid by comparison with an authentic sample. The second crop on recrystallisation from the same solvent separated as colourless needles, m. p. 211—212° alone or mixed with myristicic acid.

Demethylation of Mixture M.—Mixture M (1·0 g.), acetic anhydride (10 c.c.), and hydriodic acid (*d* 1·7; 10 c.c.) were refluxed for 1 hr., then poured into an ice-cold solution of sodium hydrogen sulphite. The resulting yellow solid melted indefinitely between 282° and 310° and on examination by circular paper chromatography at 33—34° with water-saturated phenol as solvent and dilute aqueous ammonia as spray showed two rings with R_F 0·44 and 0·66 which corresponded to those given under the same conditions by robinetin and fisetin respectively.

Separation of the Mixture.—The adsorbent was prepared by rinsing alumina (Brockmann standard, E. Merck) with 0·1N-hydrochloric acid, washing it with distilled water, and drying it at 300—400° for 1 hr. A solution of mixture M (1·5 g.) in dry benzene (150 c.c.) was placed on the column which was eluted with benzene and then ethyl acetate. The m. p. of successive fractions rose progressively from 132° to 205°. Finally elution with hot ethyl alcohol gave a small quantity of oil which was discarded.

Fractions of m. p. >198°, on crystallisation from alcohol, yielded kanugin as plates, m. p. and mixed m. p. 207—208°.

Two fractions (m. p. *ca.* 145° and 150°) were combined and crystallised from ethyl acetate. The first crop melted between 124° and 146° and the second at 116—127°. The first, when chromatographed again, yielded two fractions, m. p. 122—140° and 136—144° respectively. The latter crystallised from ethyl acetate–light petroleum as needles, m. p. 147° (Found: C, 65·4; H, 4·1; OMe, 19·1. $C_{18}H_{14}O_6$ requires C, 66·2; H, 4·3; 2OMe, 19·0%). This *demethoxykanugin* gave a positive methylenedioxy-test, a reddish-pink colour with magnesium and hydrochloric acid, and no ferric reaction. It was insoluble in aqueous alkali but dissolved in concentrated sulphuric acid to a yellow solution with a greenish fluorescence and was immediately precipitated on dilution with water. However, it was more soluble in ethyl alcohol and benzene than was kanugin and its solution in alcohol did not fluoresce. Like that of kanugin, its solution in concentrated hydrochloric acid was bright yellow.

Demethoxykanugin (0·1 g.) and 8% absolute-alcoholic potassium hydroxide (10 c.c.) were refluxed for 8 hr. and worked up as described above. The bicarbonate-soluble portion crystallised from dilute alcohol as colourless needles, m. p. 229—230°, identical with piperonylic acid, and the insoluble part was 2-hydroxy-4 : ω-dimethoxyacetophenone.

Demethoxykanugin (0·1 g.) was demethylated by hydriodic acid (*d* 1·7) and acetic anhydride, and the product crystallised from ethyl acetate–light petroleum as yellow needles, m. p. 320—325°. This product gave an olive-brown colour with alcoholic ferric chloride and was soluble in sodium carbonate to an orange-yellow solution and in concentrated sulphuric acid to a yellow solution with a green fluorescence. The acetate prepared by means of acetic anhydride and pyridine crystallised from alcohol as needles, m. p. 201—202° alone or mixed with fisetin tetra-acetate.

Synthesis of Demethoxykanugin.—7-Hydroxy-3-methoxy-3′ : 4′-methylenedioxyflavone [3] (0·5 g.), anhydrous acetone (40 c.c.), dimethyl sulphate (0·1 c.c., 1 mol.), and ignited potassium carbonate (0·5 g.) were refluxed for 4 hr. Acetone was distilled off and the residue treated with water. The precipitate crystallised from ethyl alcohol as needles, m. p. 146—147° (0·5 g.) (Found: C, 66·5; H, 4·5. Calc. for $C_{18}H_{14}O_6$: C, 66·2; H, 4·3%). The mixed m. p. with the natural sample was undepressed.

THE UNIVERSITY, DELHI-8, INDIA.

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