385. *The Isolation and Constitution of Marmin, a New Coumarin from* Aegle marmelos, *Correa.*

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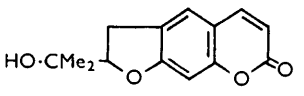
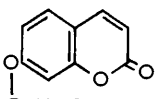
Umbelliferone, skimmianine, a new coumarin (marmin), and γ-sitosterol have been isolated from the immature bark of *Aegle marmelos* Correa. The constitution of marmin has been established as 7-(3 : 7-dihydroxy-3 : 7-dimethyloctyloxy)coumarin.

Aegle marmelos (Fam. *Rutaceae*), commonly known as bael, is a large or medium-sized tree of wide occurrence in India. It is reputed to give a valuable drug and produces besides essential oil various coumarins and alkaloids in its various parts.[1-3] The constituents vary considerably with the maturity of the specimens. The mature trunk-bark produces umbelliferone, γ-fagarine[1] (4 : 8-dimethoxyfuroquinoline), and marmesin[1] (I) whereas the



(I)



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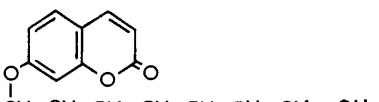

(III)

contents of an immature species are a new coumarin, marmin, $C_{19}H_{26}O_5$, m. p. 124°, umbelliferone and a different alkaloid, skimmianine (4 : 7 : 8-trimethoxyfuroquinoline). With the maturity of the bark the yields of marmin and skimmianine decrease, the contents of marmesin, γ-fagarine, and essential oil increasing. In highly mature bark there is no marmin or skimmianine.

The isolation and constitution of marmin are described in the present communication.

Hydrochloric acid washings of ethereal trunk-bark extracts of *A. marmelos* yielded a basic portion from which skimmianine (yield, 0·003%) was isolated. Chromatography of the residue obtained from the non-basic ether extract yielded γ-sitosterol (0·015%), marmesin (0·012%), and marmin (0·07%); umbelliferone (0·02%), retained in the column, could only be taken out with boiling ethanol. Marmin has $[\alpha]_D^{30}$ +25, pK_a 10·11, and an ultraviolet absorption spectrum similar to that of umbelliferone methyl ether; this and the infrared spectrum disclose a δ-hexenolactone grouping. Marmin does not consume perphthalic acid, is indifferent towards ferric ion, gives a negative Angeli–Rimini test, is free from methoxy, methylenedioxy, ketone, and aldehyde functions, and is shown to contain a coumarin nucleus by its behaviour towards alkali. Since it fails to produce an α-glycol with oxalic acid and does not react with diethylamine and potassium chloride, the presence of an epoxide group is excluded. It contains two active hydrogen atoms, which in absence of a methylene group, appear to be associated with hydroxyl groups. The latter were indifferent towards phthalic anhydride, 3 : 5-dinitrobenzoyl chloride, and toluene-*p*-sulphonyl chloride, from which the hydroxyl functions in marmin appeared to be tertiary (cf. below). Treatment with acetic acid, phosphorus oxychloride, phosphoric oxide (in benzene), aqueous oxalic acid, or acetic anhydride at their b. p.s. resulted in a cleavage to umbelliferone together with an oil (terpene), which was extremely sensitive to heat and acids and could not be characterised. Umbelliferone was also obtained from marmin during its thermal decomposition and catalytic hydrogenation, which recalled the

[1] Dutta, Proc. Acad. Sci., United Province, Agra, Oudh, India, 1935, 56; Späth, Bose, Grüber, and Guha, *Ber.*, 1937, **70**, 1021; Chatterjee and Saha, *J. Indian Chem. Soc.*, 1957, **34**, 228; Chatterjee and Bose, *ibid.*, 1952, **29**, 425; Chakravarti and Das Gupta, *J.*, 1958, 1580; Chatterjee and Srimany, XVIth Congr. Internat. Pure Appl. Chem., Paris, 1957, Part II, p. 199; Chatterjee, Bose, and Srimany, *J. Org. Chem.*, in the press; Chatterjee and Roy, Proc. 44th Indian Sci. Congr., 1957, Part III, p. 124; Chatterjee and Mitra, *J. Amer. Chem. Soc.*, 1949, **71**, 606; Chaudhury, Ghosh, and Chatterjee, Proc. 46th Indian Sci. Congr., 1959, Part III, p. 142.

[2] Mookerjee, *Current Sci.*, 1943, **12**, 209.

[3] Chakravarty, *J. Indian Chem. Soc.*, 1944, **21**, 401.

behaviour of geranyloxy- and farnesyloxy-coumarins.[4] Marmin suffered spontaneous hydrolysis with glacial acetic acid, like coumarins containing isoprenoid ether groups,[5] to umbelliferone and a terpenaceous oil, from which it followed that marmin was an umbelliferone ether (II).

Treatment of marmin with periodic acid or lead tetra-acetate, even at elevated temperature, failed to produce an aldehyde or ketone, which established that none of the two tertiary hydroxyl groups and the potential hydroxyl group of the ether (II) were vicinal. However, treatment with chromic acid afforded umbelliferone, acetone, and lævulic acid. This, together with the presence of two tertiary hydroxyl groups and expectation of a head-to-tail terpene structure, indicates that marmin has structure (III).

Experimental

M. p.s were determined with a Kofler block. Ultraviolet spectra refer to alcohol solutions in a Beckman spectrophotometer Model DU. The microanalyses were carried out by Mr. W. Manser, Zürich, Switzerland, and Mrs. C. Dutta, University College of Science and Technology, Calcutta. During chromatography the eluants were collected in fractions of 15 c.c. each unless otherwise stated.

Extraction.—Milled trunk-bark (4 kg.) of *Aegle marmelos* Correa was extracted (Soxhlet) with ether (5 l.) for 96 hr. The extracts were concentrated to 200 ml. The concentrate which gave positive Mayer's and Dragendorff's tests for alkaloids was washed with 4N-hydrochloric acid (4 × 50 ml.) until the washings gave negative alkaloid tests.

The hydrochloric acid extract, upon basification with sodium carbonate, liberated skimmianine (0·12 g.) which crystallised from ethyl acetate and acetone in prisms, m. p. and mixed m. p. 175—176° (Found: C, 65·0; H, 5·1; N, 5·30; OMe, 36·1. Calc. for $C_{14}H_{13}O_4N$: C, 64·9; H, 5·0; N, 5·4; OMe, 35·9%).

The ether extract from which alkaloids had been removed was evaporated. The dark residue was chromatographed in benzene (50 ml.) on alumina (1·7 × 40 cm.; 500 g.). In the fractions 11—15 (benzene) appeared crude γ-sitosterol (0·59 g.), m. p. 140—145°. Fractions 16—22 yielded crude marmesin (0·4 g.), m. p. 183—186°. Eluates 23—32 with benzene–ethyl acetate (1 : 1) gave crude marmin (2·8 g.), m. p. 121—123°; umbelliferone (0·8 g.) was finally extracted with boiling ethanol.

γ-Sitosterol was purified by chromatography again over alumina with 1 : 1 benzene–light petroleum (b. p. 40—60°) as eluant. From absolute methanol it formed plates, m. p. and mixed m. p. 146—147° $[\alpha]_D^{25}$ −41·7° (in chloroform) (Found: C, 84·1; H, 12·0. Calc. for $C_{29}H_{50}O$: C, 84·05; H, 12·1%).

Marmesin was obtained pure by several crystallisations from alcohol from which it separated in rods, m. p. and mixed m. p. 190° (Found: C, 68·4; H, 5·7. Calc. for $C_{14}H_{14}O_4$: C, 68·3; H, 5·7%), $\lambda_{max.}$ in ethanol 226, 248, 260, and 335 mμ (log ε 3·98, 3·58, 3·47, 4·16).

Recrystallisation from ethyl acetate–methanol and sublimation at 160°/0·01 mm. afforded pure umbelliferone (0·7 g.), m. p. 232° (Found: C, 66·7; H, 3·6. Calc. for $C_9H_6O_3$: C, 66·7; H, 3·7%), $\lambda_{max.}$ in methanol 216, 327 mμ (log ε 4·09, 4·16).

Chromatography again of crude marmin (2·5 g.) on alumina (100 g.) as above gave, in the fractions eluted with benzene, 0·2 g. of oil; the eluates (200 ml.) with 1 : 1 benzene–ethyl acetate (1 : 1) afforded, after several crystallisations from ethyl acetate, pure *marmin*, m. p. 124° (Found: C, 68·6; H, 7·5; active H, 0·76; *C*-Me, 4·6%; *M*, 325. $C_{19}H_{26}O_5$ requires C, 68·3; H, 7·8; *C*-Me, 4·5%; *M*, 334). In ethanol it showed intense absorption at 213 (no maximum) and 324 mμ (log ε 4·23). Marmin has infrared absorption bands in Nujol at 2·84 (OH), 5·81 (lactone), 6·2 (Ph), 7·18 (side-chain Me), 8·8 and 10·0 μ (phenol ether).

Thermal Decomposition of Marmin.—When marmin (0·5 g.) was heated, a sublimate was obtained at 190°/0·01 mm. It crystallised from ethyl acetate in needles, m. p. 232° alone or mixed with umbelliferone (Found: C, 66·7; H, 3·6. Calc. for $C_9H_6O_3$: C, 66·7; H, 3·7%).

Catalytic Hydrogenation of Marmin.—Marmin (0·3 g.) in ethanol (15 ml.), in presence of Adams platinum oxide (0·05 g.), took up 1 mol. of hydrogen. The solution was diluted with water (50 ml.) and extracted with ether. The extract was washed with 5% aqueous sodium

[4] Dean, " Progress in the Chemistry of Natural Products," ed. Zechmeister, Julius Springer, Vienna, 1952, Vol. IX, p. 225.

[5] Späth and Holzen, *Ber.*, 1933, **66**, 1137; 1935, **68**, 1123.

hydroxide (3 × 25 ml.). The alkaline extract upon acidification produced dihydroumbelliferone, m. p. and mixed m. p. 133° (Found: C, 65·7; H, 4·9. Calc for $C_9H_8O_3$: C, 65·85; H, 4·85%). The ether residue, when washed with water and dried (Na_2SO_4), gave a terpenaceous oil.

The above experiment was repeated in glacial acetic acid solution with the same results.

Action of Acetic Anhydride on Marmin.—Marmin (0·25 g.) was treated with acetic anhydride–pyridine, kept at 0° for 48 hr., then poured into ice-cold water. A benzene solution of the resinous product was chromatographed over acid-washed alumina. Elution with the same solvent gave a terpenaceous oil. Subsequent washings with ethanol yielded umbelliferone.

Dehydration of Marmin.—Marmin (0·10 g.) in dry benzene (5 ml.) was refluxed with phosphorus oxychloride or phosphoric oxide (0·5 g.) for 2 hr. The benzene solution was decanted and concentrated; umbelliferone, m. p. 232°, separated. The mother-liquor on evaporation gave an oil having a terpene-like odour.

Acid-hydrolysis of Marmin.—(*a*) Marmin (0·5 g.), in glacial acetic acid (2 ml.), was treated with 2 drops of concentrated sulphuric acid, refluxed for 1 hr., then poured into ice-cold water, made alkaline, and extracted with ether. The alkaline mother-liquor (A) was worked up for umbelliferone. The ethereal solution was freed from solvent, and the residue warmed for 5 hr. with 20% sulphuric acid (20 ml.). The hydrolysate was extracted with ether which was washed with 10% alkali solution, then water, and dried. The ethereal solution, on evaporation, left some oil with an odour of geraniol. The alkaline washings were slightly fluorescent but on acidification and extraction with ether did not yield any product.

(*b*) Marmin (0·5 g.) was refluxed in glacial acetic acid (2 ml.) for 2 hr. The product was made alkaline and extracted with ether. The aqueous alkaline solution (B) was worked up for umbelliferone. The ether extract was washed with water, dried, and evaporated, giving an oil with a fragrant odour. This was refluxed with 0·5% methanolic potassium hydroxide for 1 hr. The cold solution was diluted with water and shaken with ether (3 × 50 ml.). These ethereal extracts, on concentration, left an oil with a geraniol-like smell. An attempt to prepare the allophanate was not successful.

The alkaline solutions A and B had a strong blue fluorescence. They were cooled and acidified with hydrochloric acid. A precipitate appeared which was taken up in ether. The ether solution was washed and dried. Evaporation gave umbelliferone (0·4 g.), which crystallised from ethyl acetate in needles, m. p. and mixed m. p. 232° (Found: C, 66·8; H, 3·9%).

Chromic Acid Oxidation of Marmin.—An acetic acid solution (50 ml.) of marmin (4·0 g.) was treated with chromic acid (4·0 g.) in 50% acetic acid (50 ml.) and kept at room temperature for 3 days. The solution was cooled, neutralised with 50% potassium hydroxide solution, and steam-distilled. The distillate was collected in a 5% acetic acid solution of *p*-nitrophenylhydrazine (50 ml.), giving acetone *p*-nitrophenylhydrazone, reddish-yellow needles (from methanol), m. p. and mixed m. p. 148° (0·8 g.) (Found: C, 56·0; H, 5·8; N, 21·6. Calc. for $C_9H_{11}O_2N_3$: C, 55·9; H, 5·9; N, 21·65%).

In a similar experiment acetone was collected over 10% sodium hydroxide solution containing freshly distilled benzaldehyde (4—5 drops). The solution was kept at 0° overnight, then extracted with ether, washed, and dried. The ethereal extract, on concentration, gave dibenzylideneacetone, yellow needles (from methanol), m. p. 110—111° (Found: C, 87·0; H, 6·0. Calc. for $C_{17}H_{14}O$: C, 87·2; H, 6·0%).

The alkaline solution left after the removal of acetone was cooled and acidified (Congo Red) with 4N-sulphuric acid, then saturated with salt and extracted with ether (6 × 50 ml.) which was washed with water, dried, and freed from the solvent. The residual liquid was distilled in a vacuum. The fraction of b. p. 138°/40 mm. yielded lævulic acid. This was purified by chromatography in 1 : 1 benzene–light petroleum (b. p. 40—60°) over " Celite " (1·7 × 30 cm.), with elution by the same solvent mixture. Fractions 5—8 (50 ml. each) gave laevulic acid. An aqueous solution with a saturated 2N-sulphuric acid solution of 2 : 4-dinitrophenylhydrazine produced the yellow dinitrophenylhydrazone, m. p. and mixed m. p. 205—206° (from alcohol) (Found: C, 44·6; H, 4·1; N, 19·0. Calc. for $C_{11}H_{12}O_6N_4$: C, 44·6; H, 4·05; N, 18·9%).

From the residue left after the distillation of lævulic acid umbelliferone was isolated on sublimation at 90°/0·01 mm.

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