642. *Matteucinin (a New Flavanoid Glycoside) and Other Constituents of the Ericaceae of Hong Kong.*

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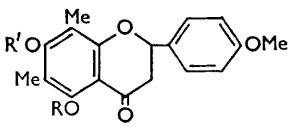
A new flavanoid glycoside, matteucinin (I), and glycosides of aromadendrin, farrerol, matteucinol, myricetin, and quercetin occur in Hong Kong species of the Ericaceae. Matteucinin is a matteucinol 7-(glucosidyl-β-glucoside).

Earlier work [1-4] has shown that species of Hong Kong Ericaceae yield various triterpenoid and flavonoid compounds. Matteucinol [1] was obtained from an ethereal extract of the leaves of *Rhododendron simsii*, and farrerol [3] similarly from *R. farrerae*. These flavanones occur in the free state.

Methanol extracts of the leaves (which were first extracted with light petroleum and then with ether) of these endemic plants [2] have now yielded products, all occurring as glycosides, as follows: *Enkianthus quinqueflorus*, quercetin; *Rhododendron simiarum*, aromadendrin and quercetin; *R. westlandii*, myricetin and quercetin; *R. farrerae*, farrerol; and *R. simsii*, matteucinol. Paper chromatography, infrared spectroscopy, and chemical methods were used in establishing the identity of the aglycones. A new glycoside has been isolated from the methanol extract of *R. simsii* and for it we propose the name matteucinin since its aglycone is matteucinol [5] which was first obtained from the Japanese fern *Matteucia orientalis*.[6] The methanol extract of *R. championae* yielded no flavonoid compound.



(I) R = H, R' = $C_{12}H_{21}O_{10}$. (II) R = R' = H.
(III) R = H, R' = Me. (IV) R = Me, R' = H.

Matteucinin (I), $C_{30}H_{38}O_{15}$, gave (−)-matteucinol (II) on hydrolysis with sulphuric acid or emulsin (β-linkage), and in both hydrolysates glucose (as the only sugar present) was identified by paper chromatography and by its osazone. Determination of matteucinol gravimetrically, and glucose colorimetrically, after acid-hydrolysis of matteucinin showed that two glucose units are present in the glycoside. Matteucinin, matteucinol, and matteucinol 7-methyl ether (III) give green colours with ferric chloride, whereas matteucinol 5-methyl ether (IV) and the product obtained on methylation of matteucinin with methyl sulphate give yellow-brown colours. This suggested that the 5-hydroxyl group of matteucinol is free in matteucinin, and this was supported in that matteucinin does not react with diazomethane and yet forms an octa-acetyl derivative, and was finally proved by methylation of the glycoside or its octa-acetyl derivative to a product which on hydrolysis yielded matteucinol 5-methyl ether (shown to differ from matteucinol and its 7-methyl ether).

Therefore, matteucinin is a matteucinol 7-(glucosidyl-β-glucoside).

Experimental

Analyses were by Dr. Zimmermann, Melbourne. Paper used for chromatography was Whatman No. 1, and the solvent was phenol saturated with water. M. p.s were taken on a gas-heated copper block (except where otherwise stated). Methanol extracts for all species

[1] Arthur and Hui, *J.*, 1954, 2782.
[2] Arthur and Hui, *J.*, 1954, 4683.
[3] Arthur, *J.*, 1955, 3740.
[4] Arthur, Lee, and Ma, *J.*, 1956, 1461.
[5] Fujise, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1929, **11**, 111.
[6] Munesada, *J. Pharm. Soc. Japan*, 1924, **505**, 185.

were made from leaves which were first extracted with light petroleum (b. p. 60—80°) and then with ether. The weights of leaves and yields of products refer to air-dried unextracted leaves.

Extraction of Flavonoid Compounds (from All Species).—Air-dried leaves were extracted with light petroleum, then with ether, and finally with cold methanol during several days. The methanol extract, when evaporated under reduced pressure, left a brown tar. This was boiled with aqueous-methanolic 0·3—2N-sulphuric acid for at least ½ hr. Removal of the methanol left a tar which was extracted repeatedly either with ether or chloroform. Removal of the solvent left usually a yellow or brown solid or a tar (*A*).

(1) Enkianthus quinqueflorus. The tar *A* from 1·25 kg. of leaves was extracted with benzene (Soxhlet). The insoluble portion gave, after 3 recrystallisations from aqueous ethanol, yellow needles of quercetin (identical with the sample from *R. simiarum*) (0·8 g., 0·06%), m. p. 309—311° (vac.) (decomp.), R_F 0·42 at ~20° [penta-acetate (from aqueous acetone) m. p. and mixed m. p. 189—191°; 3,7,3′,4′-tetramethyl ether (from methanol), m. p. and mixed m. p. 155—157°].

(2) Rhododendron simiarum. Methanol was added to the tar *A* (from 7 kg. of leaves). Yellow needles of crude quercetin (2·3 g., 0·03%) separated. Three recrystallisations from aqueous ethanol gave quercetin, m. p. 304—311° (vac.) (decomp.) (Found: C, 57·4; H, 4·0. Calc. for $C_{15}H_{10}O_7,H_2O$: C, 56·3; H, 3·8%), R_F 0·42 at ~20°, 0·54 at ~30° [penta-acetate (prepared by acetic anhydride or acetyl chloride in pyridine), needles (from aqueous acetone), m. p. 191—193° (Found: C, 57·7; H, 4·2; Ac, 41·9. Calc. for $C_{25}H_{20}O_{12}$: C, 58·6; H, 4·0; 5Ac, 42·0%); 3,7,3′,4′-tetramethyl ether (diazomethane in ether), yellow needles (from aqueous acetone), m. p. 155—157° (Found: C, 63·3; H, 5·2; OMe, 34·2. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·1; 4OMe, 34·6%)].

The methanol filtrate deposited a product (1·2 g., 0·02%) which, after four recrystallisations from aqueous acetone, gave colourless needles of aromadendrin, m. p. 241—242° (Pyrex tube), m. p. 223—225° (soda-glass tube) [7] (Found: C, 55·3; H, 4·9; loss at 80°/vac. over P_2O_5, 11·3. Calc. for $C_{15}H_{12}O_6,2H_2O$: C, 55·6; H, 5·0; $2H_2O$, 11·1. Found on dried material: C, 62·4; H, 4·4. Calc. for $C_{15}H_{12}O_6$: C, 62·5; H, 4·2%), R_F 0·83 at ~25°. The infrared spectrum was identical with that of a sample kindly supplied by Dr. W. E. Hillis. Acetylation by various procedures did not afford a pure derivative; methylation with excess of diazomethane gave an amorphous product; [8] a monomethyl ether, obtained by use of ethereal diazomethane for only a few minutes, separated as needles (from aqueous acetone), m. p. 178—181° (decomp.) (Found: C, 63·6; H, 4·9; OMe, 10·8. Calc. for $C_{16}H_{14}O_6$: C, 63·6; H, 4·7; 1OMe, 10·3%).

(3) Rhododendron westlandii. Paper chromatography of the tar *A* revealed the probable presence of two flavonoids, one of which was shown to be quercetin (R_F 0·42 at ~20°, 0·54 at ~30°; identical values were obtained for authentic quercetin under these conditions). Tar *A*, obtained from 3 kg. of leaves by Hergert's method,[9] was extracted with benzene (Soxhlet) for 6 hr. The insoluble residue was triturated with warm acetone and then crystallised from ethanol. Yellow needles separated which on two recrystallisations from aqueous ethanol gave myricetin, m. p. >350° (brown at about 310°) (Found: C, 53·8; H, 4·1. Calc. for $C_{15}H_{10}O_8,H_2O$: C, 53·6; H, 3·6%), R_F 0·34 at ~30°, 0·21 at ~20°. The infrared spectrum was identical with that of a sample kindly provided by Dr. H. L. Hergert. The hexa-acetate (acetyl chloride and pyridine), m. p. 214—216°, separated as needles from aqueous acetone (Found: C, 57·1; H, 4·0; Ac, 45·5. Calc. for $C_{27}H_{22}O_{14}$: C, 56·8; H, 3·9; 6Ac, 45·3%). The 3,7,3′,4′,5′-pentamethyl ether (diazomethane in ether) separated as pale yellow needles, m. p. 139—140°, after five recrystallisations from methanol (Found: C, 61·8; H, 5·1; OMe, 38·4. Calc. for $C_{20}H_{20}O_8$: C, 61·9; H, 5·2; 5OMe, 40·0%).

(4) Rhododendron farrerae. Leaves (0·63 kg.) were extracted with cold methanol (2 × 1·5 l.) during 7 days. Removal of solvent from the extract left a dark brown semi-solid which was extracted exhaustively with ether. The dark green ethereal solution was treated with charcoal, then evaporated to dryness. Cream-coloured needles (0·03 g., 0·005%) separated. A sample gave on paper chromatography a single spot identical with that obtained from authentic farrerol (R_F 0·93 at ~20°). The scarlet ether-insoluble semi-solid residue yielded material *A* as cream-coloured needles (0·1 g., 0·02%), m. p. 216—222°, which gave a green colour with ferric chloride solution and an R_F value identical with that of farrerol.

[7] Hillis, *Austral. J. Sci. Res.*, 1952, **5**, 379.
[8] Uoda, Fukushima, and Kondo, *J. Agric. Chem. Soc. Japan*, 1943, **19**, 467.
[9] Hergert, *J. Org. Chem.*, 1956, **21**, 534.

(5) Rhododendron simsii. Leaves (1 kg.) were extracted with cold methanol (2 × 3 l.) during 7 days. Removal of the methanol left a dark green semi-solid which was repeatedly extracted with ether. The ethereal solution was treated with charcoal and then evaporated to dryness. The cream-coloured residue crystallised from methanol as colourless needles (0·12 g., 0·01%) of matteucinol, m. p. and mixed m. p. 173—174°, R_F 0·95 at ~20° (identical with that of an authentic sample). To the ether-insoluble semi-solid residue was added water (1·4 l.). After 3 days, the aqueous extract was decanted from the dark brown water-insoluble tar and was heated on the steam-bath with 2N-sulphuric acid (400 ml.) and methanol (400 ml.) for 30 min. The methanol was removed and the liquid which remained was extracted with ether. The ethereal extract was treated with charcoal and evaporated to a brown oil. The dark water-insoluble tar (above) was triturated with methanol. The solid deposited was collected and recrystallised twice from methanol and then twice from water. Light yellow needles (4·0 g., 0·40%) of *matteucinin* (I), m. p. 139—141° after sintering at 135°, m. p. 145—147° (Kofler), separated; they had R_F 0·89 at ~20°, $[\alpha]_D^{20}$ −28·9° (*c* 1·0 in $COMe_2$) (Found: C, 53·2, 53·4; H, 6·4, 6·4; OMe, 4·6, 4·9%; *M*, 580. $C_{30}H_{38}O_{15},2H_2O$ requires C, 53·4; H, 6·2; 1OMe, 4·6%; *M*, 674). Matteucinin was freely soluble in acetone, dioxan, pyridine, and hot methanol and very sparingly soluble in ether and in cold water. The cream-coloured needles, obtained on crystallisation of matteucinin from water, became deep yellow when heated, but resumed the cream colour when left in air.

Hydrolysis of Matteucinin.—(a) *With acid.* A solution of matteucinin (0·43 g.) in 0·7N-sulphuric acid (60 ml.) and methanol (20 ml.) was heated on the steam-bath for 1·5 hr. A cream-coloured product separated. Methanol was removed. The product was collected after 12 hr., and, after 3 recrystallisations from methanol, it gave needles of matteucinol, m. p. and mixed m. p. 173—174°, R_F 0·95 at ~20° (identical with an authentic sample). A sample of the aqueous filtrate (which reduced Fehling's solution and ammoniacal silver nitrate solution, and which gave a positive Molisch test) yielded glucose phenylosazone, m. p. and mixed m. p. 203—204°, on treatment with acetic acid and phenylhydrazine on the steam-bath for 11—12 min. A second sample of the aqueous filtrate, after neutralisation with barium carbonate in the cold, was concentrated in a vacuum-desiccator and on paper chromatography gave a single spot (developed with an ammonium molybdate spray [10]), R_F 0·46 at 22°, 0·36 at 15°, identical with values for D-glucose.

(b) *Enzymically.* A suspension of matteucinin (0·3 g.) in water (20 ml.) and emulsin solution [11] (60 ml.) was held at 50° for 5 hr., then left at room temperature for 20 hr. The precipitate was collected and extracted (Soxhlet) with ether. The ethereal solution on evaporation yielded matteucinol (0·05 g.), m. p. 173—174° (from methanol). The residue after one recrystallisation yielded matteucinin (0·15 g.), m. p. 139—141°. In the aqueous filtrate glucose was identified as the osazone and by a paper chromatogram in which it gave a single spot identical with that of an authentic sample.

Quantitative Determination of Aglycone and Sugar from Matteucinin.—Matteucinin was hydrolysed by acid as above but water only was used as solvent. Matteucinin (0·2590, 0·2300 g.) yielded matteucinol (0·1182, 0·1095 g.) (45·6, 47·6%; matteucinol monoglucoside dihydrate requires 61·3; matteucinol diglucoside dihydrate requires 46·6%). Extraction of the aqueous filtrate with ether yielded less than 3 mg. of solids which gave faint positive tests in the Molisch reaction and with magnesium and hydrochloric acid in ethanol. The glucose (0·1133 g., 49·3%) in an aqueous filtrate obtained from hydrolysis of matteucinin (0·2300 g.) was determined colorimetrically [12] with chromotropic acid, by comparison with a standard glucose solution (a monoglucoside dihydrate requires 35·2%; a diglucoside dihydrate requires 53·4%).

Octa-O-acetylmatteucinin. Matteucinin (0·1 g.) was boiled with pyridine (2 ml.) and acetic anhydride (2 ml.) for 1 hr. The *octa-acetate*, isolated in the usual manner and recrystallised four times from methanol as needles (0·015 g.), had m. p. 231—233° (Found: C, 57·2; H, 5·7; OMe, 3·46; Ac, 33·7%; *M*, 555. $C_{46}H_{54}O_{23}$ requires C, 56·7; H, 5·6; 1OMe, 3·2; 8Ac, 35·3%; *M*, 974). The same product was obtained after 60 hr. at room temperature.

Methylation of Matteucinin and its Octa-acetate.—Neither compound was methylated by

[10] El Khadem and Mohammed, *J.*, 1958, 3320.
[11] Mann and Saunders, "Practical Organic Chemistry," 3rd edn., Longmans, Green & Co., London, 1952, p. 414.
[12] Paech and Tracey, "Modern Methods of Plant Analysis," Vol. II, Springer Verlag, Berlin, 1955, p. 42.

diazomethane in ether or methanol–ether. Matteucinin (0·25 g.) in acetone (50 ml.) was heated with methyl sulphate (2 ml.) and potassium carbonate (4 g.) on the steam-bath until it no longer gave a green colour with ferric chloride solution (6 hr.). After being filtered, the solution was distilled and the brown oil obtained was boiled under reflux for 2 hr. with 0·8N-sulphuric acid (80 ml.) and methanol (20 ml.). The methanol was removed at reduced pressure and the aqueous solution extracted with ether. The ethereal extract was evaporated. The yellow oil obtained slowly deposited crystals which, on recrystallisation from methanol, yielded matteucinol 5-methyl ether, m. p. 140°, which depressed the m. p. of matteucinol 7-methyl ether to 64—66°.

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