1183. *Constituents of* Mikania cordata *(Burm. f.) B. L. Robinson (Compositae). Isolation of Mikanin, Epifriedelinol, and Fumaric Acid; the Structure of Mikanin*

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Mikanin, a new flavone, was isolated together with epifriedelinol from the root, and together with fumaric acid from the leaves and stems, of *Mikania cordata*. It is shown to be 3,5-dihydroxy-4′,6,7-trimethoxyflavone.

The herbaceous creeper, *Mikania cordata*, is one of the ground-covering crops grown in rubber estates in the early years after planting the rubber seedlings.

Much work has been carried out by Watson and his colleagues [1] on the interaction between the cover crops and the rubber trees on the one hand and the soil on the other. They reported that rubber trees grown together with *M. cordata* showed low leaf-nitrogen and -phosphorus, depressed rooting in the litter layer and A horizon, and relatively small canopies. Wong [2] has shown that substances present in *M. cordata* inhibit the growths of rubber seedlings, tomatoes, *Pueraria phaseoloides* and *Fomes lignosus* (*in vitro*).

We now report the isolation of a new flavone, mikanin, from the plant material, and discuss its structure. Together with mikanin, epifriedelinol [3] was isolated from the root, and fumaric acid from the leaves and stems.

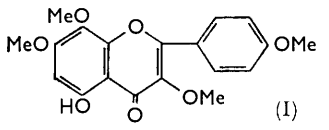
Mikanin, $C_{18}H_{16}O_7$, is a bright yellow compound, m. p. 222—224°. Its solution in ethanol gives an intense greenish-violet colour with ferric chloride, and the usual colour reactions of a flavone. It contains three methoxy-groups and forms a diacetate, m. p. 187—189°; reaction with diazomethane or one mole of dimethyl sulphate gives a monomethyl ether, m. p. 174—175°; prolonged heating with excess of dimethyl sulphate gives a dimethyl ether, m. p. 157—158°; demethylation gives a pentahydroxyflavone, m. p. 328—330°. The monomethyl ether is different from flinduletin [4] (I) and the dimethyl ether from tangeretin [5] (II). The drastic conditions required for dimethylation show that mikanin is a 5-hydroxyflavone.

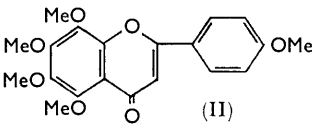
The ultraviolet spectrum of mikanin in ethanol shows $\lambda_{max.}$ at 256, 270, 361, and inflexion at 339 mμ (log ε 4·31, 4·26, 4·27, and 4·25). The spectrum is not affected by fused

[1] G. A. Watson, P. W. Wong, and R. Narayanan, *J. Rubber Res. Inst. Malaya*, 1964, **80**, 123.
[2] P. W. Wong, *J. Rubber Res. Inst. Malaya*, 1964, **18**, 231.
[3] P. R. Jefferies, *J.*, 1954, 473.
[4] R. F. C. Brown, P. T. Gilham, G. K. Hughes, and E. Ritchie, *Austral. J. Chem.*, 1954, **7**, 181.
[5] L. J. Goldsworthy and R. Robinson, *Chem. and Ind.*, 1957, 47.

sodium acetate, showing the absence of a hydroxyl group on C-7;[6] but in the presence of aluminium chloride the long-wavelength band undergoes a bathochromic shift of 60 mμ, indicating a 3-hydroxyflavone.[7]

The n.m.r. spectrum of mikanin in dimethylsulphoxide shows two hydroxyl protons at


(I)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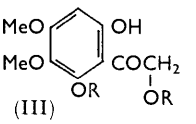
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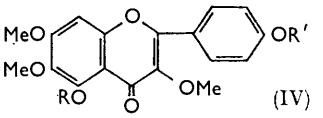
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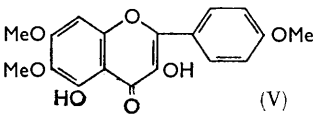
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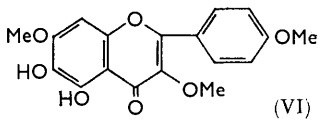
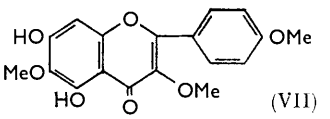

(VI)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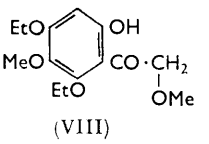
(VIII)

τ −2·4 and 0·2, one aromatic proton (singlet) at τ 3·2 and a pair of doublets at τ 1·8 and 2·9 (J = 8 c./sec.) due to four aromatic protons. That of the dimethyl ether in deuterochloroform displays fifteen methoxy-protons at τ 6·05, one aromatic proton (singlet) at τ 3·25 and a pair of doublets at τ 1·90 and 3·00. The pairs of doublets in both cases belong to simple AB spectra, characteristic of 4′-substituted B rings in flavonoids.[8]

Dimethylmikanin is identical with dimethylpenduletin (IV; R = R′ = Me) which was prepared from pendulin (IV; R = H, R′ = glucosyl) by the method of Flores and Herrán.[9] Also, alkaline degradation of dimethylmikanin yields anisic acid and an acetophenone, m. p. 70—71°, in agreement with the expected 2-hydroxy-ω,4,5,6-tetramethoxyacetophenone (III; R = Me), previously obtained from the fully methylated vogeletin.[10]

From the above observations, mikanin could be 3,5-dihydroxy-4′,6,7-trimethoxyflavone (V), and the third naturally-occurring flavone with this oxygenation pattern.

Unlike most 3-hydroxyflavones, mikanin in aqueous sodium hydroxide does not turn brown on heating in air. More chemical evidence was therefore sought to exclude the two other possible structures (VI) and (VII).

Compound (VI) is known,[11] and is not mikanin (m. p.s of the compounds and their diacetates differ). Similarly, structure (VII) is excluded because alkaline degradation of diethylmikanin, $C_{22}H_{24}O_7$, m. p. 94—95°, gives an acetophenone, $C_{14}H_{20}O_6$, m. p. 61—62°, which is different from the 4,6-diethoxy-2-hydroxy-ω,5-dimethoxyacetophenone (VIII) expected. The latter was synthesised from 2,4,6-trihydroxy-ω-methoxyacetophenone[12] by diethylation, followed by Elbs persulphate oxidation and monomethylation.

Mikanin therefore must possess structure (V), and the acetophenone derived from its diethyl ether is a new compound, ω,6-diethoxy-2-hydroxy-4,5-dimethoxyacetophenone (III; R = Et). Attempts to synthesise mikanin are in progress.

Experimental

Melting points, unless otherwise stated, were taken on a Kofler hot-stage apparatus. The light petroleum used had b. p. 56—70°.

[6] L. Jurd and R. M. Horowitz, *J. Org. Chem.*, 1957, **22**, 1618.
[7] L. Jurd and T. A. Geissman, *J. Org. Chem.*, 1956, **21**, 1395.
[8] T. J. Batterham and R. J. Highet, *Austral. J. Chem.*, 1964, **17**, 428.
[9] S. E. Flores and J. Herrán, *Tetrahedron*, 1958, **2**, 308.
[10] S. Rangaswami and K. H. Rao, *Proc. Indian Acad. Sci.*, 1959, **49***A*, 241.
[11] A. C. Jain, T. R. Seshadri, and K. R. Sreenivasan, *J.*, 1955, 3908.
[12] R. Robinson and K. Venkataraman, *J.*, 1929, 63.

Isolation of Mikanin and Epifriedelinol from Root Powder.—The root powder (1·2 kg.) was continuously extracted with (*a*), light petroleum (5 l.) for 1 week, then (*b*), methanol for 1 week.

(*a*) The solids which appeared in the light petroleum extract and on the walls of the flask were filtered off. They were extracted with hot ethyl acetate, and concentration of the solution gave bright yellow *crystals* (240 mg.), m. p. 220—222°. Recrystallisation from benzene or chloroform–methanol raised the m. p. to 222—224° (Found: C, 62·6; H, 4·9; 5·0; OMe, 24·8. $C_{18}H_{16}O_7$ requires C, 62·8; H, 4·7; 3OMe 27·0%).

The filtered petroleum extract was concentrated to a thick gummy residue. Careful rubbing with light petroleum gave flakes (620 mg.) m. p. 297° (282—283° in evac. tube) (from benzene), $[\alpha]_D^{28}$ ($CHCl_3$) +19·5° (Found: C, 83·85; H, 12·2. Calc. for $C_{30}H_{52}O$: C, 84·0; H, 12·2%). The compound gave a pink colour in the Liebermann–Burchardt reaction, and formed with acetic anhydride and pyridine an acetate, m. p. 291—292° (from methanol–benzene), $[\alpha]_D^{26.5}$ ($CHCl_3$) +42·0° (Found: C, 81·7; H, 11·65. Calc. for $C_{32}H_{54}O_2$: C, 81·6; H, 11·6%). The infrared (i.r.) spectrum was identical with that of epifriedelinol. Jefferies [3] gives epifriedelinol m. p. 279—283°, $[\alpha]_D^{13}$ +24° and the acetate, m. p. 290—294°, $[\alpha]_D^{13}$ +45°.

(*b*) Solids also formed on the walls of the flask containing the methanol extract. These were worked up as for extract (*a*) and furnished mikanin (150 mg.), m. p. 220—222°.

The methanol filtrate from the solids was evaporated almost to dryness. Rubbing with benzene and ethyl acetate left an insoluble residue. This gave, on continuous extraction with methanol, a yellow solid, m. p. 212—214°, which left an ash on combustion. The yellow solid was dissolved in dilute aqueous sodium hydroxide. Filtration and acidification gave a precipitate (1·5 g.), which crystallised from benzene–chloroform to give mikanin, m. p. 222—223°.

Isolation of Mikanin and Fumaric Acid from the Leaves and Stems.—The powdered material (3 kg.) was exhaustively extracted with (*a*), light petroleum, and (*b*), methanol.

(*a*) The petroleum extract gave a dark waxy residue from which no mikanin was isolated.

(*b*) The methanol extract, on progressive concentration and cooling, gave a brownish solid (24·5 g.) separated by filtration, and a semi-solid. The latter was rubbed with water and then with benzene, leaving a brownish solid (6·5 g.). The solids were combined; they contained inorganic material (mainly potassium salts), mikanin, and fumaric acid.

Most of the mikanin could be isolated by sublimation of the solids at 215—220°/0·4 mm., and the fumaric acid was obtained from the non-sublimable residue by extraction with hot water, and extraction of the acidified extract with ethyl acetate.

Alternatively, the solids were boiled with excess of hot water, filtering from a small amount of insoluble material. The extract was heated for 10 min. with a few drops of hydrochloric acid to give a yellow precipitate of crude mikanin, which was filtered off; on cooling, the filtrate deposited crystalline fumaric acid. A further amount of fumaric acid could be obtained by extracting the mother-liquor with ethyl acetate. The fumaric acid, recrystallised from water, had m. p. 291—292° (evac. tube), and was characterised by its i.r. spectrum. The total amounts of mikanin and fumaric acid obtained were 3·1 and 6·1 g., respectively.

Mikanin Diacetate.—A mixture of mikanin (88 mg.), acetic anhydride (1·2 ml.), and pyridine (1·0 ml.) was warmed on a steam-bath for a few minutes until a clear yellow solution was formed. The mixture was left at room temperature overnight. Addition of water then gave a precipitate which crystallised from acetone–methanol as colourless *needles* (90 mg.), m. p. 187—189° (Found: C, 61·6; H, 4·8; OMe, 19·6. $C_{22}H_{20}O_9$ requires C, 61·7; H, 4·7; 3OMe, 21·7%) giving a negative ferric reaction in ethanol.

Monomethylmikanin.—(*a*) *Using diazomethane.* A solution of mikanin (75 mg.) in chloroform–ether (1 : 1 v/v, 70 ml.) was treated with an ethereal solution of diazomethane (prepared from 2 g. of nitrosomethylurea). The reddish-brown solution was left at room temperature overnight. The solvents were removed under reduced pressure, and the *residue* crystallised from acetone–ethanol as yellow plates (16 mg.), m. p. 174—175° (Found: C, 63·5; H, 5·3. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·1%), giving an intense green ferric reaction in ethanol.

(*b*) *Using 1 mole of dimethyl sulphate.* A mixture of mikanin (100 mg.), dimethyl sulphate (50 mg.), anhydrous potassium carbonate (3 g.), and acetone (60 ml.) was heated under reflux for 4 hr. The mixture was filtered, and the filtrate evaporated to dryness. The residue crystallised from acetone–methanol as pale yellow prisms (70 mg.), m. p. 173—174° (Found: C, 63·9; H, 5·25%), giving an intense ferric reaction in ethanol. The i.r. spectrum was identical with that of monomethylmikanin and different from that of flinduletin.[4]

Dimethylmikanin.—Mikanin (800 mg.), acetone (800 ml.), potassium carbonate (1·6 g.),

and dimethyl sulphate (1·6 ml.) were heated under reflux on a steam-bath for 48 hr., when the solution gave a negative reaction with ferric chloride. The solution was filtered, and the solvent removed. Water was added to the oily residue, which solidified overnight. Recrystallisation from ethanol gave colourless needles, m. p. 157—158° (Found: C, 64·5; H, 5·6. Calc. for $C_{22}H_{20}O_7$: C, 64·5; H, 5·4%).

Pentahydroxyflavone.—Acetic anhydride (5 ml.) was added to freshly distilled, ice-cold hydriodic acid (7 ml.). Mikanin (200 mg.) was added, and the mixture heated under reflux for 1 hr. The cooled mixture was poured into water (25 ml.) and the brownish solid (180 mg.) filtered off. The solid was sublimed at 230—235°/0·04 mm. and the sublimate recrystallised twice from ethanol–acetone. Yellow crystals, m. p. 328—330°, were obtained (Found: C, 59·5; 59·7; H, 3·7, 3·6. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%).

Alkaline Degradation of Dimethylmikanin.—A solution of dimethylmikanin (0·69 g.) and potassium hydroxide (2·5 g.) in absolute ethanol (50 ml.) was heated under reflux for 6½ hr. Processed in the usual way, the acidic fraction furnished anisic acid (0·23 g.), identified by m. p., mixed m. p. and i.r. spectrum. The phenolic fraction afforded 2-hydroxy-ω, 4,5,6-tetramethoxyacetophenone (0·14 g.), m. p. 70—71° (lit.,[10] 71—72°) (Found: C, 56·4; H, 6·3. Calc. for $C_{12}H_{16}O_6$: C, 56·2; H, 6·3%), giving an intense olive-green ferric reaction in ethanol.

Diethylmikanin and its Alkaline Degradation.—Treatment of mikanin (1 g.) with diethyl sulphate (3·4 ml.) in boiling acetone (300 ml.) containing anhydrous potassium carbonate (12 g.) for 23 hr. gave a *product* which crystallised from benzene–light petroleum as colourless needles (0·78 g.), m. p. 94—95° (Found: C, 66·2; H, 6·2. $C_{22}H_{24}O_7$ requires C, 66·0; H, 6·0%).

Alkaline degradation of the product (0·75 g.) by the method described above furnished anisic acid (0·17 g.), and an *acetophenone* which crystallised from light petroleum as pale yellow prisms, m. p. 61—62°, giving an intense brown-green ferric reaction (Found: C, 58·9; H, 7·2. $C_{14}H_{20}O_6$ requires C, 59·1; H, 7·1%).

4,6-*Diethoxy*-2-*hydroxy*-ω-*methoxyacetophenone*.—Ethylation of 2,4,6-trihydroxy-ω-methoxyacetophenone[12] (1·0 g.) with diethyl sulphate (1·55 ml.) and anhydrous potassium carbonate (6 g.) in boiling acetone (100 ml.) for 5 hr. furnished 4,6-diethoxy-2-hydroxy-ω-methoxyacetophenone[13] (1·0 g.), which crystallised from acetone–methanol as colourless needles, m. p. 111—112° (Found: C, 61·5; H, 7·1. Calc. for $C_{13}H_{18}O_5$: C, 61·4; H, 7·1%).

4,6-*Diethoxy*-2,5-*dihydroxy*-ω-*methoxyacetophenone*.—A solution of potassium persulphate (3·1 g.) in water (4·5 ml.) was added during 4 hr. to a stirred solution of the above synthesised ketone (2·9 g.) in pyridine (7 ml.) and aqueous sodium hydroxide (3·5 g. in 150 ml.) at 15—20°. The mixture was set aside at 18° for 24 hr. and acidified to Congo Red with concentrated hydrochloric acid. The unchanged ketone (0·85 g.) was filtered off, and the filtrate extracted once with ether. The aqueous solution was then warmed with concentrated hydrochloric acid (20 ml.) on a steam-bath for ½ hr. The resultant precipitate (0·73 g.) was collected and recrystallised from light petroleum, giving 4,6-*diethoxy*-2,5-*dihydroxy*-ω-*methoxyacetophenone* as pale yellow needles, m. p. 101—103° (Found: C, 58·05; H, 6·8. $C_{13}H_{18}O_6$ requires C, 57·8; H, 6·7%).

4,6-*Diethoxy*-2-*hydroxy*-ω,5-*dimethoxyacetophenone*.—A mixture of the foregoing ketone (0·50 g.), dimethyl sulphate (0·25 g.), anhydrous potassium carbonate (5 g.), and acetone (60 ml.) was heated under reflux for 2½ hr. The solid was filtered off and washed with hot acetone. Evaporation of the filtrates gave an oil to which water (20 ml.) was added. The mixture was left to stand at room temperature for 24 hr., and extracted with ether. Evaporation of the solvent furnished a red oil, which was dissolved in light petroleum and chromatographed on silica gel. Elution with light petroleum–ether (4 : 1) furnished 4,6-*diethoxy*-2-*hydroxy*-ω,5-*dimethoxyacetophenone* (0·14 g.), which crystallised from light petroleum as colourless prisms, m. p. 57—58° (Found: C, 59·2; H, 7·0. $C_{14}H_{20}O_6$ requires C, 59·1; H, 7·1%).

The authors thank Mr. Phui Weng Wong for the supplies of plant material; Dr. J. Herrán for a generous gift of pendulin; Dr. P. R. Jefferies for epifriedelinol, Professor George H. Stout for tangeretin, and Dr. E. Ritchie for flinduletin; Professor K. Nakanishi for obtaining the n.m.r. spectra; Mrs. H. K. Tong for microanalyses; and the National Institutes of Health, Bethesda, for a generous grant.

CHEMISTRY DEPARTMENT, UNIVERSITY OF SINGAPORE. [*Received, May 3rd*, 1965.]

[13] P. R. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 1946, **24***A*, 456.