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THE EXTRACTIVES OF *VITEX LUCENS*—I

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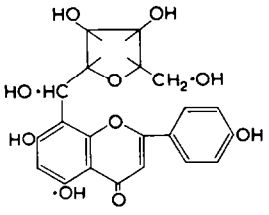
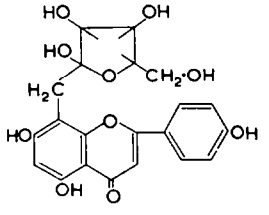
Abstract—Vitexin, isolated from *Vitex lucens*, has been formulated as $C_{21}H_{20}O_{10}$ and, in agreement with recent workers, structure (I) has been assigned to the compound. β-sitosterol has been isolated from the heartwood.

EVANS *et al.*[1] have advanced the tentative structure (I) for vitexin, isolated from New Zealand puriri wood (*Vitex lucens**).

Working on similar lines, we have arrived at the same structure for this compound. The formulation of vitexin as $C_{21}H_{20}O_{10}$ has been confirmed by the preparation of



(I)



(II)

the hepta-acetate, a hexa-acetate, a tetrabenzoate, the trimethyl and triethyl derivatives, a diethyl derivative, a ditosylate and a monotrityl derivative. Repetition of the degradative studies of previous workers[2–6] resulted, in the main, in identical products. Only those that are new or differ markedly from those of previous workers are described in this communication.

In contrast to the isolation by Evans *et al.*; vitexin has only been obtained from the wood extracts after acid hydrolysis. It is considered by us to be an artifact formed during hydrolysis, which requires conditions of aerial oxidation for the production of good yields. The view that vitexin from saponarin is also an artifact has been expressed by Geissman *et al.*[7,8] Vitexin was obtained from both the sapwood and heartwood of *Vitex lucens* by a modification of Perkin's method,[2] but not from the leaves by the same procedure. The trimethyl and triethyl derivatives were each prepared by extended heating under reflux with dimethyl sulphate or diethyl sulphate, respectively, and anhydrous potassium carbonate in acetone. These derivatives have previously been prepared from vitexin penta-acetate.[1]

As Evans *et al.* have shown, oxidation of vitexin with sodium periodate gave variable and apparently anomalous results. The initial stages of reaction showed the comparatively rapid initial uptake of 2 molecules of periodate followed by further

* Formerly *Vitex littoralis;* see Cheeseman.

[1] W. H. Evans, A. McGookin, L. Jurd, A. Robertson and W. R. N. Williamson, *J. Chem. Soc.* 3510 (1957).
[2] A. G. Perkin, *J. Chem. Soc.* **73**, 1019 (1898).
[3] A. G. Perkin, *J. Chem. Soc.* **77**, 422 (1900).
[4] G. Barger, *J. Chem. Soc.* **89**, 121 (1906).
[5] E. Péteri, *J. Chem. Soc.* 1635 (1939).
[6] T. Nakaoki, *J. Pharm. Soc. Japan* **64**, 57 (1944).
[7] T. A. Geissman and U. Krannen-Fielder, *Naturwissenschaften* **10**, 226 (1956).
[8] T. A. Geissman, L. Jurd and M. K. Seikel, *Arch. Biochem. Biophys.* **67**, 284 (1957).
[9] T. F. Cheeseman, *Manual of New Zealand Flora* (2nd Ed.) p. 763. Government Printer, New Zealand (1925).

gradual oxidation and is thus not confined to 1:2-diol fission. Small amounts of a volatile acid formed on oxidation and reported by Evans *et al.* were identified by us as formic acid (S-benzyl*iso*thiuronium salt), but this is regarded as a product of secondary oxidation during the formation of 8-formylapigenin from dehydro*seco*-vitexin, and not as evidence for the acceptance of these workers' alternative structure (II).

In contrast to Nakaoki's claim[6] to have isolated 2:4:6-trihydroxyphenylacetic acid, alkaline permanganate oxidation of vitexin led to the formation of apigenin, probably arising from the ready decarboxylation of apigenin-8-carboxylic acid. The failure to isolate Nakaoki's reported product lends further evidence to the acceptance of structure (I) for vitexin. Nakaoki, in assigning a 2:3:4:5:6-pentahydroxy-*n*-hexyl structure for the non-flavonoid moiety of vitexin, reported that it formed a diacetone compound. Repeated attempts to form an *iso*propylidene derivative however, have been unsuccessful. This could be attributed to a *trans*-diol grouping in the $C_6H_{11}O_5$ residue of (I).

Perkin's "homovitexin" has now been shown to be a dextrorotatory compound, m.p. 246–247°, isomeric with vitexin, for which the name *iso*vitexin is now preferred. Although a comprehensive examination of *iso*vitexin was not possible owing to its formation in low yield (0·04 per cent), it could be readily differentiated from vitexin by its greater solubilities and by paper chromatography in two solvent systems. *iso*Vitexin formed a hepta-acetate with acetic anhydride and pyridine, but, as with vitexin, difficulty was encountered in the attempted formation of a trimethyl derivative. The products of alkaline fusion were phloroglucinol, *p*-hydroxybenzoic acid and acetic acid.

Paper chromatography of the crude aglycone mixtures of a series of hydrolyses has now shown that *iso*vitexin is the initial product in the formation of vitexin. Further extended acid treatment of *iso*vitexin gave a good yield of vitexin. Also the yield of *iso*vitexin from hydrolysis of the original wood extract depended on the acid concentration and the time of heating, high acid concentration and extended hydrolysis resulting in the production of negligible quantities of *iso*vitexin. This behaviour is paralleled by Nakaoki's findings for acid treatment of saponarin. Although the melting point of *iso*vitexin is somewhat higher than that recorded for saponaretin[6] (m.p. 225–226°) and a positive rotation was observed for *iso*vitexin, it appears likely that the two compounds could be identical. Seikel and Geissman[10] have shown by paper chromatography that an equilibrium exists between vitexin and saponaretin in hot acid solution, and with the assumption that saponaretin is represented by the formula $C_{21}H_{22}O_{11}$, have suggested that the equilibrium might be explained as a cyclisation by dehydration and ring opening by hydration.

A crystalline sterol isolated from a light petroleum extract of the heartwood was identified as β-sitosterol.

EXPERIMENTAL

β-Sitosterol. Coarsely ground heartwood of *Vitex lucens* (14·5 kg) was extracted in a Soxhlet extractor with light petroleum (boiling range 50–65°) for 24 hr. Trituration of the concentrated extract with acetone and then chromatography of the waxy solid (1·0 g) in benzene on alumina gave β-sitosterol (plates from methanol), m.p. and mixed m.p. 136–137°, λ_{max} 208 mμ (log ε 3·72). The infra-red spectrum was

[10] M. K. Seikel and T. A. Geissman, *Arch. Biochem. Biophys.* **71**, 17 (1957).

identical with that recorded[11] (Found: C, 84·2; H, 11·5. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·1 per cent). The acetate had m.p. and mixed m.p. 127–128° (Found: C, 82·3; H, 11·3. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·5 per cent).

Vitexin. Finely milled sapwood (8·2 kg) was extracted with ethanol for 36 hr and the concentrated extract was separated from insoluble material by water extraction. The concentrated aqueous solution was heated under reflux with 1% hydrochloric acid in an open dish for 1 hr. A greenish-black polymer was deposited on heating and it was separated by repeated filtration of the hot solution. Hydrolysis was then continued with an increased acid concentration (2%) for 2 hr. Crude vitexin (2·0 per cent yield) was obtained as a microcrystalline mass by washing the tarry deposit several times with ethanol. Vitexin (1·5 per cent yield) was also obtained from an acetone extract of the heartwood by the same procedure. Paper chromatography of the concentrated hydrolysate with butanol–pyridine–water (3:1:1) showed the presence of glucose. The polymer on alkaline and acid treatment had an identical infra-red spectrum with that obtained from either asperuloside or toluquinhydrone, on similar treatment.[12]

Vitexin crystallised from 60% acetic acid in yellow rhombic plates, m.p. 263° (dec.), $[\alpha]_D^{18}$ −14·3° (*c.* 1·10 in pyridine), λ_{max} 270 and 335 mμ (log ε 4·32 and 4·33), infra-red spectrum (potassium bromide disc method) 3425, 3392, 3284, 2889, 1650 cm^{-1} (Found for sample dried over magnesium perchlorate: C, 58·5; H, 5·1. Calc. for $C_{21}H_{20}O_{10}$: C, 58·3; H, 4·7 per cent).

Vitexin hepta-acetate. Acetylation with acetic anhydride (6 hr at 100°) gave the hepta-acetate, which separated from ethanol–acetic acid as colourless rhombs, with a negative ferric chloride reaction, m.p. 255–256°, $[\alpha]_D^{18}$ −75·7° (*c.* 0·95 in ethanol), λ_{max} 225 and 300 mμ (log ε 4·21 and 4·28), infra-red spectrum 1748, 1712, 1656 cm^{-1} (Found: C, 57·7; H, 4·6; Ac, 41·5, 41·9. Calc. for $C_{35}H_{34}O_7$: C, 57·85; H, 4·7; 7Ac, 41·5 per cent).

Vitexin hexa-acetate. Acetylation of vitexin (450 mg) with acetic anhydride (1·5 g) and fused sodium acetate (2·0 g) for 8 hr at 100° gave a brown oil, which solidified on being kept overnight. The hepta-acetate (20 mg), m.p. and mixed m.p. 255–256°, was separated by crystallisation of the crude product from ethanol–acetic acid. *Vitexin hexa-acetate* (520 mg) was deposited when the mother-liquors were poured into water. The product separated from absolute ethanol as colourless needles, m.p. 157–159°, λ_{max} 271 and 305 mμ (log ε 4·49 and 4·36), infra-red spectrum 3509, 3135, 2959, 1754, 1656 cm^{-1} (Found: C, 57·4; H, 5·1; Ac, 37·9. $C_{33}H_{32}O_{16}$ requires C, 57·9; H, 4·7; 6Ac, 37·7 per cent). The derivative was soluble in cold sodium hydroxide and gave a dark-red reaction with ferric chloride. Further acetylation with acetic anhydride (6 hr at 100°) gave vitexin hepta-acetate, m.p. and mixed m.p. 255–256°.

Vitexin tetrabenzoate. Excess of benzoyl chloride was added in portions with shaking and cooling to vitexin in 10% sodium hydroxide. The product, isolated as a cream oil, solidified on standing. Purification by chromatography in benzene on neutralized alumina and crystallisation from benzene–ligroin gave colourless needles of the *tetrabenzoate*, m.p. 137°, with a negative ferric chloride reaction, infra-red spectrum 3497, 3135, 3003, 1793, 1653 cm^{-1} (Found: C, 69·0; H, 4·3; Bz, 49·1. $C_{49}H_{36}O_{14}$ requires C, 69·3; H, 4·2; 4Bz, 49·5 per cent).

[11] R. M. Ma and P. S. Schaffer, *Arch. Biochem. Biophys.* **47**, 419 (1953).
[12] L. H. Briggs and B. F. Cain, *J. Chem. Soc.* 4182 (1954).

Ditosylvitexin. A cooled solution of toluene-*p*-sulphonyl chloride (320 mg) in pyridine (2 ml) was added dropwise, with mechanical stirring, to vitexin (161 mg) in the same solvent at −10°C. After 2 hr at −10° and 5 days at room temperature, the *ditosyl derivative* (190 mg) was precipitated on contact with crushed ice. Repeated crystallisation from methanol gave short cream rods, m.p. 198° (dec.), infra-red spectrum 3425, 2959, 2717, 1653 cm^{-1} (Found: C, 56·3; H, 4·3. $C_{21}H_{20}O_{10}$ $(C_7H_6O_2S)_2$ requires C, 56·7; H, 4·35 per cent).

Monotritylvitexin. A cooled solution of triphenylmethyl chloride (180 mg) in dry pyridine (2 ml) was added, with mechanical stirring, over a period of 30 min to vitexin (156 mg) in the same solvent (2 ml) at −10°C. After 2 hr at −10° and 12 days at room temperature, the derivative (330 mg) was precipitated as a cream amorphous powder on contact with crushed ice. Three crystallisations from methanol gave a cream microcrystalline *monotrityl derivative* (102 mg), m.p. 234–235° (dec.) with shrinking at 190°, infra-red spectrum 3448, 3115, 2959, 1656 cm^{-1} (Found for sample dried at 100°: C, 70·8; H, 5·3. $C_{40}H_{34}O_{14}$ requires C, 71·2; H, 5·1 per cent).

5:7:4′-*Trimethylvitexin.* A suspension of finely powdered vitexin (500 mg) in dry acetone (40 ml), was heated under reflux with dimethyl sulphate (1·9 ml) and anhydrous potassium carbonate (3·43 g) for 86 hr. Concentration of the combined filtrate and the hot acetone washings of the salts gave 5:7:4′-trimethylvitexin as an amorphous yellow mass (35 per cent). Crystallisation from methanol gave clusters of colourless needles, m.p. 195–196° (dec.), with a negative ferric chloride reaction, insoluble in cold aqueous sodium hydroxide, λ_{max} 265 and 312 mμ (log ε 4·71 and 4·61) infra-red spectrum 3401, 2950, 1647 cm^{-1} (Found: C, 61·0; H, 5·4; OMe, 18·0. Calc. for $C_{24}H_{26}O_{10}$: C, 60·8; H, 5·5; 3OMe, 19·6 per cent). Trimethylvitexin, m.p. and mixed m.p. 195–196°, was also obtained in higher yield (50 per cent) by initial treatment of vitexin in absolute methanol with ethereal diazomethane and then further methylation of the crude product by the method described above.

The tetra-acetate, prepared with acetic anhydride–pyridine (6 hr at 100°) separated from 75% aqueous ethanol as colourless needles, m.p. 208°, λ_{max} 265 and 328 mμ (log ε 4·44 and 4·48), infra-red spectrum 2959, 2841, 1754, 1698, 1647 cm^{-1} (Found: C, 59·9; H, 5·7; Ac, 26·1; OMe, 14·1. Calc. for $C_{32}H_{34}O_{14}$: C, 59·8; H, 5·3; 4Ac, 26·8; 3OMe, 14·5 per cent).

7:4′-*Diethylvitexin.* A mixture of finely powdered vitexin (1·0 g), diethyl sulphate (1·5 ml) and potassium carbonate (3·6 g) was heated under reflux with acetone (50 ml) for 48 hr. Evaporation of the acetone solution and washings yielded a gum, which was triturated with water. The residue (710 mg), when repeatedly crystallised from ethanol, gave 7:4′-*diethylvitexin* (62 per cent) as pale-yellow needles, m.p. 276–276·5°, λ_{max} 270 and 326 mμ (log ε 4·26 and 4·21), infra-red spectrum 3413, 2941, 1656 cm^{-1} (Found: C, 61·0, 60·9; H, 6·1, 6·0; OEt, 18·85. $C_{25}H_{28}O_{10}$ requires C, 61·5; H, 5·8; 2OEt, 18·4 per cent). Diethylvitexin was soluble in 10% sodium carbonate and gave a red coloration with ferric chloride.

5:7:4′-*Triethylvitexin.* Continued ethylation of diethylvitexin gave the 5:7:4′-triethyl derivative as a yellow gum after removal of excess of diethyl sulphate with light petroleum. It separated from methanol as colourless needles, m.p. 270° (lit.[1] m.p. 270°), with a negative ferric chloride test. Triethylvitexin was also the major product (500 mg from 1 g) from prolonged ethylation (68 hr) of vitexin.

Treatment of crude triethylvitexin with acetic anhydride and fused sodium

acetate at 100° for 8½ hr gave the tetra-acetate, which separated from ethanol as colourless needles, m.p. 235·5°, with sintering at 120° (Found: C, 61·2; H, 5·7; Ac, 26·6; OEt, 18·7. Calc. for $C_{35}H_{40}O_{14}$: C, 61·4; H, 5·9; 4Ac, 25·2; 3OEt, 19·7 per cent).

Alkaline permanganate oxidation of vitexin. Potassium permanganate (535 mg) was added in portions, with stirring, to vitexin (646 mg) in 0·4% sodium hydroxide (25 ml) at room temperature. Stirring was continued for 13 hr, the mixture was set aside overnight, and the reaction was stopped by addition of methanol. Precipitated manganese dioxide was removed and most of the ethanol was removed by distilling under reduced pressure. Acidification gave a yellow precipitate (344 mg), m.p. 246° (dec.), which, after being washed with water and hot ether, on slow crystallisation from ethanol gave apigenin as a yellow microcrystalline powder, m.p. and mixed m.p. 347° (dec.), λ_{max} 269 and 335 mμ (log ε 4·30 and 4·32) (Found for sample dried at 100°: C, 60·2; H, 4·6. Calc. for $C_{15}H_{10}O_5 \cdot 1{\cdot}5H_2O$: C, 60·6; H, 4·4 per cent). The product gave solubility and colour reactions identical with those of an authentic sample.

The triacetate, prepared with acetic anhydride–pyridine (2 hr at 100°) crystallised as colourless needles from methanol, m.p. and mixed m.p. 180–181°.

Ether extraction of the acid filtrate gave an orange gum on removal of the solvent. From the benzene extract of the residue *p*-hydroxybenzoic acid (needles from ether), m.p. and mixed m.p. 209–210°, was isolated in low yield (10 mg).

iso*Vitexin*. *iso*Vitexin, obtained from the alcoholic washings of vitexin by the method of Perkin,[2] crystallised from absolute ethanol as yellow needles, m.p. 246–247° (dec.) (lit.,[2] m.p. 245–246° for "homovitexin"), $[\alpha]_D^{180}$ + 16·21° (*c.* 0·37 in ethanol), λ_{max} 270 and 337 mμ (log ε 4·43 and 4·40), infra-red spectrum 3378, 2959, 1661 cm^{-1} (Found: C, 58·7; H, 5·1. $C_{21}H_{20}O_{10}$ requires C, 58·3; H, 4·7 per cent). Solubility properties and colour reactions corresponded with those recorded for "homovitexin".[2]

iso*Vitexin hepta-acetate*. Acetylation with acetic anhydride–pyridine (2 hr at 100°) gave the *hepta-acetate*, which separated from ethanol as colourless prisms, m.p. 248°, with a negative ferric chloride reaction, infra-red spectra 1773, 1712, 1661 cm^{-1} (Found: C, 57·6; H, 5·0; Ac, 42·2. $C_{35}H_{34}O_{17}$ requires C, 57·85; H, 4·7; 7Ac, 41·5 per cent).

Conversion to vitexin. An ethanolic solution of *iso*vitexin (50 mg) was heated under reflux with 5% sulphuric acid for 8 hr. The brown solid (41 mg) obtained on dilution gave vitexin (rhombic plates from 60% acetic acid), m.p. and mixed m.p. 262–263°.

Paper chromatography. Chromatography on Whatman No. 1 paper with "Forestal solvent"[13] and butanol–acetic acid–water (4:1:5) gave the following R_F values: vitexin, 0·83 and 0·65; *iso*vitexin, 0·90 and 0·76. The spots were coloured brown-purple in ultra-violet light and yellow-green in the presence of ammonia.

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[13] E. C. Bate-Smith, *Biochem. J.* **58**, 122 (1954).