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CHEMISTRY OF THE PODOCARPACEAE—II

THE ISOLATION OF GENISTEIN FROM *PODOCARPUS SPICATUS* AND THE CONSTITUTION OF PODOSPICATIN

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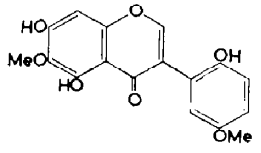
Abstract—Genistein has been isolated from the heartwood. Podospicatin has been shown to have the constitution 5:5′:7-trihydroxy-2′:-6-dimethoxy*iso*flavone.

IN a more systematic extraction of the heartwood of *Podocarpus spicatus* matairesinol and podospicatin have again been isolated and, in addition, the *iso*flavone genistein. Other constituents will be described in a further communication.

The remaining structural feature required for podospicatin[1] is the position of the two methoxyl groups. Since podospicatin gave no positive Bargellini test for three vicinal phenolic groups[2] at least one of the hydroxyl groups of the 3-phenyl component is unmethylated. Tests for *o*- or *p*-dihydroxyphenols with chloropentammino-cobaltic chloride[3] were also negative. On the basis of these colour tests podospicatin is either 5:5′:7-trihydroxy-2′:6-dimethoxy*iso*flavone or 2′:5:7-trihydroxy-5′:6-dimethoxy*iso*flavone. That the latter is correct was shown by degradation of the triethyl ether. Vigorous alkaline treatment of podospicatin triethyl ether gave a phenol and an acid. The same phenol, 3:5-diethoxy-4-methoxyphenol, would be expected from both possible formulations. The synthetic compound was shown to be identical with that obtained by degradation. The acid was shown to be 2-ethoxy-5-methoxyphenylacetic acid by comparison with a synthetic specimen. Podospicatin is, therefore, 2′:5:7-trihydroxy-5′:6-dimethoxy*iso*flavone(I).



I

Podospicatin 7-methyl and 2′:7-dimethyl ether were prepared by Simpson and Beton's method.[4]

In agreement with Swain[5] a marked shift in the ultra-violet absorption spectra of podospicatin, its 7-methyl and 2′:7-dimethyl ether, has been observed on the addition of aluminium chloride to the ethanolic solution.

[1] L. H. Briggs and B. F. Cain, *Tetrahedron* **6**, 143 (1959).
[2] G. Bargellini, *Gazzetta* **49**, 47 (1919).
[3] Y. Sashina, J. Asano and Y. Ueno, *Bull. Chem. Soc. Japan* **17**, 104 (1942).
[4] T. H. Simpson and J. L. Beton, *J. Chem. Soc.* 4065 (1954).
[5] T. Swain, *Chem. & Ind.* 1480 (1954).

EXPERIMENTAL

Microanalyses were carried out by Dr. A. R. Campbell, University of Otago. Ultra-violet spectra were measured in ethanol.

Extraction. Dry, finely ground heartwood of *Podocarpus spicatus* was successively extracted with light petroleum (b.p. 60–90°) and ether in a large Soxhlet extractor. Evaporation of the light petroleum extract to 1/25 volume precipitated a gum. After digestion with a little light petroleum this was crystallized from ethanol and repeatedly from acetic acid to give podospicatin, m.p. 213·5–214°.

Ultra-violet spectrum: λ_{max} in 0·1% ethanolic aluminium chloride solution, 275 mμ (log ε 4·34), 310 mμ (log ε 4·16).

Infra-red spectrum (KBr): 3390, 3175, 2941, 2817, 2597, 1656, 1634, 1567, 1499,1464, 1435, 1370, 1335, 1299, 1261, 1209, 1181, 1157, 1131, 1063, 1036, 991, 964, 932, 917, 856, 817, 803, 742, 718, 683 cm^{-1}.

The crystalline material, which formed after concentration of the ether extract to 1/10 volume, was repeatedly crystallized from 60% acetic acid to give rhombic crystals of matairesinol, m.p. and mixed m.p. 117–119°. Further concentration of the ether extract gave a gum which could be separated into podospicatin and matairesinol by fractional crystallization.

Acidic constituents were removed from the remaining ether extract by successive extraction with saturated sodium hydrogen carbonate, 10% sodium carbonate and 0·2% sodium hydroxide solutions followed by acidification. The material from the hydrogen carbonate extract is under investigation.

The material from the carbonate extract crystallized from 60% acetic acid to give matairesinol and a compound crystallizing in yellow needles, m.p. 293–294°. The latter has been identified with genistein, mixed m.p. 300–302° with an authentic specimen, m.p. 301–303°. (Found: C, 66·2; H, 3·7. $C_{15}H_{10}O_5$ requires: C, 66·8; H, 3·7%). The ultra-violet spectrum, λ_{max} 265 mμ (log ε 4·59) agrees with that, λ_{max} 263 mμ (log ε 4·57), recorded by Walter.[6] The melting points of the trimethyl ether, m.p. 164–165°, and the triacetate, m.p. 202–204°, agree with those reported by Seshadri[7] and Walz[8] respectively.

Alkaline fusion of podospicatin triethyl ether. A solution of podospicatin (1·485 g) in acetone was ethylated with diethyl sulphate in the presence of anhydrous potassium carbonate. The oily product was dissolved in ether, washed with sodium hydroxide solution and the solvent removed. The product (1·43 g) was mixed with sodium hydroxide (5 g) and water (2 ml) and heated to 220° for 50 min. The products were divided into acid and phenolic fractions in the usual way. The major phenolic compound crystallized from water in needles, m.p. 71–72°, (250 mg) raised to 83·5–84·5° on drying in a desiccator. (Found: C, 62·5; H, 7·3. $C_{11}H_{16}O_4$ requires: C, 62·3; H, 7·6%). The melting point was not depressed by a synthetic sample of 3:5-diethoxy-4-methoxyphenol[9] and the infra-red spectra of the respective samples were identical.

The major acid component from the alkaline fusion crystallized from water in plates, m.p. 103–104° (151 mg). (Found: C, 62·5; H, 6·6. $C_{11}H_{14}O_4$ requires: C, 62·8; H, 6·7%). The melting point was not depressed by a synthetic sample of 2-ethoxy-5-methoxyphenylacetic acid prepared by ozonolysis of 2-ethoxy-5-methoxyallylbenzene and the infra-red spectra of the respective samples were identical.

Infra-red spectrum (KBr): 3390, 3279, 2985, 2632, 2532, 1603, 1508, 1466, 1391, 1330, 1259, 1225, 1178, 1157, 1124, 1104, 1025, 994, 900, 861, 848, 814, 780, 743 cm^{-1}.

Synthesis of 2-ethoxy-5-methoxyphenylacetic acid. Quinol monomethyl ether (19 g) was allylated by refluxing with allyl bromide (24 ml) in acetone (500 ml) in the presence of anhydrous potassium carbonate (60 g) for 9 hr. The product from the acetone solution was taken up in ether and washed with sodium hydroxide solution and the solvent removed. The resultant liquid (22·5 g) failed to solidify and was submitted to Claisen rearrangement directly.

The allyl ether (22·5 g) and dimethylaniline (12 g) were refluxed for 45 min, dissolved in light petroleum and extracted successively with dilute sulphuric acid and 10% sodium hydroxide solution. The alkali soluble material, after acidification and extraction with ether, yielded 2-allyl-4-methoxyphenol as an oil, b.p. 190° (bath temp.)/8 mm (17·6 g).

The above phenol (17·5 g) was ethylated by refluxing with diethyl sulphate (18 ml) in acetone

[6] E. D. Walter, *J. Amer. Chem. Soc.* **63**, 3273 (1941).
[7] T. R. Seshadri, *Curr. Sci.* **22**, 263 (1953).
[8] E. Walz, *Liebigs Ann.* **489**, 118 (1931).
[9] T. R. Seshadri and M. Krishnamurti, *Proc. Indian Acad. Sci.* **39**, 144 (1954).

(200 ml) in the presence of anhydrous potassium carbonate (60 g) for 16 hr. The material from the acetone solution was taken up in ether and washed with sodium hydroxide solution and the solvent removed. 2-Ethoxy-5-methoxyallylbenzene was obtained as an oil, b.p. 174–178° (bath temp.)/5 mm.

The oil (700 mg) was finally ozonized in carbon tetrachloride (25 ml) and the ozonide decomposed and oxidized by solution in glacial acetic acid and treatment with a mixture of hydrogen peroxide (30%, 2·5 ml), sulphuric acid (0·1 ml) and water (5 ml) under reflux for 2 hr. The product was taken up in ether and the acidic material removed with sodium hydrogen carbonate solution. After acidification the material was repeatedly crystallized from water to give colourless plates of 2-ethoxy-5-methoxyphenylacetic acid, m.p. and mixed m.p. with the degradation acid, 101–102°. The infra-red spectra of the two acids were also identical.

Podospicatin 7-methyl ether. Podospicatin (554 mg) was methylated by refluxing with dimethyl sulphate (0·31 ml, 1·1 mole) in acetone (150 ml) in the presence of sodium hydrogen carbonate (10 g) for 21 hr, with continuous passage of carbon dioxide. The product crystallized from glacial acetic acid in pale yellow, rectangular plates, m.p. 206·5–207·5°. (Found: C, 62·8; H, 5·0; OMe, 27·0; Calc. for $C_{16}H_7O_4(OMe)_3$: C, 62·8; H, 4·7; 3 OMe, 27·0%).

Ultra-violet spectrum: (9·67 mg/l.): λ_{max} 265 mμ (log ε 4·33), 301 mμ (log ε 4·11). λ_{max} in 0·1% ethanolic aluminium chloride solution: 277 mμ (log ε 4·23), 307 mμ (log ε 4·05).

Infra-red spectrum (KBr): 3413, 3125, 2915, 2849, 1650, 1618, 1580, 1490, 1456, 1425, 1374, 1321, 1287, 1276, 1236, 1211, 1176, 1139, 1080, 1059, 1041, 1010, 976, 932, 877, 855, 826, 812, 761, 741, 719, 699 cm^{-1}.

The product was soluble in warm sodium carbonate solution and gave a green ferric chloride coloration.

Podospicatin 2′:7-dimethyl ether. A solution of podospicatin (173 mg), dimethyl sulphate (3·6 ml), ethanol (21 ml) and N-sodium carbonate solution (39 ml) was maintained at 20–21° for 20 min, diluted with water (100 ml) and acidified. After standing, the product crystallized from 60% acetic acid in pale yellow hexagonal plates, m.p. 120–121·5°. (Found: C, 63·25; H, 5·0; OMe, 30·8; Calc. for $C_{16}H_6O_3(OCH_3)_4$: C, 63·7; H, 5·1; 4 OMe, 34·6%).

Ultra-violet spectrum (78·3 mg/l.): λ_{max} 262 mμ (log ε 4·44), 300 mμ (log ε 4·25). λ_{max} in 0·1% ethanolic aluminium chloride solution, 275 mμ (log ε 4·53), 307 mμ (log ε 4·46).

Infra-red spectrum (KBr): 3546, 3257, 2967, 1650, 1623, 1580, 1497, 1458, 1357, 1299, 1232, 1215, 1179, 1142, 1079, 1060, 1040, 1018, 985, 943, 917, 861, 817, 803, 770, 739, 714, 699 cm^{-1}.

The product was soluble in sodium hydroxide solution and gave a green ferric chloride coloration.

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