EXTRACTIVES OF AUSTRALIAN TIMBERS*

III. AFROMOSIN (CASTANIN, 6,4′-DIMETHOXY-7-HYDROXYISOFLAVONE) FROM CASTANOSPERMUM AUSTRALE CUNN. ET FRAS.

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An examination of the triterpenoid extractives of *Castanospermum australe* Cunn. et Fras. has been reported by Simes (1950). Investigation of the phenolic constituents of the extract has led to the isolation of a new isoflavone, castanin, which was shown to be 6,4′-dimethoxy-7-hydroxyisoflavone (Hinterberger 1956). The structure of castanin has been briefly recorded by one of us (Simes *et al.* 1959); more recently McMurry and Theng (1960) have assigned the same structure to afromosin which was isolated from the wood of *Afromosia elata* Harms. Because of the detailed published work on afromosin, which is similar to our own studies, we now report those experiments not previously recorded. The melting points of afromosin and several of its derivatives as determined by us differ somewhat from those claimed by McMurry and Theng. Formononetin (7-hydroxy-4′-methoxyisoflavone) was also present in the phenolic fraction of the extract.

Experimental

Melting points are uncorrected. Analyses were carried out by Dr. E. Challen, University of New South Wales and by the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne. Analytical specimens were dried for 8 hr at 100°C/1 mm.

(*a*) *Isolation of Afromosin.*—The finely milled wood (5 kg) of *C. australe* was extracted with ethanol at room temperature. The alcoholic solution (40 l.) was concentrated to 1·2 l. and the black viscous residue was poured into ether (4 l.). The resulting precipitate of crude saponin was triturated with ether and dried on the steam-bath (yield 504 g). The dark red-brown, ethereal ethanolic filtrate was extracted successively with (i) 5% $NaHCO_3$ soln. (4 × 400 ml), (ii) N Na_2CO_3 (10 × 200 ml), (iii) 0·25N NaOH (2 × 80 ml), (iv) N NaOH (500 ml), and finally water. Acidification of the alkaline extracts gave respectively (i) 25·6 g, (ii) 44·6 g, (iii) 8·0 g, and (iv) 12·8 g of amorphous brown solids. The residual ethereal solution contained 15·0 g of a semisolid neutral fraction.

During the extraction of the ethereal layer with carbonate solution a finely divided Na salt separated out at the interphase. Acidification of a hot aqueous solution of the salt gave 3·8 g of the isoflavone. The combined carbonate extractives (48·4 g) were twice crystallized from ethanol and the resulting isoflavone was further purified via its Na salt which crystallized from 0·5N Na_2CO_3. Further recrystallizations from ethanol gave afromosin (castanin) as colourless needles, m.p. 233–234°C (Found: C, 68·8; H, 4·9; OCH_3, 20·2%. Calc. for $C_{17}H_{14}O_5$: C, 68·5; H, 4·7; 2 × OCH_3, 20·8%). The total yield of castanin was 2·8 g (*c.* 0·05% yield).

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(*b*) *2-Hydroxy-4-ethoxy-5-methoxyphenyl 4-Methoxybenzyl Ketone.*—A mixture of *O*-ethylafromosin (1·4 g), ethanol (40 ml), and 2N NaOH (40 ml) was refluxed for 20 min. Acidification of the cooled reaction mixture gave a solid which, on crystallization from methanol furnished the *benzyl ketone* as pale yellow plates, m.p. 108–109°C (Found: C, 68·3; H, 6·4%. Calc. for $C_{18}H_{20}O_5$: C, 68·3; H, 6·4%).

The ketone readily gave a *2,4-dinitrophenylhydrazone* as deep red plates from dioxan, m.p. 231–232°C (Found: C, 57·9; H, 4·8; N, 11·3%. Calc. for $C_{24}H_{24}O_8N_4$: C, 58·1; H, 4·9; N, 11·3%).

(*c*) *3-Ethoxy-4-methoxyphenol.*—5-Nitroguaiacol (Paul 1906) was ethylated by the procedure of Head and Robertson (1930). The resulting 3-ethoxy-4-methoxynitrobenzene (26 g) in ethanol (150 ml) was reduced by hydrogen in the presence of Raney nickel catalyst at atmospheric pressure and room temperature. After removal of the catalyst, the ethanolic filtrate was treated with a mixture of water (250 ml) and 36N H_2SO_4 (33 ml) and most of the ethanol was then distilled off. The amine was then converted into the corresponding phenol by the procedure of Head and Robertson (1930). The phenol which distilled at 154°C/2 mm crystallized from light petroleum (60–80°C) to yield 2·5 g (13%), m.p. 76°C.

(*d*) *Synthetic 2-Hydroxy-4-ethoxy-5-methoxyphenyl 4-Methoxybenzyl Ketone.*—3-Ethoxy-4-methoxyphenol (1·8 g) and an equivalent amount of 4-methoxybenzyl cyanide were dissolved in dry ether (40 ml) containing fused $ZnCl_2$ (1·8 g). The mixture was saturated at 0°C with dry HCl. After 48 hr at 0°C, the ether was decanted from the dark red semisolid which was washed with ether and then heated on a steam-bath with water (40 ml) for 45 min. The resulting solid gave the benzyl ketone as pale yellow plates, m.p. 108–109°C, from methanol, undepressed on admixture with the ketone obtained by degradation of *O*-ethylafromosin.

(*e*) *Comparison of Melting Points.*—The m.p.'s of the following compounds were found to differ somewhat from those published by McMurry and Theng (1960):

Afromosin, m.p. 233–234°C (cf. McMurry and Theng, 228–229°C).
O-Acetylafromosin, m.p. 173–174°C (165–167°C).
O-Methylafromosin, m.p. 181–182°C (174–175°C).
O-Ethylafromosin, m.p. 148–149°C (135–138°C).
6,7,4′-Triacetoxyisoflavone, m.p. 224–225°C (217°C).

(*f*) *Isolation and Characterization of Formononetin.*—Concentration of the initial ethanolic mother liquors from the purification of crude afromosin gave, on standing, a semicrystalline solid (5·5 g). Purification of this substance by repeated recrystallization from ethanol as well as via its Na salt as in (*a*) gave formononetin as colourless needles (2·6 g), m.p. 255–256°C (Found: C, 71·5; H, 4·8; OCH_3, 11·7%. Calc. for $C_{16}H_{12}O_4$: C, 71·6; H, 4·5; $1 \times OCH_3$, 11·6%). Purification of the combined NaOH extractives (20·8 g) by crystallization from ethanol gave a further quantity (3·8 g) of formononetin.

Formononetin was characterized as its acetate (m.p. 172–173°C) and its methyl ether (m.p. 163–164°C) prepared by the usual methods. The m.p.'s of these derivatives corresponded closely with those obtained by earlier workers.

The natural isoflavone was identical (m.p. and infrared spectra) with a synthetic sample prepared by the method of Baker *et al.* (1953).

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