THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES

XIV. THE CONSTITUENTS OF FLINDERSIA PUBESCENS BAIL. AND F. SCHOTTIANA F. MUELL.

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Summary

The alkaloids and other constituents of *Flindersia pubescens* Bail. and *F. schottiana* F. Muell. have been isolated. From *F. schottiana* has been obtained osthol hydrochloride and chemical work on its genesis is described. Several substances related to osthol are reported for the first time.

I. Introduction

Flindersia pubescens Bail. occurs typically on the Atherton Tableland and adjoining areas of north Queensland. It is commonly known as silver ash and the timber is used as a building material, for plywood, and for "blonde" furniture. *F. schottiana* F. Muell. is a valuable commercial timber, popularly known as southern silver ash or bumpy ash, and is found in the coastal scrubs from the Hastings River, N.S.W., northward into south-eastern Queensland. Mr. L. S. Smith of the Queensland Botanic Museum and Herbarium, Brisbane, has written as follows on the classification of the two species:

"*Flindersia schottiana* and *F. pubescens* are generally accepted as two very closely related species differing little except in length and density of the indumentum, although *F. pubescens* does tend to have slightly larger leaves and fruit . . . It is possible that when more complete botanical material is available throughout the whole range of distribution, both of the above may prove to be variants within a single polymorphic species (*F. schottiana*)."

II. Discussion

Contrary to this botanical opinion, the two species have proved to be somewhat different chemically, as shown in Tables 1 and 2. The substances were isolated by systematic extraction in the usual way.

The alkaloid, $C_{16}H_{15}O_2N$, m.p. 138 °C, appears from its spectral properties to be a furoquinoline type; but in view of the difficulty of separation of these alkaloids and the small amount available, its homogeneity must still be considered suspect. The other bases were substances of known structure, apart from maculosidine, the isolation of which at this stage allowed its structure to be resolved (Prager, Ritchie, and Taylor 1960). Details of a paper chromatographic system for furoquinolines are given in Section III.

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The isolation of osthol hydrochloride (Ia), which was readily identified by elemental analysis and direct comparison, was most surprising. The prior treatment of the extract with 5% hydrochloric acid to separate alkaloids strongly suggested that the compound was an artefact formed during this process, osthol or osthol hydrate (Ib) being possible precursors. On mechanistic grounds osthol was not seriously considered, and indeed it was recovered unchanged when treated under conditions similar to those employed in the work-up of the extraction.

TABLE 1

THE CONSTITUENTS OF F. PUBESCENS

Substance	Bark (%)	Leaves (%)	Wood (%)
Sitosterol	0·001	0·006	—
Osthol	0·34	—	—
Sesamin	0·008	—	—
Dictamnine	Trace	—	—
Flindersiamine	Trace	—	—
Kokusaginine	—	0·004	Trace
Maculosidine	—	0·005	—
Skimmianine	Trace	—	—
$C_{16}H_{15}O_2N$, m.p. 138 °C ..	Trace	—	Trace

TABLE 2

THE CONSTITUENTS OF F. SCHOTTIANA

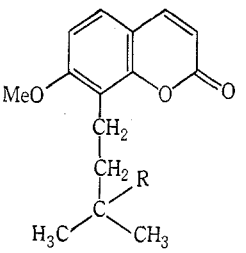
Substance	Bark (%)	Leaves (%)	Wood (%)
Sitosterol	0·02	0·0003	0·004
Osthol	0·4	0·0004	0·0001
Osthol hydrochloride	0·06	—	—
Kokusaginine	0·0004	0·005	—
Maculine	0·009	0·008	0·002

Osthol hydrate (Ib) seemed a more likely precursor, and so its synthesis was examined. Treatment of osthol hydrochloride with silver acetate gave a non-crystalline acetate, but attempted hydrolysis of this yielded mainly osthol by an elimination process. However, it was eventually found that hydrolysis of the corresponding trifluoroacetate under mild conditions yielded osthol hydrate, together with much osthol which was readily separated by chromatography.

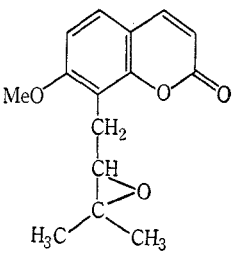
Osthol hydrate crystallized as a monohydrate, the anhydrous material being a gum for which a satisfactory analysis could not be obtained. However, its structure was confirmed by its conversion to osthol hydrochloride on treatment with dry hydrogen chloride. On the other hand, osthol hydrate was recovered completely unchanged after being shaken in ethereal solution with 5% hydro-

chloric acid for 15 hr. It therefore seems unlikely that the hydrate was the precursor of the hydrochloride in *F. schottiana*.

In a further attempt to elucidate the origin of osthol hydrochloride, two more samples of *F. schottiana* bark were extracted. In one, the constituents were separated by means of chromatography, without recourse to acid treatment, while in the other 5% hydrochloric acid was again used for the initial separation of alkaloids. In neither case was the presence of osthol hydrochloride or osthol hydrate detected. Although osthol was isolated from both specimens, the question whether osthol hydrochloride is an artefact therefore remains open.



(Ia; R = Cl)
(Ib; R = OH)



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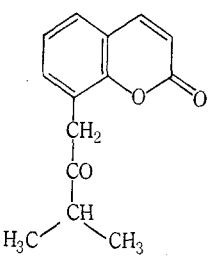

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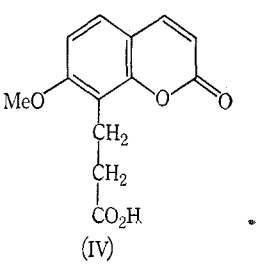

(IV)

Other routes to osthol hydrate tentatively explored involved osthol epoxide (II) and homo-ostholic acid (IV), preparations of which are reported here for the first time. (+)-Osthol epoxide occurs in orange peel oil as aurapten (Böhme and Pietsch 1939). The (±)-epoxide was prepared by selective epoxidation of osthol. However, while it was expected that reductive fission of the oxirane would give the tertiary alcohol (Newman 1956), catalytic hydrogenation and chemical methods were either ineffective or, under more forcing conditions, produced attack on the lactone ring ; on acid treatment the known ketone (III) was formed. Homo-ostholic acid was prepared by Arndt-Eistert homologation of ostholic acid. The reaction of the derived acid chloride with dimethylcadmium or methylmagnesium iodide gave unsatisfactory results, and the work was discontinued when the more direct route to the hydrate was developed.

III. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Ultraviolet spectra were measured in purified ethanol on a Hilger Uvispek. Infrared spectra of substances as mulls in paraffin were recorded on a Perkin-Elmer Infracord 137. Analyses were performed by Miss B. Stevenson of these Laboratories, and by the C.S.I.R.O. and the University of Melbourne Microanalytical Laboratories, Melbourne. The general procedure for extraction and isolation that was followed has been outlined in Part XII (Ritchie, Taylor, and Willcocks 1960). Substances isolated were identified by direct comparison (mixed m.p.'s and infrared spectra) with authentic specimens.

(*a*) *Extraction of the Bark of* F. pubescens.—The dried milled bark (26 kg) (S.N.5937, collected at Danbulla, north Queensland) was exhausted by percolation at room temperature in turn with light petroleum, ether, acetone, and methanol. The concentrated light petroleum extract deposited osthol (86 g) on standing. The alkaloids isolated with 5% HCl from the light petroleum and ether extracts (2 g and 0·5 g respectively) were combined, as were those from the acetone and methanol (0·5 g each). The alkaloids were separated by repeated chromatography on alumina, giving pure specimens of dictamnine (10 mg), flindersiamine (5 mg), and skimmianine (15 mg); a fraction of constant m.p. 186 °C proved to be bourjotine since, on careful chromatography on a large amount of alumina, flindersiamine, and skimmianine could be partly separated. The mother liquors of the bourjotine crystallization on further chromatography yielded a new *base* (0·3 g), m.p. 138 °C, after repeated crystallizations from aqueous methanol and then cyclohexane (Found: C, 76·4; H, 6·0; N, 5·9; O, 12·2%. Calc. for $C_{16}H_{15}O_2N$: C, 76·0; H, 5·9; N, 5·5; O, 12·7%). Light absorption: $\lambda_{max.}$ 215, 248, 317, and 337 mμ, log ε 4·6, 4·64, 4·24, and 4·29 respectively.

Paper chromatography of mother liquors did not indicate the presence of alkaloids other than those isolated. The bases were run on paper buffered to pH 2 with citric acid, with cyclohexane–benzene (40 : 60) as the mobile phase. Detection was by means of ultraviolet light. Typical R_F values (circular chromatography) are as follows:

Flindersiamine	0·76	Skimmianine	0·68	Maculosidine	0·62
Maculine	0·89	Dictamnine	0·92	Kokusaginine	0·63
Maculosine	0·00	$C_{16}H_{15}O_2N$, m.p. 138 °C			0·43

Negligible acidic and phenolic fractions were obtained.

Hot saponification of the neutral fraction from the light petroleum extract gave a neutral fraction from which (+)-sesamin (2 g) was obtained by crystallization from methanol, m.p. and mixed m.p. 125 °C (Found: C, 67·5; H, 5·3%; OCH_3, nil. Calc. for $C_{20}H_{18}O_6$: C, 67·7; H, 5·1%), $[\alpha]_D$ +48·2° (*c*, 0·98) (lit. +68°, indicating possibly slight racemization). Chromatography of the mother liquors on alumina yielded sitosterol (0·2 g). Likewise, the ether extract gave sesamin (0·1 g); the acetone extract, osthol (0·5 g); the methanol extract, osthol (0·2 g), sesamin (0·1 g), and sitosterol (0·1 g).

(*b*) *Extraction of the Leaves of* F. pubescens.—The leaves (15·1 kg) were extracted successively with light petroleum, ether, acetone, and methanol. The ether extract yielded sitosterol (1 g) and maculosidine (2·5 g) and kokusaginine (0·25 g). No crystalline material was isolated from the light petroleum, acetone, or methanol extracts.

(*c*) *Extraction of the Wood of* F. pubescens.—The wood (12 kg) was treated as in (*a*) above. The combined alkaloid fraction gave kokusaginine (0·2 g) and the $C_{16}H_{15}O_2N$ alkaloid isolated from the bark (0·1 g).

(*d*) *The Extraction of the Bark of* F. schottiana.—The bark (11·5 kg) (S.N.5887 collected at Whian Whian, N.S.W.) was treated as in (*a*) above.

The concentrated light petroleum extract deposited osthol (49 g) on standing; the combined light petroleum and ether alkaloid fraction (2·7 g) gave maculine (0·56 g) on chromatography, while the acetone and methanol extracts gave maculine (0·28 g) and kokusaginine (0·042 g).

The light petroleum neutral fraction (40 g) was chromatographed on alumina (800 g). Elution with benzene gave osthol hydrochloride (8·9 g) and ether-eluted sitosterol (2·0 g).

The ether neutral fraction (12 g) yielded osthol (0·5 g) and sitosterol (0·12 g), the acetone neutral fraction (7 g), osthol (0·052 g) and sitosterol (0·03 g), and the methanol extract (7·1 g), osthol (0·03 g).

(*e*) *Extraction of the Leaves of* F. schottiana.—The leaves (14·2 kg) (S.N.5887 and S.N.6019 collected at Whian Whian) were treated as in (*a*) above. The light petroleum extract gave osthol (0·32 g), sitosterol (0·04 g), maculine (0·55 g), and kokusaginine (0·07 g); the ether extract, maculine (0·43 g) and kokusaginine (0·72 g), and the methanol extract, maculine (0·03 g).

(*f*) *Extraction of the Wood of* F. schottiana.—The wood (19 kg) (S.N.5887) was treated as in (*a*) above, the acetone extraction being omitted. The light petroleum extract gave sitosterol (0·6 g) and maculine (0·2 g), the ether extract, osthol (0·03 g) and maculine (0·03 g), and the methanol extract, sitosterol (0·04 g) and maculine (0·04 g).

(*g*) *Osthol Hydrochloride.*—This crystallized as colourless blades from light petroleum, m.p. 98–99 °C (Found: C, 64·3; H, 6·2; O, 17·3; Cl, 12·4; OMe, 10·9%. Calc. for $C_{15}H_{17}O_3Cl$: C, 64·2; H, 6·2; O, 17·1; Cl, 12·5; 1×OMe, 11·0%). It was readily prepared by treating osthol in chloroform with dry hydrogen chloride.

(*h*) *Osthol Hydrate.*—Osthol hydrochloride (0·6 g) in benzene (15 ml) was shaken in the dark with silver trifluoroacetate (0·6 g) for 4 hr. Methyl iodide (1 g) was added to remove the excess silver trifluoroacetate and the mixture filtered. The filtrate was evaporated to dryness to give a clear gum with strong bands in the infrared at 1780 (trifluoroacetate) and 1730 cm^{-1} (coumarin lactone). Hydrolysis was effected by treating the ester in methanol (30 ml) with $NaHCO_3$ (200 mg) in water (2 ml). After 2 days at room temperature the solution was evaporated to dryness *in vacuo* and the residue worked up in the usual way to give a product which was chromatographed on silica gel (40 g). Elution with benzene gave osthol (0·45 g) and benzene–ether (70 : 30) gave osthol hydrate (60 mg). The recovered osthol was recycled to give, eventually, 120 mg of material, needles from ether, m.p. 83–86 °C (Found: C, 64·1; H, 7·1%. Calc. for $C_{15}H_{18}O_4.1H_2O$: C, 64·2; H, 7·1%). On drying *in vacuo* even at room temperature, water of crystallization was lost, to give a clear gum which could not be obtained completely anhydrous (Found: C, 67·6; H, 6·8%. Calc. for $C_{15}H_{18}O_4$: C, 68·6; H, 6·9%). On treatment with dry HCl in chloroform osthol hydrochloride was obtained quantitatively.

A solution of osthol hydrate (65 mg) in ether (25 ml) was shaken with 5% HCl soln. (25 ml) for 15 hr. The product obtained after work up in the usual way gave a negative Beilstein test; it was chromatographed on alumina to give negligible material on elution with benzene, but on elution with benzene–ether (50 : 50), osthol hydrate (55 mg). Similarly, osthol was recovered unchanged after treatment with 5% HCl soln. for 3 days.

(*i*) *Osthol Epoxide.*—Osthol (2 g; 0·00082 mole) in chloroform (30 ml) was kept at 0 °C for 7 days with perbenzoic acid (0·009 mole). Excess peracid was then removed with KI–$Na_2S_2O_3$, and the solution worked up in the usual way to give the epoxide (1·9 g), needles from methanol, m.p. 105–106 °C. For analysis it was sublimed at 195 °C/0·01 mm, giving needles, m.p. 117–118 °C (Found: C, 69·1; H, 6·1%. Calc. for $C_{15}H_{16}O_4$: C, 69·2; H, 6·2%).

Osthol epoxide was inert to mild hydrogenation conditions, such as palladized charcoal, but stronger conditions such as hydrogen in presence of platinic oxide/perchloric acid gave ill-defined products whose spectral properties suggested hydrogenation of the lactone ring. Sodium borohydride at room temperature in refluxing methanol had no effect; lithium aluminium hydride attacked the lactone ring.

(*j*) *Isomerization of Osthol Epoxide.*—The epoxide (0·1 g) was refluxed for 4 hr with 20% H_2SO_4 (20 ml). Extraction with chloroform gave a crude product which was chromatographed on alumina; elution with benzene–ether (99 : 1) gave the ketone (III), needles from water, m.p. 63 °C (lit. m.p. 66 °C).

(*k*) *Homo-ostholic Acid.*—Ostholic acid (1 g) was refluxed with thionyl chloride (10 ml) for 1·5 hr, when excess was distilled off *in vacuo*. The residue was taken up in benzene (15 ml) and the solution concentrated to one-half volume; addition of dry ether gave ostholyl chloride, needles from benzene–ether, m.p. 222–223 °C. An ethereal suspension of the chloride was added slowly with stirring to ethereal diazomethane (80 ml) prepared from *N*-nitrosomethylurea (10 g).

After stirring for 3 hr the precipitated diazoketone was collected and decomposed as follows: A solution of silver benzoate (0·1 g) in dry triethylamine (10 ml) was added dropwise to a solution of the diazoketone in methanol (25 ml) over 0·5 hr at 55 °C. After stirring for a further 1·5 hr and heating to 100 °C for 3 min the solution was charcoaled and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue, in benzene, chromatographed on alumina. Elution with benzene–ether (95 : 5) yielded methyl homo-ostholate (0·7 g), needles from benzene–ether, m.p. 135–136 °C (Found: C, 60·4; H, 5·1%. Calc. for $C_{14}H_{14}O_5$: C, 60·7; H, 4·9%). Hydrolysis of the ester with 3N HCl in dioxan yielded homo-ostholic acid, purified by sublimation at 220 °C/0·4 mm followed by crystallization from methanol-ethyl acetate as plates, m.p. 238–240 °C (Found: C, 62·8; H, 5·0%. Calc. for $C_{13}H_{12}O_5$: C, 62·9; H, 4·9%).

(*l*) *Further Extractions of Bark of* F. schottiana.—(i) The bark (9·1 kg) (S.N.6035) was extracted with light petroleum. The concentrated extract was chromatographed in benzene on alumina (4 kg). The material eluted with benzene (10 l.) was rechromatographed in light petroleum on alumina; elution with benzene gave a mixture of coumarins and alkaloids which were separated by chromatography on silica gel, elution with benzene giving osthol (25 g, 0·3%). Thorough examination of all mother liquors did not reveal the presence of any osthol hydrochloride or osthol hydrate.

(ii) The bark (8·6 kg) (S.N.6019) was extracted as in (*a*) above to give osthol (24 g, 0·27%).

IV. Acknowledgments

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