Structure of Podecdysone A, a Steroid with Moulting Hormone Activity from the Bark of *Podocarpus elatus* R.Br.

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THE bark of the tree *Podocarpus elatus* R.Br. contains besides crustecdysone (0·05%) (already reported[1] as a constituent of the wood) much smaller amounts of three other active compounds, podecdysones A, B, and C.[2] We now propose structure (I) for podecdysone A. This structure, based on the carbon skeleton of β-sitosterol, is of particular interest because of the widespread occurrence of C(24)-alkylated sterols in plants. In the *Calliphora* bioassay, podecdysone A shows moulting hormone activity equal to that of crustecdysone.

The molecular formula of podecdysone A (I), $C_{29}H_{48}O_7$, m.p. 262—264° (decomp), λ_{max} (ethanol) 243 mμ (ε 14,000), ν_{max} (KBr) 3470 and 1650 cm.$^{-1}$, was established by microanalysis and the appearance fo the M^+ peak at *m/e* 508 in the mass spectrum (direct inlet).

Brief acetylation of podecdysone A with pyridine–acetic anhydride at room temperature[3] gave a 2-acetate [(II), m.p. 145—147°)] which on oxidation with periodic acid afforded the same methyl ketone (III) as that obtained by periodic acid oxidation of crustecdysone 2-acetate (IV).[4] Podecdysone A thus has the same tetracyclic nucleus as crustecdysone. The C(22)-hydroxy-group of podecdysone A undergoes acetylation, but the rate (rate constant $k = 0{\cdot}2 \times 10^{-2}$min.$^{-1}$) was less than that of the 22-hydroxy-group of crustecdysone ($k = 0{\cdot}5 \times 10^{-2}$min.$^{-1}$)[3] indicating steric hindrance by an additional side-chain substituent.

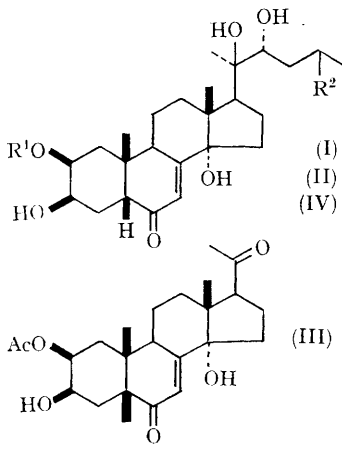
Chemical shifts of methyl resonances (δ)

Compound	Solvent	C(18)	C(19)	C(21)	C(26)	C(27)	C(26), C(27)	C(29)
Podecdysone A	[$^2H_5$]Pyridine	1·21	1·07	1·55	1·39	1·26	av. 1·33	1·10*
Crustecdysone	,,	1·22	1·08	1·59			1·37	
Podecdysone A	[$^2H_4$]Methanol	0·90	0·96	1·23	1·23	1·11	av. 1·17	1·00*
Crustecdysone	,,	0·88	0·95	1·19			1·19	

* (d, *J* 5 c./sec.)

In its mass spectrum podecdysone A has prominent ions at *m/e* 363 and 345, characteristic of easy 20,22-diol cleavage,[5] and a weak peak at *m/e* 508 which is assigned to the parent ion. This assignment is supported by the observation that the prominent ions attributed to the side-chain fragments (*m/e* 127 and 109) are exactly 28·032 mass units (C_2H_4) higher than the corresponding ions (*m/e* 81 and 99) in the spectrum of crustecdysone.

When the n.m.r. spectra of podecdysone A in two solvents are compared with those of crustecdysone (see Table), it is seen that the signals ascribed to the C(25)- and C(26)-methyl groups of podecdysone A are in contrast to those in crustecdysone non-equivalent, indicating the close proximity of an asymmetric centre. The above evidence indicates that podecdysone A differs from crustecdysone in having an ethyl group in its side-chain. The signal attributed to the methyl protons of the ethyl group appears in the n.m.r. spectrum as a broad doublet (*J* 5 c./sec.), doubtless due to virtual coupling. This signal was collapsed to a singlet by irradiation at δ 1·73. The ethyl group can be placed only at C(23) or C(24), and from the magnitude of the non-equivalence (0·12 p.p.m.) of the C(26)- and C(27)-methyl-groups, it is concluded that podecdysone A has the ethyl substituent at C(24). This assignment is the most likely on biogenetic grounds, as the phytosterols commonly carry a C(24)-alkyl substituent (see for example ref. 6). Since β-sitosterol is the major sterol of the barks of conifers,[7] it is likely that podecdysone A is synthesised from this sterol and that its 24-ethyl group has the same α-configuration (I).



(I) R^1=H, R^2=Et
(II) R^1=Ac, R^2=Et
(IV) R^1=Ac, R^2=H

(III)

The isolation of a β-sitosterol analogue of crustecdysone is of particular interest since phytosterol analogues might be expected to predominate in plants. In fact, crustecdysone or other C(27) ecdysones appear to be the main active compounds present in plants and arthropods. Early experiments[8] suggest that crustecdysone may be synthesised in plants from cholesterol rather than β-sitosterol. It is also of interest that phytophagous insects synthesise C(27) ecdysones[9] presumably from C(29) sterols.[10]

Professor K. Nakanishi, Tohoku University, has kindly informed us that both he and Professor T. Takemoto of the same University have independently isolated compounds (respectively makisterone-C and lemmasterone) from plant sources, to which they have assigned structures (side-chain configurations undefined) the same as that derived above for podecdysone A.

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