The Structure of Podecdysone B, a New Phytoecdysone

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EARLIER WE have reported[1] the isolation of podecdysones A, B, and C, three new steroids with insect moulting hormone activity from an extract of the bark of *Podocarpus elatus* R.Br., and suggested structure (I) for podecdysone A. We have since isolated three other compounds podecdysones D, E, and F from the same extract, and suggest structure (II) for podecdysone B. Comparison of podecdysone D with an authentic sample† of makisterone A (III), isolated[2] from *P. macrophyllus*, has shown that they are identical.

In the *Calliphora* bioassay for moulting hormones, podecdysone B shows about one-fifth of the activity[3] of crustecdysone. This relatively high biological activity is noteworthy, since unlike all the other active compounds reported previously, podecdysone B lacks a 7-en-6-one grouping. However, its activity may be due to *in vivo* isomerisation to such a grouping. A structure analogous to that suggested above for podecdysone B has been assigned[4] to one of the products (IV) obtained by treatment of ecdysone (V) with acid, and in fact podecdysone B may be biosynthesised from crustecdysone (VI), another constituent[5] of the extract, by dehydration.

Podecdysone B, m.p. 125—127 [λ_{max} (ethanol) 244 nm. (ϵ 13,200), ν_{max} (KBr) 3490, 1705, 1650 cm.$^{-1}$] has the molecular formula $C_{27}H_{42}O_6$ [microanalysis and mass spectral peak at *m/e* 444 ($M^+ - H_2O$)]. The mass spectrum also shows peaks due to loss of two further molecules of water, and peaks at *m/e* 99 and 81, ascribed to fragmentation of the side-chain between C-20 and C-22 as with

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TABLE 1

Chemical shifts of protons (δ)

Compound	Solvent	C-7	C-15	C-18	C-19	C-21	C-26/27
Podecdysone B	[$^2H_5$]Pyridine	—	—	1·35	1·08	1·55	1·43
Crustecydsone		—	—	1·22	1·08	1·59	1·37
Podecdysone B	[$^2H_4$]Methanol	—	5·38	1·04	0·98	1·23	1·17
Crustecdysone		5·77	—	0·88	0·95	1·19	1·19

TABLE 2

Properties of dehydration products

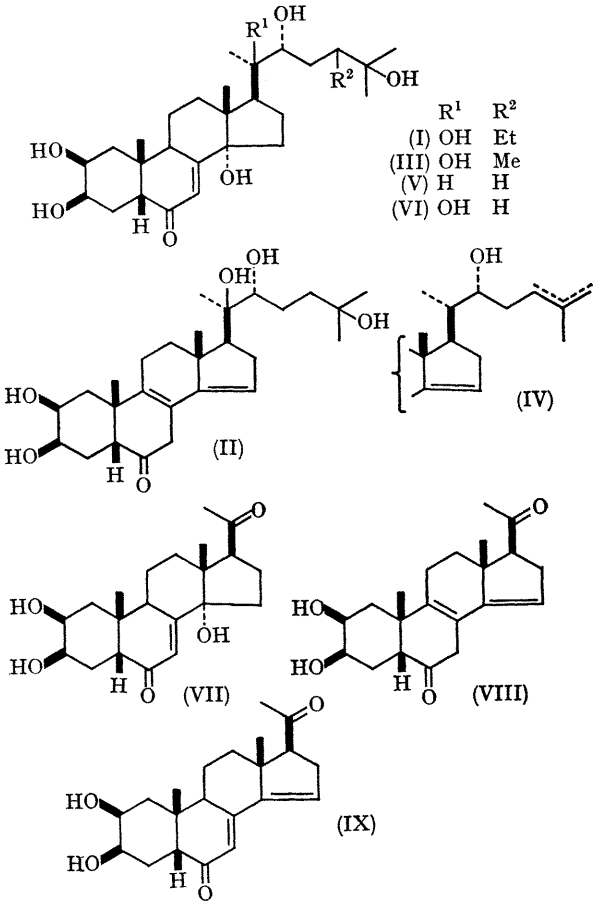
Compound	λ_{max} (ethanol)	ϵ_{max}	N.m.r. in deuteriochloroform (δ)	ν_{max} (KBr)
(VIII)	246 nm.	11,900	0·75, 1·01, 2·15 (each 3H) 5·35 (1H)	3500 1705, 1650 cm.$^{-1}$
(IX)	296 nm.	13,000	1·09, 1·11, 2·15 (each 3H) 5·62, 6·14 (each 1H)	3500, 1705 1660 ,1630, 1590 cm.$^{-1}$

crustecdysone.[6] Peaks at *m/e* 344 and 327 can be attributed to the tetracyclic fragments from this cleavage, and a peak at *m/e* 300 to C-17–C-20 fission. The i.r. and u.v. data agree well with those reported for compound (IV) [λ_{max} (ethanol) 244 nm. (ε 15,400), ν_{max} 1708, 1660 cm.$^{-1}$].

The methyl signals in the n.m.r. spectra of podecdysone B are similar in chemical shift to those of crustecdysone (Table 1), except that the signal ascribed to the C-18 methyl is at lower field (0·13—0·16 p.p.m.) than that in the spectrum of crustecdysone. In deuteriomethanol solution the vinylic C-15 proton occurs as a broad multiplet at δ 5·38, and no other vinyl proton absorption is present.

The above structure was confirmed as follows: the known[7] methyl ketone (VII) was heated under reflux in 0·5N-ethanolic HCl and the product chromatographed to yield two main products, which are assigned structures (VIII) and (IX) on the basis of their spectra (Table 2) and by analogy with the structures assigned[4] to the acid dehydration products of ecdysone. Selective acetylation[8] of podecdysone B to the 2-acetate and oxidation with periodic acid gave an acetoxymethyl ketone, which on hydrolysis with potassium hydrogen carbonate afforded a diol-dione Comparison of spectral properties (i.r., u.v., n.m.r.) and t.l.c. behaviour in several solvents showed that this product was identical with compound (VIII). The mass spectra (M^+ peak at *m/e* 344) of the two samples were also identical.

It is unlikely that podecdysone B is an artefact formed by dehydration of crustecdysone during work-up, because the compounds with the 7,14-dien-6-one structure, expected to accompany podecdysone B if dehydration[4] had occurred during isolation, were not evident. Also compounds formed by dehydration of other labile tertiary hydroxy-groups could not be detected in the extract. An attempt to resolve this question by extracting another batch of *P. elatus* bark under very mild conditions resulted instead in the isolation of a different compound, podecdysone E, with very similar chromatographic properties to podecdysone B. Evidently *P. elatus* exhibits marked phytochemical variability, as a third sample of the bark, collected from young trees, yielded no phytoecdysones at all.



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