Insect Hormones. The Structure of Ponasterone A, an Insect-moulting Hormone from the Leaves of *Podocarpus nakaii* Hay

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THE isolation[1] and determination of the structure[2,3] of ecdysone (IIa), 2β,3β,14α,22β_F,25-pentahydroxy-5β-cholest-7-en-6-one, the insect-moulting hormone, has aroused great interest leading to announcements of syntheses by two groups.[4,5] Furthermore, isolations of 20-hydroxyecdysone (IIb)[6] from silk worm[7] (ecdysterone),[8] crayfish[9] and oak-silk moth (crustecdysone)[10] have been reported. We have isolated four active substances, ponasterones -A, -B, -C, and -D from *Podocarpus nakaii* Hay (collected in Taiwan; "togariba-maki" in Japanese) and propose structure (Ia) for ponasterone A.† The aqueous layer that separated after addition of water to the concentrated ethanol extract of 4·8 kg. of dried leaves was first extracted with chloroform to give a ponasterone-A and -B mixture, and then with ethyl acetate to give a ponasterone-C and -D mixture. Both mixtures were separated by silica gel chromatography, and the respective compounds were recrystallized from ethanol to give 2 g. of ponasterone-A, 50 mg. of ponasterone-B, 500 mg. of ponasterone-C, and 20 mg. of ponasterone-D, all crystalline excepting ponasterone-B.

The molecular formula of ponasterone-A, $C_{27}H_{44}O_6$, m.p. 259—260° (decomp.), $[\alpha]_D^{15}$ + 90° (MeOH), i.r. (KBr) 3420, 1643 cm.$^{-1}$, u.v. (MeOH) 244 (12, 400),326 mμ (ϵ 130), was established by microanalyses and the appearance of the M^+ peak at *m/e* 544 in the mass spectrum (direct inlet

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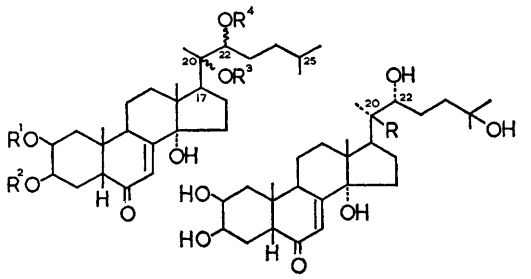
† The planar structure corresponds to 25-deoxyecdysterone, but the name "ponasterone" is retained since configurations of the side-chain hydroxyls are still unknown.

(Ia: $R^1 = R^2 = R^3 = R^4 = H$)
(Ib: $R^1 = R^2 = R^4 = Ac$; $R^3 = H$)
(Ic: $R^1 = R^2 = H$; R^3, R^4 = acetonide)
(Id: R^1, $R^2 = R^3$, R^4 = acetonide)
(Ie: $R^1 = R^2 = Ac$; R^3, R^4 = acetonide)

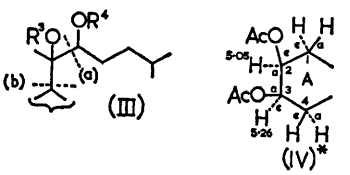


(IIa: R = H)
[IIb: R = OH (C-20 and C-22 configurations undefined)]

system) of its diacetonide (Id). Preparation of the following derivatives established the presence of four hydroxy-groups: triacetate (Ib), m.p. 119—120°, monoacetonide (Ic), m.p. 195—197° (decomp.), diacetonide (Id) m.p. 193—195°, and a monoacetonide diacetate (Ie), m.p. 157—159°. However, a fifth hydroxyl must be present because of the OH absorption in the i.r. spectra of the diacetonide (Id); moreover, the n.m.r. spectra of (Ia)—(Ie) indicated this hydroxyl to be tertiary. Of the five hydroxyls, four constitute two α-glycol systems, as shown by the consumption of 1·9 moles of sodium periodate by ponasterone-A.

Evidence for the 20,22-dihydroxy-steroid side-chain (III) was first obtained from mass spectral data: (i) ponasterone-A (III: $R^3 = R^4 = H$) had peaks at *m/e* 83 (25% intensity relative to *m/e* 18 base peak, 20 ev;‡ *a*-fission), $M - [83 + H + 18 + 18]$ (= 344, 40%), and at $M - [145(b\text{-fission}) + H + 18]$ (= 300, 42%); (ii) ponasterone-A diacetonide (III: R^3 and R^4 = acetonide) and ponasterone-A dodecadeuteriodiacetonide showed peaks due to *b*-fission at *m/e* 185 (30% relative to *m/e* 18, base peak, 70 ev) and 191 (28%), respectively. The side-chain structure was established by sodium metaperiodate oxidation of ponasterone-A to give isohexanal, 2,4-dinitrophenylhydrazone, m.p. 99°, and a noncrystalline "methyl ketone" (positive iodoform test).

Structures of the moieties (IV) and (V) were deduced from the 100 Mc./sec. n.m.r. spectra, measured by Dr. M. C. Woods from Varian, of the triacetate (Ib) (in hexadeuterioacetone) with the aid of decoupling. The presence of the partial structures (III), (IV) (OH instead of OAc) and (V), coupled with the C_{27} molecular formula and the presence of five Me peaks in the n.m.r. spectra (see Table) indicated that ponasterone-A is a steroid. Obviously partial structure (IV) can only be placed in ring A of a steroid skeleton. On the other hand, the αβ-unsaturated ketone moiety can be fitted into a steroid nucleus either as indicated in (V) or in (VI) (steroid numbering). Partial structure (V) requires the t-hydroxyl to be placed at C-14, whereas partial structure (VI) requires it to be placed at C-9. That the former was the case was indicated by treatment of ponasterone-A with MeOH–HCl, which resulted in the disappearance of starting material (checked by t.l.c.) and the formation of products having u.v. spectra maxima at 294 mμ and 241 mμ, the two maxima being assigned to the $\Delta^{7,14(15)}$-6-one and $\Delta^{8,14(15)}$-6-one chromophores, respectively. The 18-Me chemical shift (see Table) further showed that the 14-OH had an α-configuration.

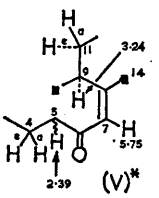


(III)

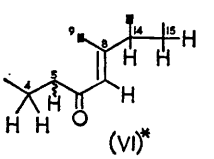
(IV)*

J (c./sec.)	
2a, 1a	12·0
2a, 1e	5·0
2a, 3e	3·0
3e, 4a	5·0
3e, 4e	4·0

J (c./sec.)	
7, 9	3·0
9, 11a	7·5
9, 11β	11·0
5, 4a	13·5
5, 4e	6·5
7, 5	<0·1



(V)*



(VI)*

(black squares denote carbons with no protons)

* N.m.r. data in hexadeuterioacetone, p.p.m. from internal tetramethylsilane.

Finally, the n.m.r. data pertaining to the ring A α-glycol group (IV) can be accounted for by either a 2β,3β-dihydroxy-5β-H structure (A/B *cis*) or a 2α,3α-dihydroxy-5α-H structure (A/B *trans*). The remarkable coincidence of the chemical shifts of the 19-methyl groups in (Ia) and (IIa)/(IIb) (see Table) suggested an A/B *cis*-arrangement; this was confirmed by the positive Cotton effect (a = 68, in dioxan) exhibited in the rotatory dispersion curve of ponasterone-A, which was almost superimposable on the rotatory dispersion curve of 22-isoecdysone.[11] In contrast, the amplitudes of A/B *trans* compounds are *ca.* 140.[11] The chemical shifts of other Me groups

‡ The peak intensity was 90% (relative to *m/e* 44, base peak) at 70 ev.

TABLE

Me chemical shifts (pyridine solution)

	C-18	C-19	C-21	C-26/27
Ecdysone (IIa)[2]	0·73	1·07	1·28	1·38
Crustecdysone (IIb)[9]	1·20	1·07	1·56	1·38
Ecdysterone (IIb)[8]	1·19	1·06	1·55	1·34
Ponasterone A (Ia)	1·16	1·03	1·51	0·82 (d, J = 6)

shown in the Table are in complete accord with structure (Ia) for ponasterone-A.

Tests with *Samia cynthia* and *Calliphora*[12] show that the activities of all four ponasterones are of the same order as that of 20-hydroxyecdysone. The ready isolation of insect-moulting hormones from plants,[13] in contrast to the extremely poor yield from insects and other sources, *viz.*, 70 mg. or less from 1 ton of material, makes it possible to supply large amounts of active substances for biological testing. The structures of the other three active constituents, which are more highly hydroxylated compounds as compared to ponasterone-A, are under investigation.

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[11] Personal communication from Dr. J. H. Fried, Syntex Corporation, to whom we are greatly indebted for providing this information and for furnishing us with rotatory dispersion data for ecdysone and related compounds.
[12] We thank the Takeda Chemical Industries, Osaka (*Samia cynthia*) and Dr. D. H. S. Horn, C.S.I.R.O., Melbourne (*Calliphora*) for carrying out these tests.
[13] Professor T. Takemoto, Department of Pharmacy, Tohoku University, has informed us of his isolation of inokosterone and iso-inokosterone, presumably 2,3,14,20,22,26-hexahydroxycholest-7-en-6-one and ecdysterone, respectively, from the crude drug *Achyranthis radix*.