5β-Hydroxy-ecdysones and a Revision of the Structure of Ponasterone C

By MASATO KOREEDA and KOJI NAKANISHI*†
(*Department of Chemistry, Tohoku University, Sendai, Japan*)

Summary Some of the spectral properties characteristic of the 5β-hydroxy-ecdysones are described and the structure of ponasterone C has been revised to (V); ponasterone B (I) is the only ecdysone having 2α,3α-dihydroxy-groups.

THE structures[1] of ponasterones B (I) and C (II), which together with ponasterone A (III)[2] belong to one of the first groups of phytoecdysones (from *Podocarpus nakaii* Hay.) have been re-investigated: (i) as these were the only ecdysones with the atypical 2α,3α-dihydroxy-groups among the nearly 30 ecdysones characterized to date; and (ii) because the optical and m.s. data of ponasterone C was very similar to those of the recently isolated ajugasterone A (from *Ajuga decumbens*),[3] which turned out to be polypodine B (IV),[4] a 5β-OH hydroxy-ecdysone.

High-resolution m.s.‡ of ponasterone C with peaks at 478 (M − 18) and 460 (M − 36) indicated a molecular formula of $C_{27}H_{44}O_8$. The series of peaks‡ at m/e 379, 361, 343, and 325 (C-20/C-22 fission followed by losses of 18, *cf.* V), also present in polypodine B and sengosterone[5] (another 5β-hydroxy-ecdysone), were in contrast to the m/e 363, 345, and 327 peaks exhibited by the more common ecdysones with only three nuclear hydroxy-groups, *e.g.*, ponasterone A (III), and demonstrated that an extra hydroxy-group is present on the nucleus. This extra group is 5β for the following reasons: (i) The olefinic 7-H n.m.r. signal (5.17 p.p.m., d, 2·5 Hz, in deuteriopyridine) was only coupled to the 9α-H signal, whereas in the common ecdysones an additional 7-H/5β-H long-range coupling is invariably observed; (ii) The 2-H, 3-H, and 19-H n.m.r. signals of ponasterone C were very similar to those of polypodine B; (iii) A 7% increase in the 2-H n.m.r. signal area resulted upon irradiation of the 9α-H signal (Nuclear Overhauser Effect) in ponasterone C 2,3,22,24-tetra-acetate (*cf.* VI); (iv) The c.d. data of ponasterone C and polypodine B benzoates (Table 1) indicated that the C-2 and C-3 hydroxy-groups adopt a 'negative' chirality[7] (*cf.* VI). Only partial structure (VI)[6] can account for experimental results

TABLE 1. *C.d. of benzoates (in ethanol)*

Benzoates	nm	Δε
Polypodine B 2,3,22,25-tetra- ..	236	−14·6
	220	+14·4
Ponasterone C 2,3,22,24-tetra- ..	236	− 8·2[a]
	222	+12·8
Ponasterone B 2,3-di-	236	−16·4
	221	+12·1

[a] This smaller than average[7] value is due to interaction between the side-chain 1,3-dibenzoates.

† Present address: Department of Chemistry, Columbia University, New York, N.Y. 10027.

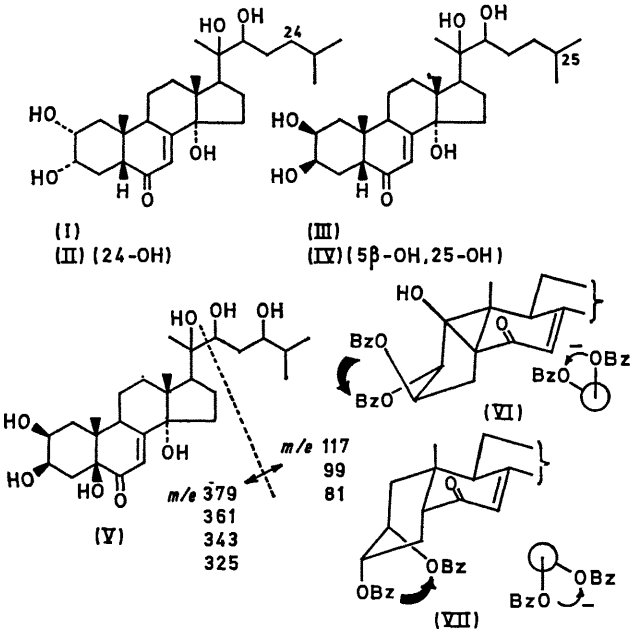
(iii) and (iv), a conclusion which is supported by preparation of a polypodine B 3,5-carbonate.[8]

The c.d. Cotton effects due to the ring B enone system (Table 2) is worthy of mention. Thus, the A/B *cis* ring-junction can be readily distinguished from the *trans*-fused system by the smaller amplitudes of the two Cotton effects.

TABLE 2. *C.d. of ring B enone (in dioxan)*

	$\pi \rightarrow \pi^*$		$n \rightarrow \pi^*$	
	nm	$\Delta\epsilon$	nm	$\Delta\epsilon$
General for 14α-OH-7-en-6-one				
A/B *cis*	242	− 3·5	340	+1·6
A/B *trans*	240	− 7·1	340	+3·3
Polypodine B	251	− 5·74	326	+2·64
Ponasterone C	251	− 5·44	324	+3·00
3β-Acetoxy-5α-hydroxyergosta-7,22-dien-6-one[a] ..	245	−12·72	349	+3·55

[a] Prepared according to A. Burawoy, *J. Chem. Soc.*, 1937, 409.



Presence of a 5β-hydroxy-group shifts the positions of both the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ Cotton-effect peaks; the same tendency has been encountered in sengosterone[5] as well. Thus, the c.d. data in Table 2 should be of diagnostic value for the characterization of further unknown ecdysones. Ponasterone C is thus represented by structure (V).

Mass spectral data of ponasterone B confirmed that only three nuclear hydroxy-groups were present in this ecdysone; this was corroborated by the M^+ peak‡ at *m/e* 504 ($C_{30}H_{48}O_6$) of its 20,22-monoacetonide. One of the C-2 or C-3 hydrogens must be equatorial and the other axial as judged from the half-band-width of 7·5 Hz and 20 Hz for these protons in the n.m.r. of ponasterone B 2,3,22-triacetate. Finally, the chirality of the 2,3-dibenzoate grouping is left-handed (Table 1). The only arrangements of hydroxy-groups satisfying these criteria are 2β,3β or 2α,3α (and ring A chair conformation), but since ponasterone A (III) is represented by 2β,3β,[2] ponasterone B is 2α,3α, and therefore the sole ecdysone having this moiety. Apparently, configurations of the C-2 and C-3 hydroxy-groups do not greatly influence the moulting hormone activity as the activity of ponasterone B is comparable to those of other ecdysones.

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