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INSECT HORMONES. V. THE STRUCTURES OF PONASTERONES B AND C[1)*]

K. Nakanishi and M. Koreeda

(Department of Chemistry, Tohoku University, Sendai, Japan)

M. L. Chang and H. Y. Hsu

(Bristol Research Institute of Taiwan, Ltd., Taipei, Taiwan)

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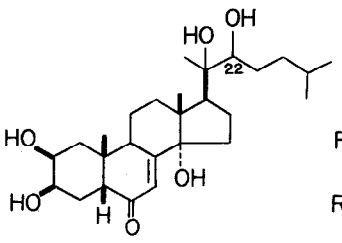
The dried leaves (6 kg) of Podocarpus Nakaii HAY. afford roughly 9.5 g of ponasterone A, 0.4 g of B, 0.1 g of C and a smaller amount of D, i.e., ca 0.2% total yield. These poly-hydroxylated steroids[2)] exhibit strong moulting hormone activity[3, 4)], and constitute the first isolation of such compounds from plant sources. Independently, Takemoto and co-workers have also isolated ecdysterone and inokosterone from plant sources[5)]; isolation of such active compounds from plants has subsequently been reported by several workers[6)], and it now appears that their occurrence is quite widespread. Following the structure determination of ponasterone A (1)[2)], we derive structures 2 and 3, respectively, for ponasterone B and C.

In contrast to the active steroids isolated so far, i.e., ecdysone[7)], 20-hydroxyecdysone[9)] (crustecdysone[8)] or ecdysterone[10)]), ponasterone A[2, 11)], inokosterone[5)], cyasterone[12)], 20,26-dihydroxyecdysone[13)], which all possess 2β,3β-hydroxyl groups, ponasterones B and C have 2α,3α-hydroxyl groups.

The high biological activity[3, 4)] of ponasterones B and C indicates that the ring A hydroxyl configurations can be varied in addition to the side-chain structure for manifestation of moulting hormone activity. The co-occurrence of 2β,3β- and 2α,3α-hydroxy steroids from the same plant is also of interest.



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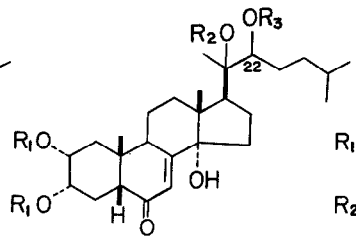
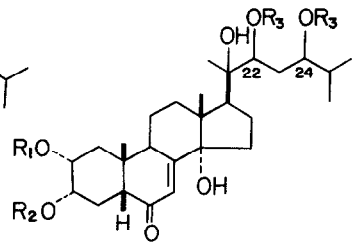
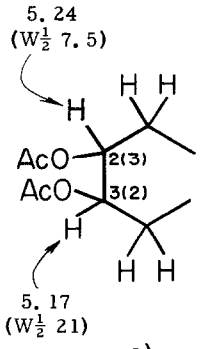


2 $R_1=R_2=R_3=H$
6 $R_1=R_3=Ac$, $R_2=H$
7 $R_1=H$, R_2/R_3=acetonide

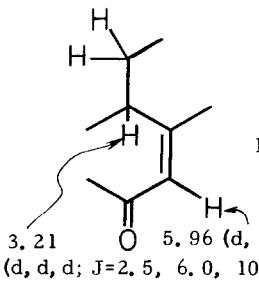


3 $R_1=R_2=R_3=H$
4 R_1, R_2=H, Ac, R_3=Ac
5 $R_1=R_2=R_3=Ac$

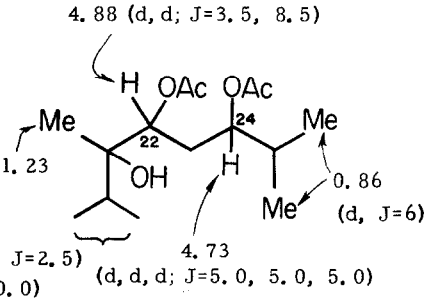
Ponasterone C (3): $C_{27}H_{44}O_7$, m. p. 270-272° (dec.), IR (KBr), 3375, 1668, 1626 cm^{-1}; UV (MeOH), 244 mμ (ε 11,000), 326 mμ (ε 100). Similar to the case of ponasterone A[2], NMR measurements[14] of the triacetate 4 and tetraacetate 5 indicated presence of the moieties I, II and III:



I[a)]



II[b)]



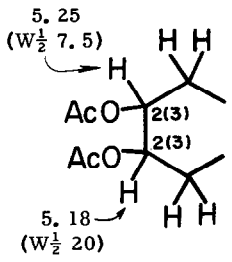
III[b)]

a: NMR data of tetraacetate 5 in $CDCl_3$, 100 Mc
b: NMR data of triacetate 4 in $CDCl_3$, 100 Mc
half-band width ($W_{\frac{1}{2}}$) and J are in cps

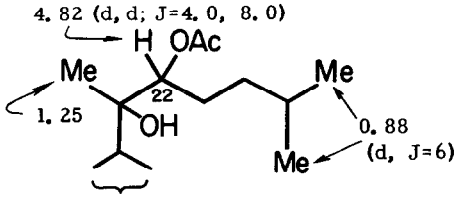
The side-chain structure was established by sodium metaperiodate oxidation of ponasterone C to give trans-isohexenal, which was identified through its 2,4-dinitrophenylhydrazone, m. p. 175°, UV ($CHCl_3$) 372 mμ, M^+ at m/e 278 (spectral data and mixed m. p. with synthetic sample); dehydration had occurred after oxidative cleavage of the α-glycol linkage.

The remaining tert-hydroxyl group was placed at C-14 since, similarly to ecdysone[7a] and ponasterone A[2], heating of ponasterone C at 80° in MeOH-HCl gave rise to two products (checked by TLC) having UV maxima at 242 mμ (8, 14-diene) and 294 mμ (7, 14-diene-6-one), the former absorption becoming stronger with prolonged heating. Thus the planar structure for ponasterone C (3) is derived.

Ponasterone B (2): IR (KBr), 3400, 1660, 1630 cm^{-1}; UV (MeOH), 241, 320 mμ. Although ponasterone B itself could not be obtained crystalline, it yielded a crystalline triacetate 6, $C_{33}H_{50}O_9$, m. p. 128-130°, and a monoacetonide 7, $C_{30}H_{48}O_6$, m. p. 240-242°, M^+ m/e 504. The great similarity in the mass spectra of the 20, 22-acetonides of ponasterones A and B suggested that they may be merely configurational isomers, a fact which was verified by spectroscopic data, especially the NMR spectrum (100 Mc) of the triacetate 6 in $CDCl_3$[14]. The side-chain structure was again established by periodate oxidation, which as in the case of ponasterone A[2], gave isohexanal, 2, 4-dinitrophenylhydrazone, m. p. 99°. The fifth hydroxyl group was also placed at C-14 on the basis of the conventional MeOH-HCl treatment described above.



IV



V

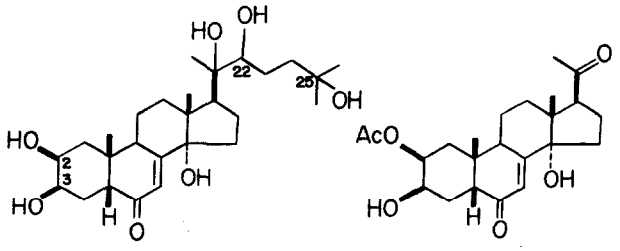
NMR data for triacetate 6 (in $CDCl_3$)

Stereochemistry: The RD curves of the 14α-hydroxy-7-en-6-one system of ecdysone and related compounds exhibit Cotton effects having the following amplitudes (a) (in dioxane)[15]; a comparison with the data of the ponasterones (abbreviated to PN in Table) indicate that the A/B ring juncture of the three ponasterones also belong to the cis series:

	A/B cis	A/B trans	PN-A	PN-B	PN-C
at ca 240 mμ ($\pi-\pi^*$)	-240	-520	-269	-180	-240
at ca 340 mμ ($n-\pi^*$)	+ 60	+140	+ 68	+ 57	+110*

* This amplitude is undecisive, but the amplitude of the $\pi-\pi^*$ band and the close similarity in the NMR data of PN-B and C (see below) leaves no doubt that they have identical configurations at C_2, C_3, C_5, etc.

The α-configuration of the 14-hydroxyl group of ponasterones B and C is based on the close similarity of the chemical shifts of the 18-methyl group to that of ponasterone A. In Table 1 the assignments of the 18- and 19-Me peaks in pyridine are based on the data of ecdysone[7a], ecdysterone[10] and crustecdysone[8] (both 8), while those in $CDCl_3$ are based on a comparison of the data of ponasterone A 2-monoacetate and its periodate cleavage product, the methyl



ecdysterone 8 "methyl ketone" 9

TABLE 1. Methyl chemical shifts of ponasterones and derivatives

	18	19	21	26/27
Ecdysterone (8)[a)10)]	1.19	1.06	1.55	1.34 (s)
Ponasterone A[a)]	1.16	1.03	1.51	0.82 (d, J=6)
Ponasterone B[a)]	1.17	1.11	1.54	0.82 (" ")
Ponasterone C[a)]	1.17	1.12	1.54	1.00 (" ")
Ecdysterone (8) 2,3,22-tri-OAc[b)16)]	0.85	1.02	1.24	1.18, 1.21
Ponasterone A 2-mono-OAc[b)11)]	0.87	0.99	1.20	0.91 (d, J=6)
Methyl ketone 9 derived from above[b)11)]	0.63	0.99	2.15	—— (" ")
Ponasterone A 2,3,22-tri-OAc[b)]	0.85	1.02	1.24	0.88 (" ")
Ponasterone B 2,3,22-tri-OAc (6)[b)]	0.83	0.93	1.25	0.88 (" ")
Ponasterone C 2,3,22,24-tetra-OAc (5)[b)]	0.86	0.93	1.24	0.90 (" ")

a: in pyridine b: in $CDCl_3$

ketone 9[11]. The 0.87 ppm peak in ponasterone A 2-monoacetate is shifted to 0.63 ppm in the methyl ketone 9, whereas the 0.99 ppm peak remains constant; therefore, it is clear that the 0.87 ppm and 0.99 ppm peaks, respectively, should be assigned to the 18- and 19-methyl groups. Assignments of the methyl peaks in ecdysterone[16] and ponasterone acetates then follow unambiguously.

Configurations of the 2- and 3-hydroxyl groups in ponasterones B and C are identical but differ from those of ponasterone A (β, β). This is evident from a comparison of the 19-methyl chemical shifts of ponasterones and derivatives having identical substituents in ring A, i.e., 2,3-dihydroxyls and 2,3-diacetoxyls (Table 1), and also from the following observation. The NMR signal shapes (in $CDCl_3$, 100 Mc), of the overlapping C-2 and C-3 carbinyl protons of ponasterone B triacetate 6 and ponasterone C tetraacetate 5 were practically identical (see partial structures IV and I): on the other hand, the C_2-H and C_3-H signals in ponasterone A 2,3,22-triacetate were clearly separated, and occurred at 5.05 ppm (in $CDCl_3$) (C_2-ax H; d, d, d; J=11.5, 4.5, 3.5) and 5.32 ppm (C_3-eq H; d, d, d; J=4.0, 3.8, 3.5).

Thus the configurations in both ponasterones B and C have to be α, β or β, α or α, α. Presence of an intramolecular hydrogen bonding was detected in the IR spectrum of ponasterone B monoacetonide 7 in dilute CCl_4 solution (0.0001 mole/l), i.e., 3600 cm^{-1} (free C-14 α-OH), 3550 cm^{-1} (free OH), 3485 cm^{-1} (bonded OH). This evidence coupled with the NMR half-band widths of the C-2 and C-3 protons, which suggested that one and only one of them was involved in an ax-ax coupling, was in agreement only with the α, α-configurations (twist-boat or chair conformation for ring A) and excluded the other two possibilities.

Although the absolute configurations at C-20 and C-22 in ecdysterone 8 and the three ponasterones have yet to be established, it is clear that they are identical in view of the similarity in the chemical shifts of C_{18}-Me and C_{21}-Me peaks (Table 1). In support of this the C_{22}-H peaks of the ponasterone and ecdysterone acetates are also very similar (in $CDCl_3$):

PN-A 2,3,22-tri-OAc	4.82 ppm	d, d; J=3.5, 9.0 cps
PN-B 2,3,22-tri-OAc (6)	4.82 ppm	d, d; J=4.0, 8.0 cps (see V)
PN-C 2,3,22,24-tetra-OAc (5)	4.88 ppm*	d, d; J=3.5, 8.5 cps (see III)
Ecdysterone (8) 2,3,22-tri-OAc[12]	4.79 ppm	d, d

* lowering in chemical shift is due to 24-acetoxyl group.

REFERENCES

1) Part IV. S. Imai, S. Fujioka, K. Nakanishi, M. Koreeda and T. Kurokawa, Steroids, November (1967).

*) Reported in part at the 20th Annual Meeting, Chemical Society Japan, April, 1967, Tokyo. This investigation was supported in part by National Institutes of Health, Public Health Service Research Grant No. CA 08394.

2) K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang and H. Y. Hsu, Chem. Comm., 915 (1966).

3) M. Kobayashi, K. Nakanishi and M. Koreeda, Steroids, 9, 529 (1967).

4) H. Hoffmeister, K. Nakanishi, M. Koreeda and H. Y. Hsu, J. Insect. Physiol., in press.

5) T. Takemoto, S. Ogawa and N. Nishimoto, Yakugaku Zasshi, 87, 325 (1967).

6) See Part IV and references cited therein.

7) a]: P. Karlson, H. Hoffmeister, H. Hummel, P. Hocks and G. Spiteller, Chem. Ber., 98, 2394 (1965); b]: R. Huber and W. Hoppe, ibid., 2403.

8) F. Hampshire and D. H. S. Horn, Chem. Comm., 37 (1966).

9) P. Hocks and R. Wiechert, Tetrahedron Letters, 2989 (1966).

10) H. Hoffmeister and H. F. Grutzmacher, Tetrahedron Letters, 4017 (1966).

11) H. Moriyama and K. Nakanishi, following paper.

12) T. Takemoto, Y. Hikino, K. Nomoto and H. Hikino, Tetrahedron Letters, 3191 (1967).

13) M. J. Thompson, J. N. Kaplanis, W. E. Robbins and R. T. Yamamoto, Chem. Comm., 650 (1967).

14) We are grateful to Dr. M. C. Woods (Varian) and Mr. I. Miura for decoupling measurements. Chemical shifts are expressed in ppm from internal TMS.

15) Personal communication from Dr. J. Fried, Syntex Corporation, to whom we are indebted for providing this information and for furnishing us with RD data for ecdysone and related compounds.

16) Assignments of the 18- and 19-methyl peaks are reversed in reference 12.