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THE STRUCTURES OF THREE ADDITIONAL PHYTOECDYSONES FROM PODOCARPUS MACROPHYLLUS, MAKISTERONE B, C AND D*

S. Imai,[a] S. Fujioka,[a] E. Murata,[a] Y. Sasakawa,[a] and K. Nakanishi[b]

[a] (Research and Development Division, Takeda Chemical Industries, Juso, Osaka)

[b] (Department of Chemistry, Tohoku University, Sendai)

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As already reported,[1,2] the dry leaves of Podocarpus macrophyllus D. DON. ("inu-maki") afforded besides ecdysterone (1) (0.01% yield), ponasterone A (2)[3] (0.05%), and a C_{28}-ecdysone designated makisterone A (0.001%), three other makisterones (B, C, D; yields 0.0001% each).[2] All exhibit strong insect moulting activity (0.5-1γ/insect) when assayed by the Chilo dipping test.[4] The mixture of makisterones B, C and D obtained by automatic liquid chromatography[2,5] gave two fractions by silica gel chromatography using $CHCl_3$/MeOH (4/1). The fraction with the smaller Rf value was acetylated and hydrolysed to give makisterone B. The fraction with the larger Rf value was acetylated and the mixture of two acetates was separated by silica gel chromatography; hydrolysis of respective acetates yielded makisterone C and D.

Makisterone B (4): m.p. 172-173° (dec.), $C_{28}H_{46}O_7$ (M^+-H_2O at m/e 476), IR (KBr), 3400, 1660, 1630 cm^{-1}; UV (MeOH), 243 mμ (ε 11,000); RD (dioxane) a = +53.2 (n → π*). Acetylation with Ac_2O/py gave the non-crystalline 2,3,22,26-tetraacetate, $C_{36}H_{54}O_{11}$ (M^+ at m/e 662).

Makisterone C (5): m.p. 263-265° (dec.), $C_{29}H_{48}O_7$ (M^+-H_2O at m/e 490), IR (KBr), 3400, 1650, 1630 cm^{-1}; UV (MeOH), 243 mμ (ε 10,900); RD (dioxane) a = +66 (n → π*); 2,3,22-triacetate, m.p. 201-203°, $C_{35}H_{54}O_{10}$ (M^+-H_2O at m/e 616).

Makisterone D (6): non-crystalline, $C_{29}H_{48}O_7$ (M^+-H_2O at m/e 490), IR (KBr), 3400, 1650, 1630 cm^{-1}; UV (MeOH) 244 mμ; RD (dioxane) a = +ca 42 (n → π*); 2,3,22,28-tetraacetate, m.p. 189-193°, $C_{37}H_{56}O_{11}$ (M^+-H_2O at m/e 656).

Skeletal structure of makisterones B, C and D. As in the case of makisterone A,[1] the structures of makisterones B, C and D from C-1 to C-22 were shown to be identical with that of ecdysterone 1 on the following grounds: (i) UV and IR (7-en-6-one); (ii) HCl/MeOH treatment to give two products absorbing at ca 240 mμ and 295 mμ (14-hydroxy-7-en-6-one);[6,7] (iii) 18-Me chemical shifts (Table 1) (14-hydroxyl is α); (iv) amplitudes of RD Cotton effects, a = +53.2, +66, +42 (A/B cis ring juncture);[7] (v) strong MS peaks at m/e 363 and 345 (=363-18) due to fission between C-20 and C-22 (20,22-dihydroxy side-chain). The configurations of the 2 and 3-hydroxyl groups are β,β in all three cases, since the C-2 and C-3 carbinyl proton signals (NMR in $CDCl_3$) of the acetates, appearing at ca 5.1 ppm (C_2-ax H, $W\frac{1}{2}$ 20 cps) and

ca 5.3 ppm (C_3-eq H, $W_{\frac{1}{2}}$ 8 cps) were clearly separated and very similar to the corresponding NMR signals of the 2,3,22-triacetates of ponasterone A and ecdysterone (β,β); when the 2,3-configurations are α,α, as in ponasterone B and C, the 2,3-carbinyl protons of the acetates appear at 5.17–5.18 ppm ($W_{\frac{1}{2}}$ 20 cps) and 5.24 ppm ($W_{\frac{1}{2}}$ 7.5 cps) and are not separated.[7] The 19-Me chemical shifts of the acetates at 1.00–1.02 ppm (Table 1) are also indicative of 2β,3β-configurations (the 19-Me appears at 0.93 ppm when the 2,3-acetoxyl functions are α-oriented).[7]

Side-chain structures. The side-chain of makisterone B is isomeric with makisterone A as shown by the appearance of similar strong peaks at m/e 131 (19%), 113 (94%) (see 8) and 95 (39%) (base peak at m/e 43) due to C-20/C-22 cleavage (cleavage-a[2]) followed by loss of H_2O elements. The presence of a terminal Me-CH-CH_2OH grouping was established by decoupling experiments with the tetraacetate; thus, irradiation at 1.40 ppm (25-H) caused one of the 0.90 ppm doublets (27-Me) to collapse to a singlet and also the ABX patterned CH_2OAc octet at 3.8–4.3 ppm to change to an AB quartet. The NMR data are closely related to those of inokosterone 7 possessing a similar structure.[8] The additional sec-Me group is at C-24 and not at C-23, since irradiation at 1.96 ppm changed the 0.90 ppm doublet (28-Me) to a singlet but did not affect the carbinyl signal at C_{22} (and C_{26}). Thus makisterone B is represented by 4.

The MS spectrum of makisterone C has strong peaks at m/e 145 (13%, see 9) and 127 (100%), which are 14 units higher than the corresponding cleavage-(a) peaks in makisterone A and B. The appearance of a strong peak at 98 (127-C_2H_5, 17%) suggested this additional CH_2 unit to be incorporated in an ethyl group, the presence of which was established by double resonance measurements. As in makisterone A (3) and ecdysterone (1), makisterone C (5) also has the terminal -C(Me)$_2$OH moiety (NMR, C-26/27-Me), and therefore the ethyl group should be attached to either C-23 or C-24. In support of its attachment to C-24, a characteristic MS peak is present at m/e 84 (10 R=Et, 84%), which corresponds to the m/e 70 peak (10 R=Me, 100%) (cleavage-b)[2] in makisterone A. It follows that the structure of makisterone C is 5, probably identical with podecdysone.[10]

The MS spectrum of makisterone D is again characterized by conspicuous peaks at m/e 145 (11, 22%) and 127 (100%), which indicate that its side-chain structure is isomeric with makisterone C. NMR data show the presence of three sec-Me groups, and moreover, decoupling measurements with the tetraacetate established the presence of a CH_3-CH(OAc) grouping. Accordingly makisterone D can be represented by 6.

It is remarkable that the leaves of P. macrophyllus contain a total of at least six closely related phytoecdysones all possessing strong insect moulting activity; the fact that these range from C_{27} to C_{29} compounds is also interesting from a biogenetic viewpoint.

Acknowledgements

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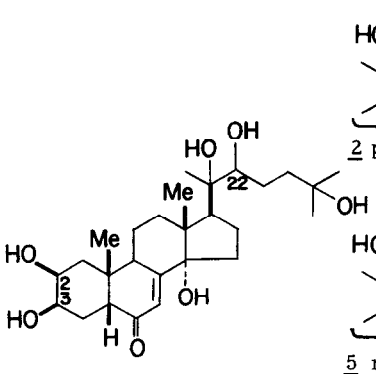
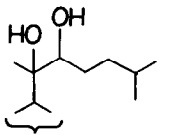
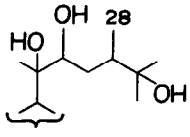


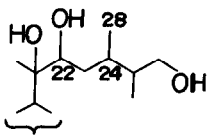
1 ecdysterone



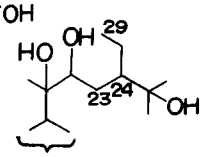
2 ponasterone A



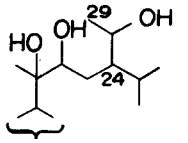
3 makisterone A



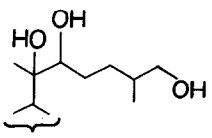
4 makisterone B



5 makisterone C



6 makisterone D



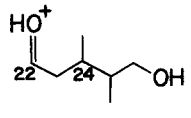
7 inokosterone

TABLE 1. Methyl chemical shifts of makisterones and related compounds.

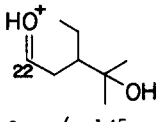
Free ecdysones	18	19	21	26/27	28	29
Ecdysterone (1)[a]	1.19	1.06	1.55	1.34, 1.34	-	-
Ponasterone A (2)[a]	1.16	1.03	1.51	0.82 (d, 6)	-	-
Makisterone A (3)[a]	1.21	1.09	1.54	1.32, 1.29	1.05 (d, 6)	-
" B (4)[b]	1.16	1.04	1.54	1.00 (d, 6)	0.90 (d, 6)	-
" C (5)[b]	1.20	1.07	1.55	1.38, 1.26	-	1.07[d]
" D (6)[b]	1.20	1.06	1.57	0.87 (d, 6)	-	1.30 (d, 6)
Inokosterone (7)[8] [a]	1.19	1.07	1.52	0.97 ?(d, 6) 1.03 (d)	-	-
Acetates[c]						
1-2,3,22-tri- (ecd.)	0.85	1.02	1.24	1.21, 1.18	-	-
2- " " (PN-A)	0.85	1.02	1.24	0.88 (d, 6)	-	-
3- " " (A)	0.83	1.01	1.23	1.18, 1.13	0.91 (d, 6)	-
4-2,3,22,26-tetra- (B)	0.85	1.02	1.23	0.90 (d, 7)	0.90 (d, 7)	-
5-2,3,22-tri- (C)	0.84	1.00	1.22	1.16, 1.14	-	0.98 (t, 6)
6-2,3,22,28-tetra- (D)	0.85	1.02	1.25	0.89 (d, 6) 0.84 (d, 6)	-	1.21 (d, 6)
7-2,3,22,26-tetra- (inoko.)[9]	0.85	1.02	1.24	0.94 (d, 6)	-	-

a): in pyridine b): in d_5-pyridine c): in $CDCl_3$

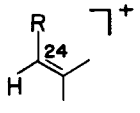
d): splitting is unclear due to overlap; however, the triplet nature is clear in the triacetate spectrum (0.98 ppm, t, 6 cps).



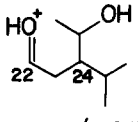
8 m/e 131 (makisterone B)



9 m/e 145 (makisterone C)



10 m/e 70 (R=Me) m/e 84 (R=Et)



11 m/e 145 (makisterone D)

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8) T. Takemoto, S. Ogawa and N. Nishimoto, Yakugaku Zasshi, 87, 325, 1474 (1967); T. Takemoto, Y. Hikino, S. Arihara, H. Hikino, S. Ogawa and N. Nishimoto, Tetrahedron Letters, 2475 (1968).

9) Private communication from Professor T. Takemoto, Tohoku University.

10) The same structure (side-chain stereochemistry undefined) was proposed by Dr. D. H. S. Horn, CSIRO, Melbourne, for podecdysone isolated from Podocarpus elatus: Conference on Insect-Plant Interaction, International Biological Program, Santa Barbara, California, March 18-22, 1968. The properties of makisterone C are very similar to those of podecdysone, but a few points remain to be clarified in the podecdysone structure (letter from Dr. Horn, May 7, 1968). Makisterone C will be called podecdysone when the structure of the latter and identity of the two compounds have been established. We are grateful to Dr. Horn for disclosure of unpublished data.

Addendum

After completion of this manuscript, we have learned that the structure derived for lemmasterone by Professor Takemoto and co-workers (this issue) is identical with 5, i. e., makisterone C and probably podecdysone (Reference 10). A joint note will be published regarding the results of a comparison of the three C_{29} phytoecdysones.