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ISOLATION OF FOUR NEW PHYTOECDYSONES, MAKISTERONE A, B, C, D, AND THE STRUCTURE OF MAKISTERONE A, A C_{28} STEROID.*

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The isolations of ecdysterone (1) and ponasterone A (2)[1] from leaves of Podocarpus macrophyllus D. DON. ("inu-maki" in Japanese) has already been reported.[2] Four additional phytoecdysones have now been isolated from the leaves (collected in summer, 1967). Interestingly, these new phytoecdysones, which we designate makisterone A, B, C and D, have steroid skeletons with 28 and 29 carbon atoms, and therefore P. macrophyllus contains at least six phytoecdysones in which the number of carbon atoms range from 27 to 29. These six phytoecdysones all exhibit strong moulting activity of the same order when ligated rice-stem borers (Chilo suppressalis, 5th instar larvae) were dipped for 10 seconds in a methanolic solution of the substances, the average activity level to produce 100% response being 0. 5–1γ/insect.[3]

Isolation of makisterones

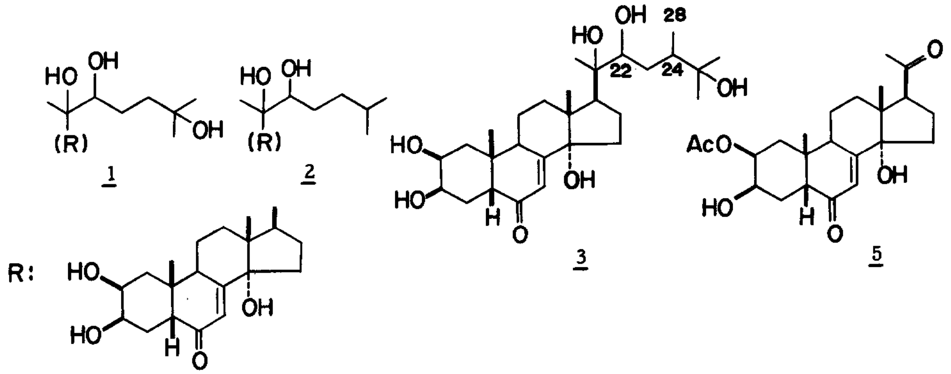
The concentrated methanol extract of the dry leaves (1. 2 tons) was treated with the same amount of water, the precipitate was removed, and the ethyl acetate extract of the filtrate was concentrated. The residue in 50% aqueous methanol was chromatographed on active carbon upon which the fraction eluted with methanol gave crude ponasterone A. The mother liquid resulting from recrystallization of ponasterone A was dried up, and the residue in ethyl acetate was chromatographed on alumina. The fraction eluted with ethyl acetate/methanol (14/1) was concentrated to dryness, and the suspension of the residue in 20% ethanol was submitted to the automatic liquid chromatography method, which utilizes an Amberlite XAD-2 column and elution with linear gradient 20%–70% aqueous ethanol; the eluates are monitored by their absorptions at 254 mμ.[4)] This retro-phase chromatography first afforded ecdysterone, then makisterone A, and finally a mixture of related substances. The last fraction was further separated into makisterone B, C and D by combinations of silica gel chromatography, acetylation and hydrolysis. The yield of the phytoecdysones from dry leaves are as follows: ponasterone A, 0.05%; ecdysterone 0.01%; makisterone A, 0.001%; makisterone B, C, D, 0.0001% each.

Makisterone A (3): m.p. 263-265° (dec.), $C_{28}H_{46}O_7$ (M^+ at m/e 494); IR (KBr), 3420, 1655, 1630 cm^{-1}, UV (MeOH), 243 mμ (ε 12,400), RD (dioxane) a = +60.3 ($n-\pi^*$). Acetylation with acetic anhydride and pyridine gave the 2,3,22-triacetate, m.p. 210-213°, M^+ at m/e 620; on the other hand, acetylation of makisterone A under conditions employed for preparing ponasterone A 2-monoacetate[5)] gave the 2-monoacetate, m.p. 217°, and 2,22-diacetate, m.p. 155-157°, together with a small amount of the non-crystalline 2,3-diacetate.[6)]

The gross skeletal structure was indicated by the following observations. The UV and IR data were characteristic of the 14-hydroxy-7-en-6-one moiety present in all other ecdysones discovered so far. Presence of the 14α-hydroxyl group was supported by the 18-methyl chemical shifts (Table 1) and the fact that brief treatment of makisterone A with MeOH/HCl gave a mixture of two compounds having absorptions at 241 mμ (8, 14-diene) and 294 mμ (7, 14-dien-6-one), respectively.[7, 8)] Prominent MS peaks were observed at m/e 363 and 345 arising from cleavage (a) in 4, as in the case of ecdysterone.[9)] Cleavage (a) also enables one to place an α-glycol grouping at C-20 and C-22 (verified below). The amplitude of the RD Cotton effect +60.3 ($n-\pi^*$), indicated the ring A/B juncture to be cis.[1, 8, 10)]

The skeletal structure was established by sodium periodate treatment of makisterone A 2-acetate, which resulted in fission between C-20 and C-22 to give 2β-acetoxy-3β, 14α-hydroxy-5β-pregn-7-en-6,20-dione (5), m.p. 235-237°; this was identical with a specimen derived from ponasterone A by similar treatment.[5)]

The clear M^+ peaks of makisterone A and the 2,3,22-triacetate suggested makisterone A to be a C_{28}-steroid. The additional sec-methyl apparent in the NMR (28-Me, Table 1) cannot be attached to C-23 since decoupling experiments which converted the 0.91 ppm doublet (triacetate, Table 1) into a singlet did not affect the shape of the C-22 carbinyl proton. It should therefore

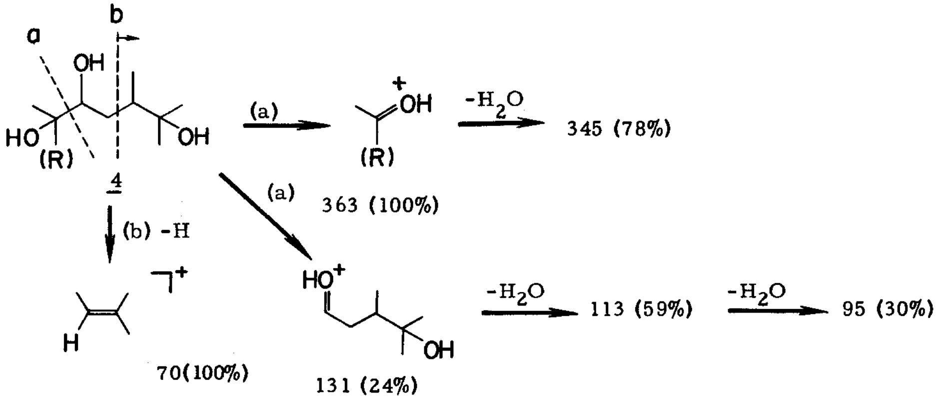


1 2 3 5

TABLE 1. Methyl chemical shifts

	18	19	21	26/27	28
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, J=6)	-----
Makisterone A (3)[a]	1.21	1.09	1.54	1.32, 1.29	1.05 (d, J=6)
1 — 2,3,22-tri-OAc[b]	0.85	1.02	1.24	1.18, 1.21	-----
2 — " "[b]	0.85	1.02	1.24	0.88 (d, J=6)	-----
3 — " "[b]	0.83	1.01	1.23	1.13, 1.18	0.91 (d, J=6)

a): in pyridine b): in $CDCl_3$



be attached to C-24, and this is corroborated by appearance of the strong m/e 70 peak due to cleavage (b) in 4; peaks at m/e 131, 113 and 95 also indicate the presence of an extra methyl group in the side-chain. Location of the extra methyl group at C-24 is obviously the one encountered in other phytosterols as well.

The evidence given above thus leads to structure 3 (side-chain stereochemistry undefined) for makisterone A, the first C_{28}-phytoecdysone.

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