INSECT MOULTING HORMONES: DACRYSTERONE, A NEW PHYTOECDYSONE FROM *DACRYDIUM INTERMEDIUM*

By G. B. RUSSELL* and J. G. FRASER*

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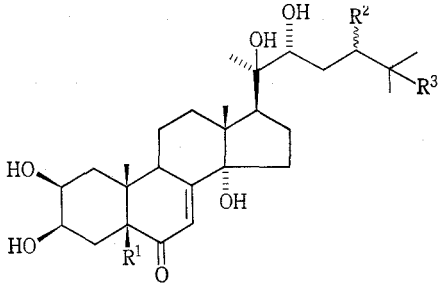
Abstract

Dacrysterone, a new phytoecdysone, has been isolated from the bark of *Dacrydium intermedium* and shown to be 5β-hydroxymakisterone A (6). Makisterone A also occurs in this plant.

In a recent communication[1] the isolation of several phytoecdysones from the bark of the tree *Dacrydium intermedium* Kirk, was reported. Among the compounds isolated were β-ecdysone (1) and its 2-cinnamate and pterosterone (2) together with the 5β-hydroxy analogues of these ecdysteroids, polypodine B (3) and its 2-cinnamate and ponasterone C (4), respectively. We have now isolated two more compounds which conform to this pattern. They are the known C_{28} ecdysone, makisterone A (5), previously isolated from *Podocarpus macrophyllus*[2] and the crab *Callinectes sapidus*[3] and 5β-hydroxymakisterone A (6) which as a new compound we have designated dacrysterone.



	R^1	R^2	R^3
(1)	H	H	OH
(2)	H	OH	H
(3)	OH	H	OH
(4)	OH	OH	H
(5)	H	Me	OH
(6)	OH	Me	OH

Makisterone A was crystallized from several fractions following chromatography of the bark extract.[1] Its identity was indicated from its R_F (0·16, $CHCl_3$: EtOH 4 : 1) on thin-layer chromatography and its colour reaction with the vanillin spray reagent.[4] Following the isolation of makisterone A a search was made for its

* Applied Biochemistry Division, D.S.I.R., Palmerston North, New Zealand.

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5β-hydroxyl derivative and this was obtained on rechromatography of residues from the isolation of pterosterone. The R_F of dacrysterone was 0·20 and this value is consistent with the behaviour of other 5β-hydroxyecdysones in relation to their 5β-hydrogen analogues[5] on thin-layer chromatography. Dacrysterone like makisterone A also gave the mauve-brown colour with the vanillin spray reagent.

The mass spectrum of makisterone A showed a molecular ion at *m/e* 494, prominent peaks at *m/e* 363 and 345 arising from cleavage of the C20–C22 bond, and peaks at *m/e* 131, 113, and 95 due to the fragmentation of the side chain.[2] Further evidence for the C24 methyl is provided by the appearance of the ion at *m/e* 70 arising from cleavage of the C23–C24 bond.[2] Dacrysterone in common with polypodine B and ponasterone C did not give a molecular ion but showed peaks due to successive loss of four molecules of water. The peaks at *m/e* 379 and 361, due to loss of the side chain, indicated that the extra hydroxyl group was attached to the tetracyclic nucleus, while ions at *m/e* 70, 95, 113, and 131 were consistent with those in the spectrum of makisterone A and confirmed the nature of the side chain.

The u.v. and i.r. spectra of dacrysterone were characteristic of the 14α-hydroxy 7-en-6-one moiety present in all phytoecdysones. The shift in carbonyl stretching vibration from 1660 cm^{-1} (KBr) in makisterone A to 1690 cm^{-1} in dacrysterone indicated an oxygen function adjacent to and near the plane of the carbonyl group in the latter compound. This shift to shorter wavelengths has been observed with other 5β-hydroxyecdysones.[5,6]

The n.m.r. spectrum of makisterone A gave signals consistent with those published for this compound.[2] The n.m.r. spectrum of dacrysterone gave a similar pattern of signals but assignment of the methyl protons indicated that the C19 methyl signal was shifted downfield by 0·11 p.p.m. This downfield shift is also observed for the 5β-hydroxyecdysones, polypodine B,[7] ponasterone C,[8] and sengosterone[5] when compared to their 5β-hydrogen analogues. Further comparison of the spectra of makisterone A and dacrysterone indicated that the latter had no signal attributable to the 5β-hydrogen which occurs at δ 2·87 as a double doublet in the former.

The c.d. curves of makisterone A and dacrysterone show the positive and negative Cotton effect peaks due to the n–π* and π–π* transitions respectively, of the ring B enone moiety.[5] Comparison of the curve of dacrysterone with that of makisterone A indicates that the positive Cotton effect peak of dacrysterone (λ 328 nm) shows a hypsochromic shift of 11 nm, an increase in the molecular ellipticity, and a disappearance of the fine structure. The presence of the 5β-hydroxy group in polypodine B,[9] ponasterone C,[10] and sengosterone[5] has been observed to incur these changes. These results establish that the 5β-hydrogen in makisterone A is replaced by a 5β-hydroxyl and confirm the structure of dacrysterone as shown in (6).

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Experimental

N.m.r. spectra were measured on a Varian T60 spectrometer in deuteropyridine and chemical shifts are relative to tetramethylsilane (δ 0·00). I.r. spectra were measured with KBr discs and u.v. spectra were in ethanol solutions. Mass spectra were recorded on an AEI MS9 spectrometer using the direct probe. Plates for thin-layer chromatography were prepared using Kieselgel HF_{254} (Merck) (0·23 mm thickness) and were developed in chloroform–ethanol (4 : 1).

The bulk extraction and isolation of the bark ecdysones has been previously described.[1] Chromatography of the ecdysone-rich material gave a series of fractions which were subjected to further purification. The fractions which eluted after the recovery of polypodine B (*E*) and before those containing β-ecdysone (*F*) were rechromatographed on silicic acid (Unisil, Clarkson Chemical Co., 50 g, 5% water) with chloroform–ethanol (17 : 3) to give makisterone A, 0·4 g, m.p. 263–265° (lit.[2] 263–265°) (Found: C, 67·0; H, 9·1. $C_{28}H_{46}O_7,\frac{1}{2}H_2O$ requires C, 66·9; H, 9·4). λ_{max} 242 nm (ε 11700). The n.m.r. spectrum showed three proton singlets at δ 1·18 (C18); 1·04 (C19); 1·53 (C21); 1·30, 1·28 (C26,27); 1·03, d, *J* 6 Hz (C28). The mass spectrum showed peaks at *m/e* 494 (<1%), 476 (1), 458 (2), 448 (8), 363 (18), 346 (22), 345 (58), 344 (13), 156 (18), 131 (12), 113 (base peak), 95 (45), 91 (30), 85 (30), 83 (48), 70 (69). The c.d. spectrum (*c*, 0·041 in dioxan) gave: $[\theta]_{385}$ 0, $[\theta]_{339}$ +5474°, $[\theta]_{275-290}$ 0, $[\theta]_{247}$ −11350°, $[\theta]_{230}$ 0.

The mother liquors and residues arising from the isolation of pterosterone (fraction *D*) were rechromatographed on silicic acid (Unisil, 50 g, 5% water) with chloroform–ethanol (9 : 1) to give dacrysterone, 0·2 g, m.p. 283–285° (Found: C, 65·5; H, 8·9. $C_{28}H_{46}O_8$ requires C, 65·9; H, 9·1). λ_{max} 240 nm (ε 11400). The n.m.r. spectrum showed three proton singlets at δ 1·22 (C18); 1·15 (C19); 1·56 (C21); 1·32 (C26,27); 1·07, d, *J* 6 Hz (C28). The mass spectrum showed peaks at *m/e* 492 (1%), 474 (2), 456 (3), 438 (3), 379 (50), 361 (47), 343 (23), 325 (25), 189 (20), 140 (22), 131 (13), 113 (base peak), 105 (26), 95 (66), 93 (20), 91 (25), 85 (28), 83 (23), 81 (23), 70 (95). The c.d. spectrum (*c*, 0·02 in dioxan) gave: $[\theta]_{370}$ 0, $[\theta]_{328}$ +9282°, $[\theta]_{275-285}$ 0, $[\theta]_{253}$ −16700°, $[\theta]_{240}$ 0.

Acknowledgments

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