Structure of Podecdysone C, a Steroid with Moulting Hormone Activity from the Bark of *Podocarpus elatus* R. Br.

THOMPSON et al.[1] isolated from the tobacco hornworm *Manduca sexta* a small amount (ca. 3 mg) of a substantially pure moulting hormone (THE-III) more polar than β-ecdysone (20-hydroxy-α-ecdysone) (I)[2]. On the basis of the spectral data of THE-III in comparison with that of a model compound and its co-occurrence with β-ecdysone they proposed structure (II) (20, 26-dihydroxy-α-ecdysone) for this hormone. Subsequently we have isolated a compound, podecdysone C[3], with closely similar properties from the polar extracts of the bark of the tree, *Podocarpus elatus* and have undertaken degrative procedures to establish the relationship of podecdysone C to THE-III and β-ecdysone.

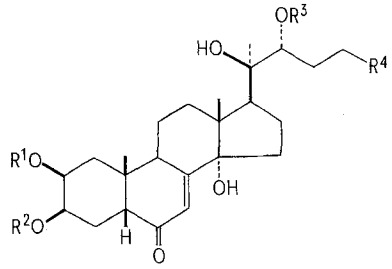
Chromatographic purification of the crude extract of the bark (100 kg)[2] provided podecdysone C as a gum (50 mg) which could not be crystallized but appeared to be pure from its behaviour in TLC and its UV-absorption (λ_{max} 242, ε 11,000, EtOH). Podecdysone C has an Rf-value of 0.17 (c.f. β-ecdysone 0.35) in TLC using unactivated silica gel (Merck type HF 254) with chloroform – 96% ethanol (70:30) as solvent and appears as a mauve spot changing (5 h) to apple-green with the vanillin-sulphuric acid spray reagent[4]. Podecdysone C is more polar than β-ecdysone, isolated earlier from the same extract[2], and like it shows in its IR-spectrum (KBr) a strong hydroxyl absorption at 3486 cm^{-1} and unsaturated ketone absorption at 1652 cm^{-1}. The chemical shifts of the methyl resonances in the NMR-spectrum of podecdysone C are essentially the same as those reported for THE-III (see Table). The mass spectra of THE-III and podecdysone C measured with an LKB Model 9000 mass spectrometer were found to be virtually identical. The spectra show prominent peaks at *m/e* 460, 442 and 424 corresponding to the parent ion with loss of 2, 3 and 4 molecules of water respectively. Peaks at *m/e* 363 (M-133) and 345 (M-133–18) are attributed to ions formed by C 20–C 22 bond cleavage, and cleavage together with loss of a molecule of water.

Reaction of podecdysone C with acetone in the presence of phosphomolybdic acid afforded a triacetonide[5], which in conformity with this formulation showed in its mass spectrum ions at *m/e* 616 (M^+), 558 (M-58) and 500 (M-2×58) corresponding to the parent ion and ions attributed to the loss of 1 and 2 molecules of acetone respectively. Acetylation (24 h) of podecdysone C afforded 2 derivatives considered from NMR to be podecdysone C 2, 3, 22, 25, 26-penta- and 2, 3, 22, 26-tetra-acetates. The mass spectrum of the tetra-acetate showed prominent peaks at *m/e* 586 (M-60-18), 568 (M-60-2×18) and 550 (M-60-3×18). Partial hydrolysis of the tetra-acetate with potassium hydrogen carbonate in aqueous methanol and chromatography of the product afforded podecdysone C 3, 22-diacetate (III) which in its mass spectrum showed prominent peaks at *m/e* 544 (M-2×18), 526 (M-3×18) and 484 (M-60-2×18). The chemical shift values of the C18, C19 and C 21 methyl groups of the diacetate showed a close correspondence with those of β-ecdysone 3, 22-diacetate (IV)[2] (see Table). Oxidation of the diacetate with periodate afforded, from its NMR-spectrum, a methyl ketone (V) which showed in its mass spectrum prominent peaks at *m/e* 590 (M^+), 562 (M-28). Reaction of this ketone with methyl magnesium iodide, acetylation of the products and separation by TLC provided a product which was identical (TLC, MS) with β-ecdysone 2, 3, 22-triacetate[2]. Podecdysone C is thus shown to be a 26-hydroxy-β-ecdysone and it remains but to define its stereochemistry at C 25, which may not be the same as that of THE-III.

In the *Calliphora* bioassay[6] podecdysone C showed an activity of about 1/10 that of β-ecdysone.

Chemical shifts of methyl resonances (δ) measured in [$^2H_5$] pyridine

	18-H_3	19-H_3	21-H_3	27-H_3
Podecdysone C	1.21	1.06	1.57	1.47
THE-III (II)	1.22	1.08	1.58	1.48
Podecdysone C 3,22-diacetate (III)	1.16	1.08	1.61	1.48
β-Ecdysone 3, 22-Diacetate (IV)	1.17	1.07	1.61	1.35



	R^1	R^2	R^3	R^4
I	H	H	H	$-C(CH_3)_2OH$
II	H	H	H	$-C(CH_3)OH-CH_2OH$
III	H	Ac	Ac	$-C(CH_3)OH-CH_2OH$
IV	H	Ac	Ac	$-C(CH_3)_2OH$
V	H	Ac	Ac	$-CO-CH_3$

Résumé. Le Podecdysone C, un stérol extrait de l'écorce de l'arbre *Podocarpus elatus* R. Br. et qui fonctionne comme hormone de mue est identique au THE-III (26-hydroxy-β-ecdysone, II) extrait du ver du tabac *Manduca sexta*.

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