Isolation of Deoxycrustecdysone, Deoxyecdysone, and α-Ecdysone from the Fern *Blechnum minus*

By Y. K. CHONG, M. N. GALBRAITH, and D. H. S. HORN

(*Division of Applied Chemistry, C.S.I.R.O., Box* 4331, *G.P.O., Melbourne, Victoria,* 3001, *Australia*)

Summary The structure of deoxycrustecdysone (I) has been confirmed following its isolation from the fern *Blechnum minus*, together with α-ecdysone (III) and the new compound deoxyecdysone (II).

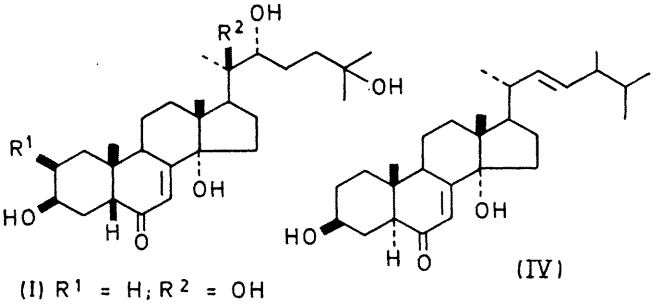
THE arthropod moulting hormone deoxycrustecdysone (I) was first isolated in very small amount from an extract of the marine crayfish *Jasus lalandii*,[1] and detected in an extract of the pupae of the moth *Antherea pernyi*.[1,2] We now report that the Australian fern *Blechnum minus* (R.Br.) Ettingsh, contains a relatively large amount of deoxycrustecdysone (yield 0·01%). In addition the fern contains a less polar compound, deoxyecdysone, to which we assign structure (II), and the insect moulting hormone α-ecdysone (III), already isolated from several ferns.[3]

Deoxycrustecdysone (I), m.p. 250—252°, shows the typical u.v. and i.r. absorptions of the ecdysones [λ_{max} (ethanol) 243 nm (ε 12,100), ν_{max} (KBr) 3450 and 1645 cm^{-1}]. The molecular formula $C_{27}H_{44}O_6$ was established by microanalysis and mass spectrometry (M^+ peak at *m/e* 464). The mass spectrum of (I) from fern shows only minor differences when compared with that of (I) from crayfish,[1] and has prominent peaks in the high-mass region which are all sixteen mass units less than the corresponding peaks in the spectrum of crustecdysone (β-ecdysone), and very prominent peaks at *m/e* 99 and 81 due to side-chain fragments. The chemical shifts of methyl resonances in the n.m.r. spectrum are in agreement with the values found[1] for the crayfish material in two solvents (Table). Also the deoxycrustecdysone from both sources showed identical behaviour (R_F 0·27) in thin-layer chromatography [silica gel with chloroform-96% ethanol (4 : 1) as solvent].

Treatment of deoxycrustecdysone with an excess of periodic acid in ethanol gave a single product, the n.m.r. spectrum of which was closely similar to that of 2β, 3β,14α-trihydroxypregn-7-ene-6,20-dione obtained[4] by selective periodate oxidation of crustecdysone, confirming the presence of a single side-chain vicinal-diol grouping in the molecule.

Overnight acetylation of deoxycrustecdysone in pyridine–acetic anhydride furnished a product which showed two additional methyl peaks in its n.m.r. spectrum at δ 2·04 and 2·10, indicating the presence of two readily acetylated hydroxy-groups. The signal assigned to the C-3 proton appears as a broad band at δ 5·03 with peak-width at half-height ($W_{\frac{1}{2}}$) of 8 Hz, as expected[5] for an equatorial proton. No low-field signal assignable to an axial C-2 proton is present. The methyl signals of deoxycrustecdysone diacetate are closely similar in chemical shift to those found for crustecdysone 3,22-diacetate.[5] The o.r.d. spectrum of deoxycrustecdysone measured in dioxan solution shows a positive Cotton effect (*a* + 43), and is closely similar to that of 2-deoxy-3-epicrustecdysone.[6] These results provide strong support for the 2-deoxycrustecdysone structure,[1] and further confirmation was obtained from o.r.d. measurements under equilibrating conditions.

When treated with base under mild conditions α-ecdysone (III) affords[7] a mixture of 5α- and 5β-isomers in a ratio of 1 : 4; the 5β-isomer predominating because of steric interaction between the 2β-hydroxy-group and the 10-methyl group in the 5α- isomer. In the absence of a 2β-hydroxy-group the 5α-isomer can be expected to predominate.[8] O.r.d. amplitude measurements obtained with deoxycrustecdysone and the model compound (IV) at room temperature in aqueous methanolic potassium carbonate showed that at equilibrium the ratio of 5α : 5β-isomers was 3 : 2, confirming the structural assignment. A 2α-hydroxy-structure, which could be consistent with the n.m.r. data, can be ruled out because the 5β-isomer would be extremely unstable due to interaction between the 2α-hydroxy-group and the C-9 hydrogen atom.



(IV)

(I) R^1 = H; R^2 = OH
(II) R^1 = H; R^2 = H
(III) R^1 = OH; R^2 = H

Chemical shifts of methyl resonances (δ)

	Solvent	18-H_3	19-H_3	21-H_3	26/27-H_3
Deoxycrustecdysone:					
(i) from crayfish[1]	[$^2H_5$]Pyridine	1·21	1·04	1·57	1·35
(ii) from *B. minus*	,,	1·21	1·05	1·57	1·36
(i) from crayfish[1]	[$^2H_4$]-Methanol	0·87	0·93	1·17	1·17
(ii) from *B. minus*	,,	0·89	0·96	1·20	1·20
Deoxyecdysone	[$^2H_5$]-Pyridine	0·74	1·05	1·28[a]	1·38
α-Ecdysone	,,	0·71	1·05	1·26[a]	1·36

[a] Doublet; *J* 7 Hz.

ν_{max} 3450 and 1650 cm^{-1}, has an R_F value of 0·33 on t.l.c. in the above solvent system. Microanalysis and mass spectrometry (M^+ peak at m/e 448) established the molecular formula as $C_{27}H_{44}O_5$, and the mass spectrum exhibited prominent peaks at m/e 430 ($M - 18$), 412, (indicating the ready loss of only two molecules of water), 361, 332, 314, and 284, all of which are sixteen mass units lower than corresponding prominent peaks in the spectrum of α-ecdysone. Peaks at m/e 99 and 81 can be assigned to the same side-chain fragments as those produced by α-ecdysone. Thus deoxyecdysone has the same side-chain as α-ecdysone but one less hydroxy-group on its tetracyclic nucleus.

The n.m.r. spectrum (Table) of deoxyecdysone shows methyl signals with chemical shifts closely similar to those of α-ecdysone. Acetylation of deoxyecdysone gave a diacetate, which in its n.m.r. spectrum has acetate-methyl signals appearing as a six-proton singlet at δ 2·03, and a signal assigned to the C-3 proton at δ 5·06 ($W_{\frac{1}{2}}$ 8 Hz). Periodic acid did not react with deoxyecdysone under conditions used to degrade deoxycrustecdysone. The o.r.d. spectrum of deoxyecdysone measured in dioxan is virtually superimposable on that of deoxycrustecdysone, and thus deoxyecdysone can be assigned structure (II).

In the *Calliphora* bioassay (using abdomens of *C.stygia*[9]) deoxyecdysone showed activity approximately equal to that of α-ecdysone, while deoxycrustecdysone was slightly more active. These results confirm that the 2-hydroxy-group of the majority of ecdysones, while important for the stabilization of the 5β-isomer, is not essential for biological activity. As (I) and (II) occur naturally and are highly active in insects, and as studies[10] indicate that 22- and 25-deoxyecdysone are unlikely to be normal precursors of crustecdysone in *Calliphora*, it is likely that the biosynthesis of ecdysones proceeds through 2-deoxy-intermediates.

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