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2-DEOXY-3-EPIECDYSONE FROM THE FERN *BLECHNUM VULCANICUM*

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Key Word Index—*Blechnum vulcanicum*; Blechnaceae; ferns; ecdysteroids; 2-deoxy-3-epiecdysone; ecdysone.

Abstract—2-Deoxy-3-epiecdysone and ecdysone were isolated from fronds of the fern *Blechnum vulcanicum*. The structure and stereochemistry of the epiecdysteroid were deduced from spectral data.

INTRODUCTION

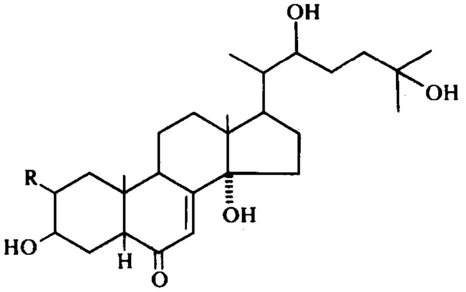
Ferns have proved to be a rich source of ecdysteroids [1] and extracts of four New Zealand Blechnaceae have been shown to possess insect moulting hormone activity [2]. When *Blechnum vulcanicum*, a small fern growing in montane forests in New Zealand, was investigated for its ecdysteroid content, ecdysone (1) and a compound assumed to be 2-deoxyecdysone (2), were isolated. Comparison of this material with an authentic sample of 2-deoxyecdysone from *Cheilanthes serberi* (D. H. S. Horn, personal communication) indicated that the *Blechnum* compound was incorrectly identified. We report here the results of spectroscopic studies on the new phytoecdysteroid (3).

RESULTS AND DISCUSSION

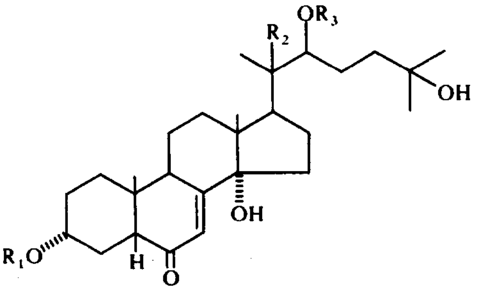
Ecdysone (1) and 2-deoxy-3-epiecdysone (3) were isolated from *Blechnum vulcanicum* as described in the Experimental. Ecdysone was identical to an authentic sample (TLC, MS) and gave spectral data (^{1}H NMR, ^{13}C NMR, UV, IR) in agreement with that reported for this compound [1,3].

The crystalline 2-deoxyecdysteroid fraction, initially isolated by column chromatography, appeared homogeneous by TLC having the same R_f and colour reaction with the vanillin–H_2SO_4 spray reagent [1] as authentic 2-deoxyecdysone (2) isolated from *Cheilanthes serberi* (D. H. S. Horn, personal communication). However, HPLC showed the crystalline material to be a mixture (1:9) of **2** and a less polar component (3). Moreover, acetylation of the material gave as a major product, a diacetate (5), which was readily distinguished from 2-deoxyecdysone diacetate by TLC.

Preparative HPLC of the crystalline material gave a pure sample of 3. UV and IR spectra of 3 showed absorptions typical of ecdysteroids and its molecular formula was $C_{27}H_{44}O_5$ (M^+ 448, MS). The mass spectrum was indistinguishable from that of 2-deoxyecdysone [4]. Overnight acetylation of 3 in Py–Ac_2O gave a diacetate (5) which showed two acetate methyl signals at δ 2.00 and 2.03 in the ^{1}H NMR spectrum ($CDCl_3$). The C-3 H resonance appeared as a broad band at δ 4.73, indicative of an axial proton, overlapping with the C-22 H signal at δ 4.90. Brief acetylation for 30 min followed by chromatography gave a monoacetate (6) as shown by a single acetate methyl signal at δ 2.00 in the ^{1}H NMR spectrum. On a TLC plate the colour produced (dark blue) by this acetate with the vanillin spray reagent was the same as the parent steroid, indicating an unmodified side-chain, whereas the diacetate **5** gave a light blue spot. The ^{1}H NMR spectrum of **6** confirmed the presence of an axial proton, with a signal at δ 4.70 of peak-width at half-height of 20 Hz, well clear of the C-22 H signal at δ 3.68 ($W_{\frac{1}{2}}$ = 12 Hz).



1 R = OH
2 R = H



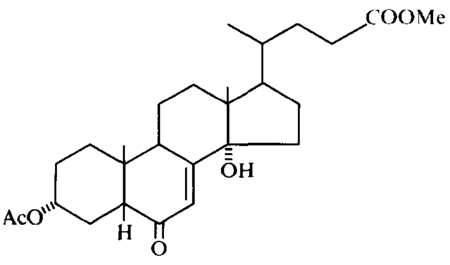
	R_1	R_2	R_3
3	H	H	H
4	H	OH	H
5	Ac	H	Ac
6	Ac	H	H
7	Ac	OH	H

Of the several possibilities for the presence of an equatorial OH in a deoxyecdysone, the C-2 OH compounds can be excluded by biogenetic considerations. This leaves the possibility that **3** has either a 3α-OH-5β-H or a 3β-OH-5α-H configuration. The ^1H NMR spectrum of **3** in d_5-pyridine shows C-18 and C-21 methyl signals (Table 1) at the same chemical shifts as for ecdysone and 2-deoxyecdysone but the C-19 methyl signal corresponds to that for synthetic 2-deoxy-3-epi-20-hydroxyecdysone (**4**) [5]. The C-19 methyl chemical shifts in $CDCl_3$ of the acetates **5** and **6** (Table 1) compare well with those of the 3α-OAc-5β-steroids **7** and **8** [5, 6] in contrast to that of the 3β-OH-5α-steroid **9** [7], the 3β-OAc-5α-steroid **10** [1] and the 3β-OH-5β-steroid **11** [7].

Final confirmation for the 2-deoxy-3-epiecdysone structure was obtained from ^{13}C NMR data. Resonances corresponding to those for carbons in rings B, C, D and the side-chain of ecdysone [3] are observed, but there are differences in ring A carbon resonances (Table 2). The presence of only one remaining methine carbon resonance at 69.1 ppm supports a ring A deoxyecdysone structure. The chemical shift of the C-19 resonance (δ 23.9) confirms a 5β-H configuration, as a 5α-H configuration would give a signal at higher field (δ 14) [8]. Using published data for 3β-acetoxy-5β-ergosta-7,22 dien-6-one [8] in $CDCl_3$ and comparing data for 3β-acetoxy- and 3α-hydroxy-5β-steroids [9], the chemical shifts for ring A carbon resonances were estimated for 3α-hydroxy-5β-ergosta-7,22-dien-6-one (Table 2) and found to be in good agreement with those observed for **3**. The possibility of a 3β-OH, 2α-OH or 2β-OH structure could be eliminated on the basis of similar estimates.

The isolation of 2-deoxy-3-epiecdysone from *Blechnum vulcanicum* raises the possibility that other ecdysteroids from *Blechnum* species may contain the C-3 epimers. 3-Epiedysteroids have been isolated from insect tissue [10, 11] but have not previously been reported as plant constituents.

The ecdysteroid content of three related ferns was tentatively established by a TLC survey of the leaf extracts. *Blechnum nigrum* contained 20-hydroxyecdysone and both *B. minus* and *B. penna-marina* contained 2-deoxyecdysone, ecdysone, 2-deoxy-20-hydroxyecdysone, 20-hydroxyecdysone and ponasterone A. These ecdysteroids are being isolated to confirm the respective identifications.



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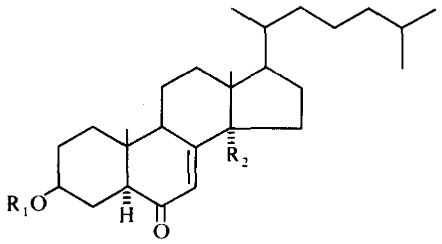
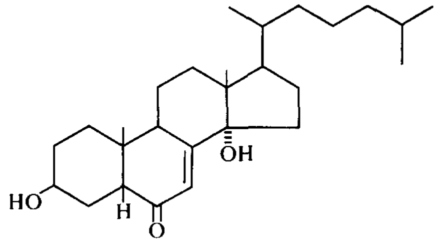


9 $R_1 = H, R_2 = OH$
10 $R_1 = Ac, R_2 = H$



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EXPERIMENTAL

Mps were determined on a Kofler microhotstage and are uncorr. Column chromatography was performed on Si gel (Mallinckrodt CC7), TLC on Merck plates (Si gel $60F_{254}$) with $CHCl_3$–EtOH (9:1) and the compounds were visualized as coloured spots by spraying with vanillin–H_2SO_4 soln and heating at 120° for 10 min. HPLC was performed on a radially compressed column of Si gel (Waters Assoc.).

Table 1. Chemical shifts (δ) of methyl proton resonances

	C-18 H_3	C-19 H_3	C-21 H_3
		In d_5-pyridine	
Ecdysone (**1**)	0.73	1.08	1.28*
2-Deoxyecdysone (**2**)	0.74	1.05	1.28*
2-Deoxy-3-epiecdysone (**3**)	0.73	0.99	1.28*
2-Deoxy-3-epi-20-hydroxyecdysone (**4**)	1.23	0.99	1.59
		In $CDCl_3$	
2-Deoxy-3-epiecdysone 3,22-diacetate (**5**)	0.67	0.92	0.93*
2-Deoxy-3-epiecdysone 3-acetate (**6**)	0.70	0.93	0.95*
2-Deoxy-3-epi-20-hydroxyecdysone 3-acetate (**7**)	0.86	0.92	1.22
3α-Acetoxy ketodiol (**8**)	0.68	0.93	
3β,14α-Dihydroxy-5α-cholest-7-en-6-one (**9**)	0.70	0.87	
Deoxyviperidone 3-acetate (**10**)	0.63	0.87	0.94*
3β,14α-Dihydroxy-5β-cholest-7-en-6-one (**11**)	0.69	1.00	

* Doublet, $J = 6$ Hz.

Table 2. ^{13}C NMR resonances for ring A carbons (δ)

	Carbon Nos						
	1	2	3	4	5	10	19
Ecdysone (1)*	38.2	68.3	68.3	32.5	51.5	38.9	24.7
2-Deoxy-3-epiecdysone (3)*	35.6	31.4	69.1	34.3	57.2	36.8	23.9
3β-Acetoxy-5β-ergosta-7,22-dien-6-one† [8]	29.3	25.5	67.7	29.6	51.5	36.2	24.2
3α-Hydroxy-5β-ergosta-7,22-dien-6-one (estimated)‡	35.5	31.4	69.7	34.9	56.8	36.5	24.1

* In d^5-pyridine.

† In $CDCl_3$.

‡ Changes in ring A carbon shielding values from 3β-OAc- to 3α-OH- were estimated by comparing data for 3β-acetoxy-5β-pregn-16-en-20-one with those for 3α-hydroxy-5β-androstan-17-one [9].

Plant material. Fronds of *Blechnum vulcanicum* were collected from the Taupo area, *B. minus*, *B. nigrum* and *B. penna-marina* were collected from the Ruahine Ranges, New Zealand. Voucher specimens are deposited in the Herbarium, Botany Division, DSIR, Christchurch, New Zealand. The plant material was air-dried and milled prior to extraction.

Extraction and isolation. B. vulcanicum (3.8 kg) was extracted in a Soxhlet with MeOH. The MeOH extract was concd *in vacuo* to a small vol. and this partitioned (×3) between petrol and $MeOH-H_2O$ (4:1). The aq. phases were combined, concd *in vacuo* and the residue (96 g) was percolated through Al_2O_3 (1 kg) with EtOH–EtOAc (1:1). The eluate (10:1) was concd *in vacuo* and repartitioned (×3) between petrol–Et_2O–MeOH–H_2O (8:5:3:1). The aq. phases were concd *in vacuo* to give a syrup (36 g) which was chromatographed on Si gel with a $CHCl_3$–EtOH gradient system, beginning with $CHCl_3$. Three hundred 10-ml fractions were collected. Fractions eluted with $CHCl_3$–EtOH, (23:2) and showing similar spots on TLC were combined to give a gum (4.5 g) which was rechromatographed on Si gel (100 g) with $CHCl_3$–EtOH, (19:1), to give impure 2-deoxy-3-epicdysone (1.5 g), mp 237–249°. Fractions eluted with $CHCl_3$–EtOH (9:1) were rechromatographed with the same solvent to give ecdysone (2.8 g), mp 241–243° (lit. 237–239° [1]). HPLC with CH_2Cl_2–2-PrOH–MeOH (87:10:3), of the impure 2-deoxy-3-epiecdysone (20 mg) gave pure **3**.

Ecdysone (1). This showed physical and spectral data identical with the lit. data [1, 3] and was identical with an authentic sample (TLC, MS).

2-*Deoxy*-3-*epiecdysone* (**3**). Mp 264–265°; $[\alpha]_D^{20}$ +98° ($CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 243 (4.061); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1650 (CO). ^1H NMR (60 MHz, d_5-Py, TMS): δ 0.73 (3 H, S, C-18 Me); 0.99 (3 H, S, C-19 Me); 1.23 (3 H, *d*, $J = 6$ Hz, C-21 Me); 1.38 (6 H, S, C-26, 27 Me); 6.20 (1 H, *m*, C-7). ^{13}C NMR (20 MHz, d_5-pyridine, TMS, chemical shifts in ppm, signal multiplicity obtained by off-resonance decoupling experiments): 201.9, (*s*, C-6), 165.8 (*s*, C-8), 121.3 (*d*, C-7), 83.8 (*s*, C-14), 74.0 (*d*, C-22), 69.7 (*s*, C-25), 69.1 (*d*, C-3), 57.2 (*d*, C-5), 48.3 (*d*, C-17), 47.6 (*s*, C-13), 43.0 (*d*, C-20), 42.5 (*t*, C-24), 36.8 (*s*, C-10), 35.6 (*t*, C-1), 34.3 (*t*, C-4), 34.0 (*d*, C-9), 31.9 (*t*, C-15), 31.6 (*t*, C-12), 31.4 (*t*, C-2), 30.3 (*q*, C-27), 30.0 (*q*, C-26), 26.7 (*t*, C-16), 25.6 (*t*, C-23), 23.9 (*q*, C-19), 20.8 (*t*, C-11), 15.8 (*q*, C-18), 13.7 (*q*, C-21). MS (probe) 70 eV, *m*/*z* (rel. int. %): 448 (M^+, 0.2), 430 (2), 412 (5), 397 (5), 396 (3), 379 (3), 361 (1), 332 (8), 314 (11), 299 (5), 283 (6), 284 (9), 263 (8), 251 (5), 99 (100), 81 (54).

2-*Deoxy*-3-*epiecdysone*-3,22-*diacetate* (**5**). Acetylation of **3** (3 mg) in pyridine–Ac_2O (1:1, 1 ml) at room temp. overnight, addition of ice, concd *in vacuo* and chromatography with $CHCl_3$ gave **5**, 2 mg, mp 178–179°. ^1H NMR (60 MHz, $CDCl_3$, TMS): 0.67 (3 H, *s*, C-18 Me); 0.92 (3 H, *s*, C-19 Me); 0.93 (3 H, *d*, $J = 6$ Hz, C-21 Me); 1.23 (6 H, *s*, C-26, 27 Me); 2.00 (3 H, *s*, MeCO); 2.03 (3 H, *s*, MeCO); 4.73 (1 H, *m*, $W_{\frac{1}{2}} = 28$ Hz, C-3); 4.90 (1 H, *m*, $W_{\frac{1}{2}} = 14$ Hz, C-22); 5.87 (1 H, *m*, C-7). MS (probe) 70 eV, *m*/*z* (rel. int. %): 454 (7, M − AcOH − H_2O), 436 (16, M − AcOH − 2 H_2O), 421 (6), 394 (9, M − 2 AcOH − H_2O), 376 (23, M − 2AcOH − 2H_2O), 361 (17), 327 (21), 326 (47), 267 (46), 266 (60), 109 (63), 99 (7), 95 (98), 93 (69), 91 (71), 81 (100).

2-*Deoxy*-3-*epiecdysone*-3-*acetate* (**6**). Acetylation of **3** (5 mg) in pyridine–Ac_2O (1:1, 2 ml) at room temp. for 30 min, work-up and chromatography with $CHCl_3$–EtOH (97:3) gave **6**, 2 mg, mp 171–172°. ^1H NMR (60 MHz, $CDCl_3$, TMS): 0.70 (3 H, *s*, C-18 Me); 0.93 (3 H, *s*, C-19 Me); 0.95 (3 H, *d*, $J = 6$ Hz, C-21 Me); 1.24 (6 H, *s*, C-26, 27 Me); 2.00 (3 H, *s*, MeCO); 3.68 (1 H, *m*, $W_{\frac{1}{2}} = 12$ Hz, C-22); 4.70 (1 H, *m*, $W_{\frac{1}{2}} = 20$ Hz, C-3), 5.88 (1 H, *m*, C-7). MS (probe) *m*/*z* (rel. int. %): 472 (1, M − H_2O), 454 (5, M − 2 H_2O), 412 (3, M − AcOH − H_2O), 394 (8, M − AcOH − 2 H_2O), 374 (8), 343 (8), 267 (27), 266 (23), 253 (16), 251 (21), 217 (33), 99 (98), 95 (50), 93 (35), 91 (45), 81 (100).

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NOTE ADDED IN PROOF

Since this paper was received for publication, the isolation of 2-deoxy-3-epiecdysone from insect tissue has been reported (Isaac, R. E., Rees, H. H. and Goodwin, T. W. (1981) *Chem. Commun.* 418).