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ECDYSTEROID XYLOSIDES FROM *LIMNANTHES DOUGLASII*

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Key Word Index—*Limnanthes douglasii*; Limnanthaceae; seeds; limnantheoside A [20-hydroxyecdysone-3-β-D-xylopyranoside]; limnantheoside B [ponasterone A-3-β-D-xylopyranoside]; ecdysteroid; phytoecdysteroid.

Abstract—Bioassay/RIA-guided phytochemical examination of seeds of members of the genus *Limnanthes* demonstrates the presence of moderate to high levels of phytoecdysteroids. *Limnanthes douglasii* has afforded two novel ecdysteroid glycosides: limnantheoside A [20-hydroxyecdysone-3-β-D-xylopyranoside] and limnantheoside B [ponasterone A-3-β-D-xylopyranoside], together with 20-hydroxyecdysone and ponasterone A. HPLC/RIA data support the additional presence of small amounts of ecdysone and an analogous ecdysone glycoside. The levels of ecdysteroids in individual seeds and plants of *L. douglasii* are very variable. The HPLC behaviour of ecdysteroid glycosides, including these two new xylosides, in both RP- and NP-systems using different mobile phases is also discussed. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Limnanthes douglasii R. Br., commonly known as 'Poached egg plant' or 'Meadowfoam' is a quick-growing early spring annual from California and southern Oregon [1]. This species, and the other members of the small family (Limnanthaceae) to which it belongs, have not been investigated for phytoecdysteroids before. However, reports on the composition of its seed-oil [2–6] and flavonoid/flavonoid glycosides from the leaves and flowers [7, 8] are available. In order to gain more information about the distribution and chemistry of phytoecdysteroids, we have undertaken a bioassay/RIA-based screening of large numbers of plant seed extracts and, as a part of that on-going investigation [9–11], we wish to report on the presence of phytoecdysteroids in the genus *Limnanthes* and on the identities of the major ecdysteroids obtained from seeds of *L. douglasii*.

RESULTS AND DISCUSSION

Seed extracts of *Limnanthes* spp. were assessed for the presence of ecdysteroid agonists and antagonists (Table 1). All extracts contained RIA-positive material detected with ecdysteroid-specific antisera and most were positive in the agonist bioassay. None of the extracts was positive in the antagonist bioassay. Taken together, these results are highly indicative of the presence of significant amounts of phytoecdysteroids in seeds of *Limnanthes* spp.

Initial chromatographic analysis of the seed extracts of *L. douglasii* by RP-HPLC revealed a broad area of RIA-positive material (Fig. 1) with RIA-positive peaks at the retention times of 20-hydroxyecdysone (20E), ecdysone (E) and ponasterone A (PoA) which coincided with UV-absorbing (242 nm) peaks. The cross-reactivity factors of the Black antiserum for E, 20E and PoA (1, 68 and 113, respectively [10]) are such that it would exaggerate the quantitative importance of the E peak relative to the 20E and PoA peaks. Assessment of the same separation with the DBL-1 antiserum (cross-reactivities; E:20E:PoA = 1:1.3:6.9) showed one major RIA-positive peak co-chromatographing with 20E, with a minor peak at the retention time of E. Each of these peaks separated into two components on NP-HPLC (Fig. 2), one peak corresponding to the expected ecdysteroid (20E or E) and one to a component of greater polarity. The biological activity (agonist bioassay) of the polar component from the '20E peak' was at least 100-fold less than that of 20E. The biological activity of the polar

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Table 1. Seed extracts of *Limnanthes* spp. tested for ecdysteroid agonists and antagonists

Species	Radioimmunoassay (μg ecdysone equivalents/g seed)		Bioassay	
	Black	DBL-1	Agonist	Antagonist
Limnanthes alba Hartw. ex Benth. (P)	1.29	38.4	+	—
Limnanthes alba Hartw. ex Benth. (F)	83.7	572.2	+	—
Limnanthes alba ssp. *versicolor* Hartw. ex Benth. (P)	6.03	325.1	++	—
Limnanthes bakeri Howell (P)	88.8	820.1	++	—
Limnanthes douglasii R. Br. (S)	20.0	1223	++(+)	—
Limnanthes douglasii R. Br. (S)	8.20	367.0	++	—
Limnanthes douglasii R. Br. (P)	9.75	417.7	++	—
Limnanthes douglasii ssp. *douglasii* R. Br. (P)	5.44	37.92	+	—
Limnanthes douglasii ssp. *nivea* R. Br. (P)	9.75	341.3	++	—
Limnanthes douglasii var. *sulphurea* R. Br. (C)	26.0	400.0	++	—
Limnanthes floccosa Howell (P)	19.5	356.4	++	—
Limnanthes floccosa Howell (P)	25.7	398.6	++	—
Limnanthes floccosa Howell (P)	10.2	134.5	+	—
Limnanthes floccosa ssp. *bellingeriana* Arroyo (P)	75.9	504.4	—	—
Limnanthes floccosa ssp. *pumila* Arroyo (P)	5.64	169.3	+	—
Limnanthes gracilis Howell (P)	6.96	161.7	++	—
Limnanthes gracilis Howell (P)	10.07	296.0	++	—
Limnanthes gracilis ssp. *parishii* Howell (P)	9.30	246.4	++	—
Limnanthes montana Jepson (P)	7.82	511.9	++	—

The sources of the seeds are indicated in brackets after the species name: C = Chiltern Seeds; F = Palmengarten, Frankfurt-am-Main; P = WRPIS, Pullman; S = Suttons Seeds.

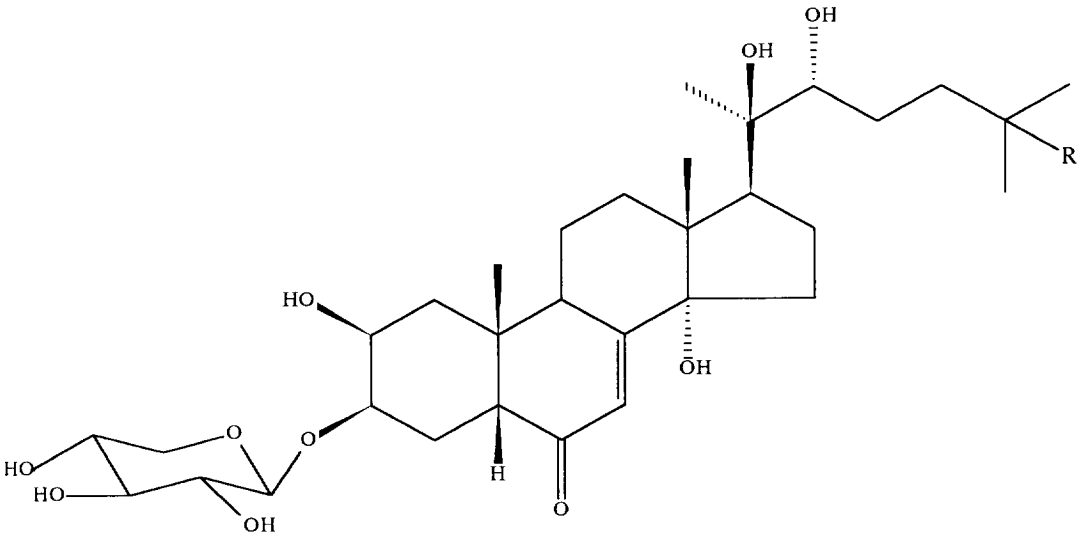
Bioassay results: active as neat extract (+), 10-fold dilution (++) or 100-fold dilution (+++): — signifies not active.

component from the 'E peak' was too low to be detectable.

RP-HPLC analysis of the seed extracts of the other *Limnanthes* samples revealed very similar patterns with a major UV-absorbing peak co-chromatographing with 20E and minor ones co-chromatographing with E and PoA, although the exact quantitative relationship between the three peaks varied between the samples. Activity in the agonist bioassay was associated only with the peak co-chromatographing with 20E.

From a methanol extract of the seeds of *Limnanthes douglasii* two novel ecdysteroid glycosides-20-hydroxyecdysone-3-β-D-xylopyranoside (**1**) and ponasterone A-3-β-D-xylopyranoside (**2**), named, respectively, as limnantheoside A and limnantheoside B, along with 20E [12, 13], and PoA [13] have been isolated by RP/NP-HPLC in combination with ecdysteroid bioassay [14, 15] and ecdysteroid-specific radioimmunoassay (RIA) [16]. All the known compounds were characterized by direct comparison of their HPLC and spectroscopic characteristics with those published in the literature and with samples previously isolated in our laboratories. The two novel compounds were characterized by spectroscopic means.

Compounds **1** and **2** were readily recognized as phytoecdysteroids from the positive responses in the bioassay and RIA. Their UV absorption spectra were also indicative of ecdysteroids. The chemical ionization/desorption mass spectrum of compound **1** revealed the molecular mass 612 suggesting the empirical formula $C_{32}H_{52}O_{11}$. A major mass fragment ion at m/z 481 representing $[M+H-132]^+$ strongly suggested the presence of a pentose unit in the molecule. In its ^1H NMR (Table 2) and ^{13}C NMR (Table 3) spectra, ^1H and ^{13}C signals, respectively, for the protons and carbons of the steroidal ring system were similar to those of 20E with the exceptions that the signals for H-3 (δ 4.15) and C-3 (δ 75.3) were much more deshielded (Tables 2 and 3) and thus suggested the attachment of the pentose at C-3. The ^1H NMR spectrum, revealing the signals (δ 3.29–4.48) for the protons from four oxymethine and an oxymethylene units suggested the presence of a pentose, the identity of which was deduced as a β-D-xylose from the characteristic signal for the anomeric proton at δ 4.48 ($d, J = 7.8$ Hz) [17] and ^1H–^1H coupling patterns (Table 2). COSY-45 and TOCSY NMR spectra further confirmed all the ^1H–^1H correlations. The ^{13}C NMR spectrum (Table 3), in addition to the signals for the carbons from the aglycone part (20E), displayed five additional oxygenated carbon signals (δ 66.3–δ 101.8) which were assigned to four oxymethine carbons and an oxymethylene carbon of the xylose unit. The chemical shift for C-1′ (δ 101.8) supported the identity of this xylose unit [18], for which attachment at C-3 was confirmed from the ^1H–^{13}C long range (^3J) coupling between the anomeric proton (H-1′) and C-3 in the PFG-HMBC [19] spectrum (Table 4). H-3 also showed ^3J coupling with C-1′ which was a weak coupling owing to the fact that the dihedral



(1) R = OH; **(2)** R = H

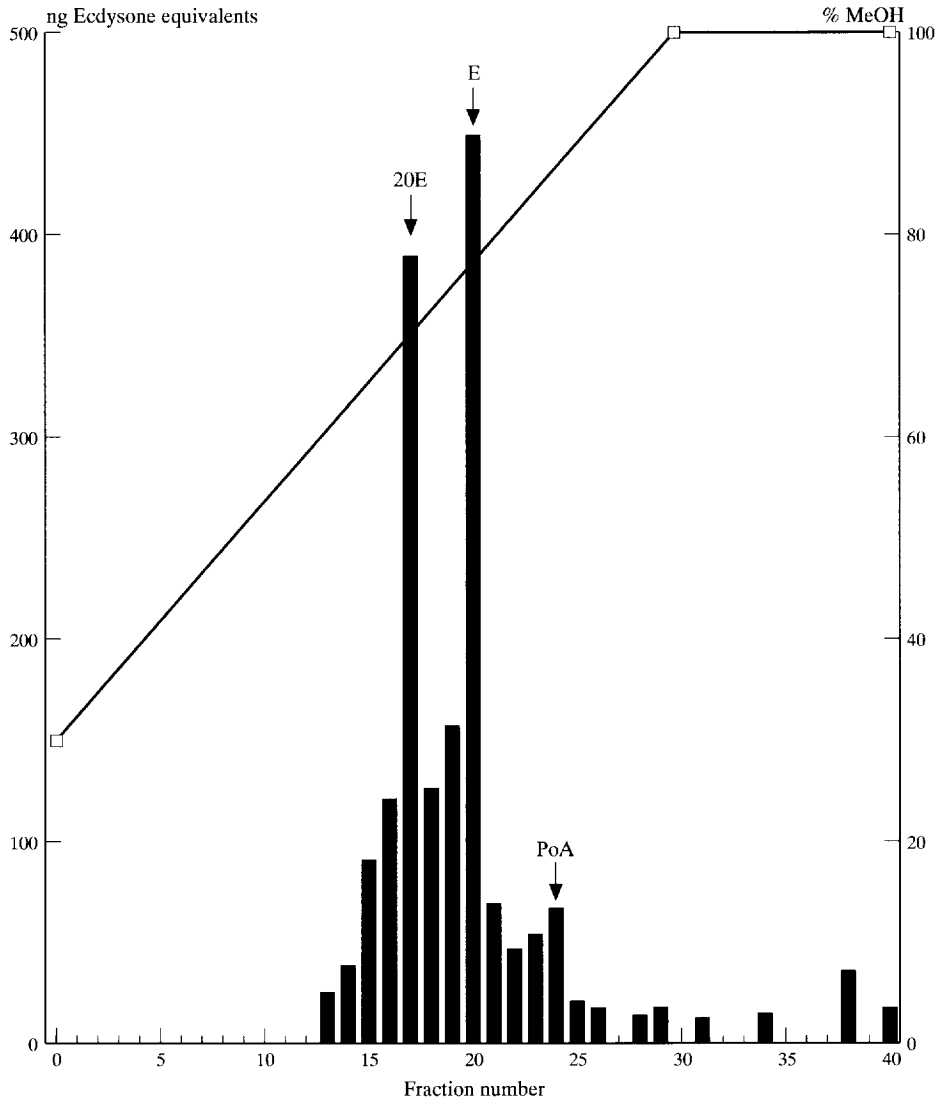


Fig. 1. RP-anal. HPLC separation of a seed extract of *Limnanthes douglasii*. Fractions of 1 min duration were collected and assessed for ecdysteroid content by RIA using the Black antiserum. The retention times of reference 20-hydroxyecdysone, ecdysone and ponasterone A (20E, E and PoA, respectively) are indicated.

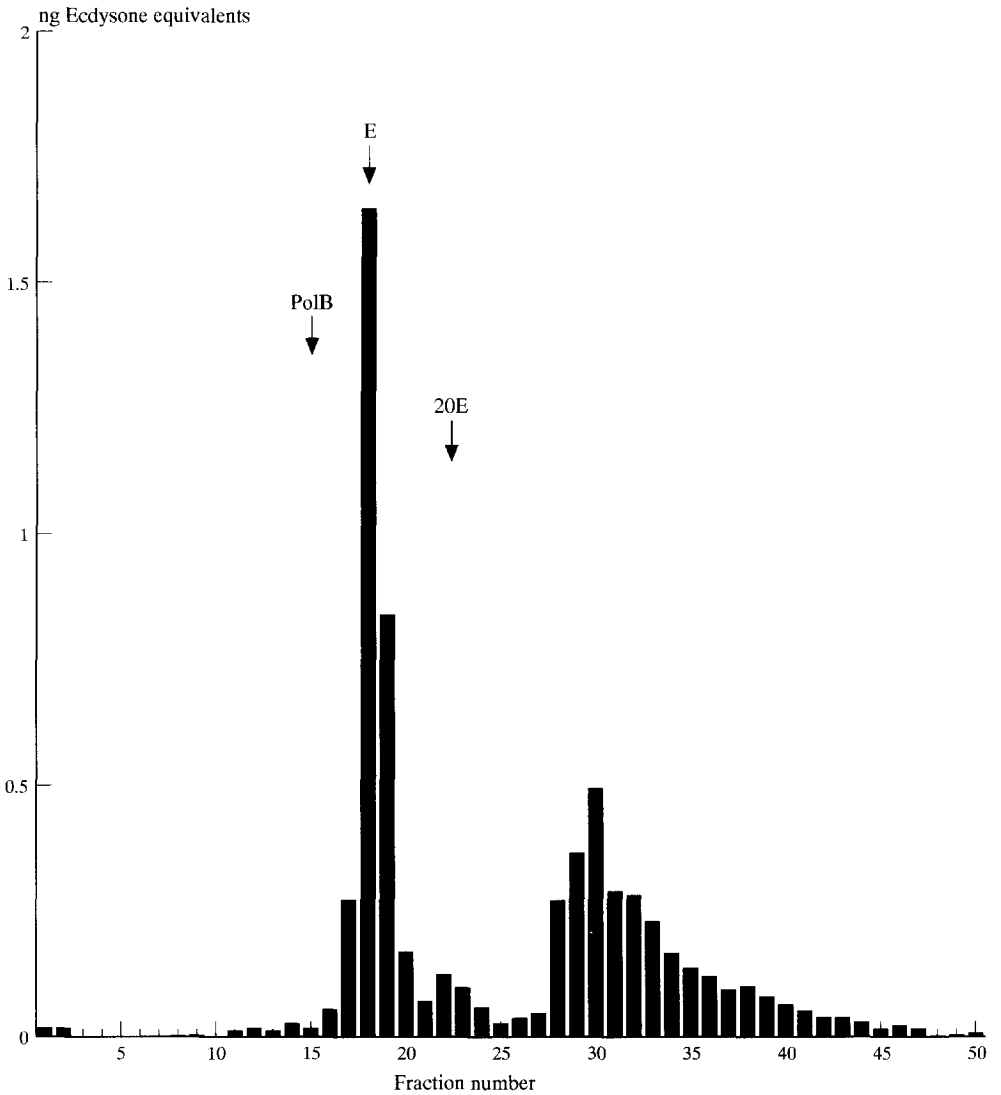


Fig. 2. NP-HPLC separation of the material from *Limnanthes douglasii* which co-chromatographed with ecdysone on RP-HPLC. Separation was performed on NP-semiprep column eluted with 6% MeOH in CH_2Cl_2 at 1.5 ml min^{-1}. Fractions of 0.75 min duration were collected and assessed for ecdysteroid content by RIA with the Black antiserum. The retention times of reference E, 20E and 5β,20-dihydroxyecdysone (PolB) are indicated.

Table 2. ^{1}H NMR spectral data of compounds **1**, **2** and 20E (500 MHz, coupling constant *J*, Hz, in parentheses)

Proton	**1**	**2**	20E
1_{ax}	1.46 *t* (13.0)	1.46 *t* (13.0)	1.38 *t* (13)
1_{eq}	1.90	1.90	1.88
2_{ax}	4.03 *m* (*w*1/2 = 22)	4.03 *m* (*w*1/2 = 22)	3.99 *m* (*w*1/2 = 22)
3_{eq}	4.15 *m* (*w*1/2 = 8)	4.15 *m* (*w*1/2 = 8)	4.07 *m* (*w*1/2 = 8)
4_{ax}	1.75	1.75	1.75
4_{eq}	1.90	1.90	1.75
5	2.43 *dd* (3.5, 13.5)	2.43 *dd* (3.5, 13.5)	2.36 *t* (13)
7	5.98 *d* (2.5)	5.98 *d* (2.5)	5.97 *d* (2.5)
9_{ax}	3.12 *m* (w1/2 = 22)	3.12 *m* (*w*1/2 = 22)	3.11 *m* (*w*1/2 = 22)
11_{ax}	1.75	1.75	1.75
11_{eq}	1.86	1.86	1.86
12_{ax}	1.95	1.95	1.95
12_{eq}	1.75	1.75	1.75
15a	2.04*	2.05*	2.05*
15b	1.65*	1.65*	1.65*
16a	1.85†	1.85†	1.90†
16b	1.80†	1.80†	1.80†
17	2.33 *t* (9.6)	2.33 *t* (9.6)	2.34 *m*
22	3.43 *dd* (1.4, 10.7)	3.45 *dd* (1.4, 10.7)	3.43 *d* (10)
23a	1.33	1.25	1.33
23b	1.65	1.60	1.65
24a	1.52 *dt* (3.4, 12.8)	1.37	1.51 *dt* (3.4, 12.8)
24b	1.80	1.26	1.80
25	—	1.55	—
18-Me	0.87 *s*	0.87 *s*	0.87 *s*
19-Me	1.00 *s*	1.00 *s*	1.00 *s*
21-Me	1.25 *s*	1.23 *s*	1.22 *s*
26-Me	1.23 *s*	1.23 *s*	1.24 *s*
27-Me	1.24 *s*	0.91 *d* (6.8)	1.24 *s*
$1'_{ax}$	4.48 *d* (7.8)	4.48 *d* (7.8)	—
$2'_{ax}$	3.33 *dd* (7.8, 9.3)	3.33 *dd* (7.8, 9.3)	—
$3'_{ax}$	3.47 *t* (9.3)	3.47 *t* (9.3)	—
$4'_{ax}$	3.65 *ddd* (5.5, 9.2, 10.5)	3.65 *ddd* (5.5, 9.2, 10.5)	—
$5'_{ax}$	3.96 *dd* (5,5, 11.6)	3.96 *dd* (5.5, 11.6)	—
$5'_{eq}$	3.29 *dd* (10.5, 11.6)	3.29 *dd* (10.5, 11.6)	—

Solution in D_2O referenced to TSP-d4; *W*1/2 = width at half-height in Hz; ax = axial; eq = equatorial; *,† = interchangeable between same signs.

Table 3. ^{13}C NMR spectral data of compounds **1**, **2** and 20E (125 MHz)

C	1[a]	2[a]	20E[b]
1	37.2	37.2	37.5
2	67.6	67.6	68.7
3	75.3	75.3	68.5
4	29.1	29.1	32.8
5	51.3	51.3	51.8
6	209.6	*	206.4
7	122.1	122.1	122.1
8	167.9	*	167.9
9	34.8	34.8	35.1
10	38.9	38.9	39.3
11	21.0	21.0	21.5
12	31.8	31.8	32.5
13	48.3	48.3	48.7
14	86.3	86.3	85.3
15	31.3	31.3	31.8
16	20.9	20.9	21.5
17	50.1	50.1	50.5
18-Me	18.0	18.0	18.0
19-Me	24.0	24.0	24.4
20	78.1	78.9	77.9
21-Me	20.3	20.5	21.1
22	78.3	77.9	78.4
23	27.0	29.8	27.4
24	41.6	36.9	42.4
25	72.9	28.4	71.3
26-Me	28.5	22.6	29.1
27-Me	29.2	23.5	29.6
1′	101.8	101.8	—
2′	73.9	73.9	—
3′	77.0	77.0	—
4′	70.5	70.5	—
5′	66.3	66.3	—

* Not observed; solutions in [a]D_2O and [b]CD_3OD referenced to TSP-d4.

angle H-3 → C-3 → O → C-1′ was possibly close to 90°. Unequivocal assignments of all the protons and carbon resonances were achieved through COSY-45, TOCSY, PFG-HMQC and PFG-HMBC experiments. Thus, the structure was assigned unambiguously as **1**.

Compound **2** having the molecular mass 596 accounted for $C_{32}H_{52}O_{10}$ showed ^1H and ^{13}C NMR signals (Tables 2 and 3) identical to ponasterone A and a xylose unit. The attachment of xylose at C-3 was confirmed in a similar fashion to that for **1** and, thus, the structure of this compound was assigned unambiguously as **2**.

The HPLC behaviour of these two xylosides together with other 20E-glycosides and their parent ecdysteroids were analysed on NP- and RP- columns, using three different solvent systems (Table 5). This analysis resulted in several important points: (*a*) the presence of a sugar unit had a greater effect on polarity on NP than on RP; (*b*) a glucose moiety enhanced polarity more than a xylose (lack of the 6′-CH_2OH is of importance, as it corresponds to a well-exposed primary alcohol group); (*c*) these effects on polarity were dependent on the position of the ether link, as seen for the series of 20E-glucosides. However, the limited effect in the RP mode was previously observed for 20E glucosides [20, 21] and also for E-25-glucoside [22]. Surprisingly, the addition of a xylose unit at C-3 has much less effect on apparent polarity than the addition of an OH at C-25 on the side-chain and the 3-β-D-xylosides showed hardly any changes in retention times relative to their corresponding aglycones on RP. Thus, analysing plant extract for ecdysteroids only by RP mode may lead to a considerable misinterpretation of HPLC data. Although the sugars increased polarity in the NP mode, compound **2** still eluted before 20E in both systems tested. It is evident that glycosides could be present in the eluting region of free ecdysteroids.

Phytoecdysteroids have not been reported either from any other species of the genus *Limnanthes* or of the family Limnanthaceae to date. Glycosides of phytoecdysteroids have been reported from a number of plant species [13], but this is the first report of phytoecdysteroid xylosides.

Considerable variation in the concentrations of phytoecdysteroids between batches of seed of *L. douglasii* (Table 1) led us to determine the amounts in individual seeds. There is a poor correlation between ecdysteroid content and seed weight (Fig. 3). In addition to seed, all other parts of plants of *L. douglasii* (leaves, roots, buds and flowers) at all stages of development contain significant levels of phytoecdysteroids (L. N. Dinan, unpublished results). Investigation of ecdysteroid levels in seedlings of *L. douglasii* also showed extensive variation between individuals (data not shown). Genetic variation has been found for every characteristic examined in *Limnanthes* (G. Jolliff, personal communication). The extreme variability in ecdysteroid levels between individuals of this species may reduce the possibility of insect adaptation to the defence capacity of these chemicals.

EXPERIMENTAL

UV spectra were in MeOH. NMR spectra were obtained on a Bruker AMX500 instrument using standard Bruker microprograms. The $1/2J$ was 100 ms for long range correlations in PFG-HMBC and 3.5 ms for PFG-HMQC. The chemical shifts are expressed in ppm. CIMS were obtained with a Riber 10-10B apparatus (Nermag S.A.) using a chemical desorption mode with NH_3 as a reagent gas. Sep-Pak Vac 35cc (10 g) C_{18} cartridge (Waters) were used for initial fractionation of extract. HPLC separation was performed in a Gilson model 811 HPLC coupled with Gilson 160 diode array detector and using Gilson Unipoint computer program. RP, NP, RP-prep. RP-anal., NP-anal. and NP-semiprep. stand, respectively, for reversed-phase, Technoprep 10C8 preparative C_8 column, Spherisorb 5 ODS-2 analytical C_{18} column,

Table 4. ^{1}H–^{13}C PFG-HMQC direct correlation (^{1}J) and ^{1}H–^{13}C PFG-HMBC long-range correlation (^{2}J and ^{3}J) in compound **1**

Proton	δ ^{13}C		
	^{1}J	^{2}J	^{3}J
H_2-1	37.2 (C-1)	67.6 (C-2), 38.9 (C-10)	75.3 (C-3), 34.8 (C-9), 51.3 (C-5)
H-2	67.6 (C-2)	37.2 (C-1), 75.3 (C-3)	
H-3	75.3 (C-3)	67.6 (C-2)	101.8 (C-1′)
H_2-4	29.1 (C-4)		
H-5	51.3 (C-5)	29.1 (C-4), 209.6 (C-6), 38.9 (C-10)	34.8 (C-9), 24.0 (C-19)
H-7	122.1 (C-7)		51.3 (C-5), 34.8 (C-9), 86.3 (C-14)
H-9	34.8 (C-9)		
H_2-11	21.0 (C-11)		
H_2-12	31.8 (C-12)		
H_2-15	31.3 (C-15)		
H_2-16	20.9 (C-16)		
H-17	50.1 (C-17)	48.3 (C-13), 78.1 (C-20)	31.8 (C-12), 18.0 (C-18), 20.3 (C-21)
H-22	78.3 (C-22)	78.1 (C-20)	
H_2-23	27.0 (C-23)		
H_2-24	41.6 (C-24)	72.9 (C-25)	
Me-18	18.0 (C-18)	48.3 (C-13)	86.3 (C-14), 50.1 (C-17), 31.8 (C-12)
Me-19	24.0 (C-19)	38.9 (C-10)	51.3 (C-5), 34.8 (C-9), 37.2 (C-1)
Me-21	20.3 (C-21)	78.1 (C-20)	50.1 (C-17), 78.3 (C-22)
Me-26	28.5 (C-26)	72.9 (C-25)	41.6 (C-24), 29.2 (C-27)
Me-27	29.2 (C-27)	72.9 (C-25)	41.6 (C-24), 28.5 (C-26)
H-1′	101.8 (C-1′)	73.9 (C-2′)	75.3 (C-3), 66.3 (C-5′), 77.0 (C-3′)
H-2′	73.9 (C-2′)	101.8 (C-1′), 77.0 (C-3′)	70.5 (C-4′)
H-3′	77.0 (C-3′)	73.9 (C-2′), 70.5 (C-4′)	101.8 (C-1′), 66.3 (C-5′)
H-4′	70.5 (C-4′)	77.0 (C-3′), 66.3 (C-5′)	73.9 (C-2′)
H_{ax}-5′	66.3 (C-5′)	70.5 (C-4′)	
H_{eq}-5′	66.3 (C-5′)	70.5 (C-4′)	77.0 (C-3′), 101.8 (C-1′)

Spectra obtained in D_2O.

Table 5. HPLC data for ecdysteroid glycosides and corresponding free ecdysteroids

Compounds	NP-anal.		RP-anal. 18% ACN-iPrOH (5:2) in 0.1% TFA
	CIW 80:40:3	DIW 125:50:5	
20E	12.3	10.1	13.9
1	23.2	21.3	13.3
20E-2-D-glucoside	31.1	50.4	10.2
20E-3-β-D-glucoside	32.4	47.0	10.5
20E-22-β-D-glucoside	32.6	40.0	7.5
20E-25-β-D-glucoside	35.9	40.2	9.1
Ponasterone A	5.4	4.4	155.1
2	8.6	7.2	146.3

CIW = cyclohexane–isopropanol–water; DIW = dichloromethane–isopropanol–water; ACN-iPrOH = acetonitrile–isopropanol; flow rate = 1 ml min^{-1} in each case.

Zorbax-SIL analytical silica column and Apex II Diol 5 μm (Jones Chromatography) semiprep column throughout this text. Chromatographic separations were monitored simultaneously at two wavelengths, 242 and 280 nm.

Radioimmunoassay. RIA was performed according to the procedure described previously [16] using ecdysteroid-specific antisera, DBL-1 and Black, which were donated by Prof. J. Koolman (University of Marburg, Germany). The cross-reactivities of these antisera with a number of phytoecdysteroids are given elsewhere [10].

Bioassay. The biological activities (ecdysteroid agonist or antagonist) of extracts and HPLC fractions were determined with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line [15].

Plant material. The seeds of *Limnanthes douglasii* were donated by Sutton Seeds, Torquay, UK. Seeds of other species in the genus were generously provided by the Western Regional Plant Introduction Station

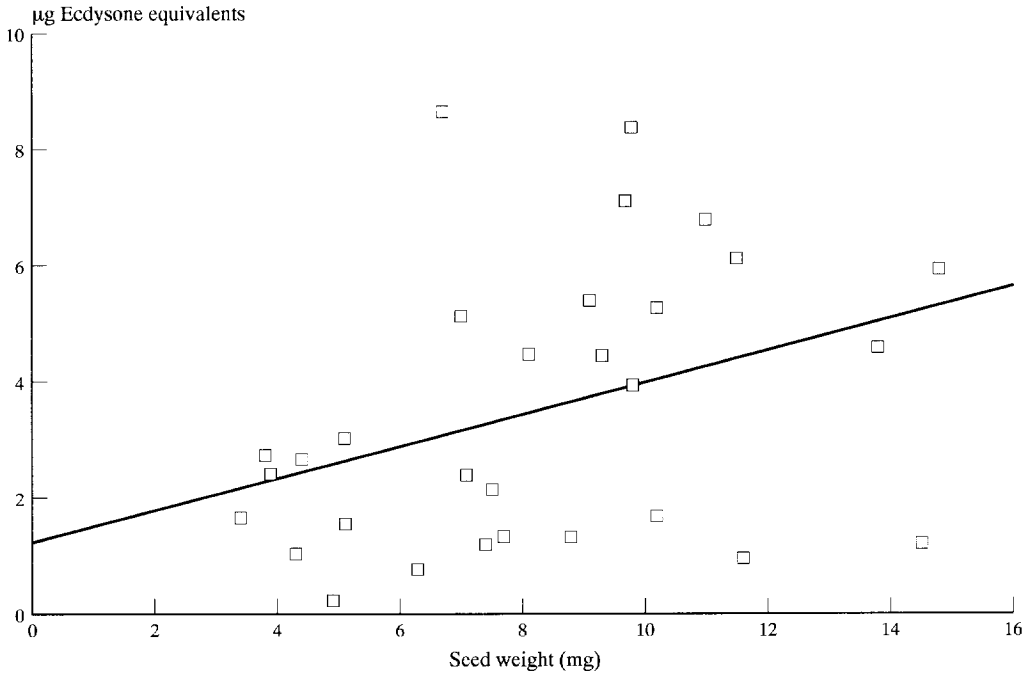


Fig. 3. The relationship between ecdysteroid content and seed weight for individual seeds ($n = 30$) of *Limnanthes douglasii*. Ecdysteroid content was assessed by RIA (DBL-1 antiserum). The regression equation is $y = 1.18 + 0.28x$, with a correlation coefficient of 0.371.

(Pullman, WA, U.S.A.) and the Palmengarten (Frankfurt-am-Main, Germany), or purchased from commercial suppliers.

Micro-extraction of plant material. Plant portions were freeze-dried for 4 days and seeds were ground in a pestle and mortar. Samples (< 25 mg) were extracted three times with MeOH (1 ml) at 55°. The pooled extracts were mixed with 1.3 ml water and 2 ml hexane. The aqueous MeOH phase was analysed for ecdysteroid content by RIA, bioassay and HPLC.

Analytical HPLC. Portions of seed extracts (1 µg ecdysone equivalents with the DBL-1 antiserum) were separated by RP-anal. column eluted at 1 ml min^{-1} with a gradient from 30 to 100% MeOH in H_2O over 30 min, followed by elution with MeOH for a further 10 min. Fractions (1 ml) were collected and monitored by agonist bioassay and RIA.

Large-scale extraction. Ground seeds (50 g) were extracted (4 × 24 hr) with 4 × 200 ml MeOH at 50° with constant stirring using a magnetic stirrer. Extracts were pooled and H_2O added to give a 70% aq. methanolic soln. After being defatted with *n*-hexane the extract was concentrated using a rotary evaporator at a maximum temperature of 45°.

Isolation of compounds. Sep-Pak fractionation of the concentrated extract (redissolved in 10% aq. MeOH) using MeOH-H_2O step gradient, followed by bioassay/RIA revealed the presence of ecdysteroids in the 60% MeOH-H_2O fraction which was then subjected to HPLC using a prep. RP-column (isocratic elution with 55% MeOH-H_2O, 5 ml min^{-1}) to yield 5 fractions. Fractions 2 (R_t 18–20 min) and 3 (R_t 33–36 min) were found to be bioassay/RIA positive. Further NP-HPLC analyses of fraction-2 on NP-semiprep column (isocratic elution with 6% MeOH in CH_2Cl_2, 2 ml min^{-1}) produced 20E (12.2 mg, R_t 13.1 min) and **1** (10 mg, R_t 19.2 min). Similar purification of fraction 3 yielded ponasterone A (0.3 mg, R_t 5.2 min) and **2** (2.8 mg, R_t 10.8 min).

Limnantheoside A [*20-hydroxyecdysone-3-β-D-xylopyranoside*] (**1**). Gum. UV λ_{max} nm (log ε): 241 (3.96). ^1H NMR (Table 1). ^{13}C NMR (Table 2). Found: $[M]^+$ 612; $C_{32}H_{52}O_{11}$; CIMS *m/z*: 630 $[M+NH_4]^+$, 613 $[M+H]^+$, 595 $[M+H-H_2O]^+$, 577 $[595-H_2O]^+$, 559 $[577-H_2O]^+$, 541 $[559-H_2O]^+$, 496, 481 $[M+H-C_5H_8O_4]^+$, 461, 463, 445, 427, 363, 345, 301.

Limnantheoside B [*ponasterone A-3-β-D-xylopyranoside*] (**2**). Gum. UV λ_{max} nm (log ε): 242 (4.00). ^1H NMR (Table 1). ^{13}C NMR (Table 2). Found: $[M]^+$ 596; $C_{32}H_{52}O_{10}$; CIMS *m/z*: 614 $[M+NH_4]^+$, 597 $[M+H]^+$, 579 $[M+H-H_2O]^+$, 561 $[579-H_2O]^+$, 543, 512, 481, 465 $[M+H-C_5H_8O_4]^+$, 447, 429, 363, 345, 191, 166, 150, 116, 102.

20-Hydroxyecdysone. Amorphous. HPLC, UV, ^1H NMR (Table 2, ^{13}C NMR (Table 3) and CIMS data as reported [12, 13].

Ponasterone A. Amorphous. HPLC, UV, ^1H NMR, ^{13}C NMR and CIMS data as reported [13].

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