Phytochemistry, Vol. 25, No. 10, pp. 2375–2379, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SESQUITERPENE LACTONES FROM *LACTUCA LACINIATA*

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(*Received* 4 *February* 1986)

Key Word Index—*Lactuca laciniata*; Compositae; sesquiterpene lactones; 9α-hydroxyzaluzanin C; 9α-hydroxy-11,13α-dihydrozaluzanin C; lactucopicriside; lactulide A; lactuside A; lactuside B.

Abstract—From the roots of *Lactuca laciniata*, six new sesquiterpene lactones, 9α-hydroxyzaluzanin C, 9α-hydroxy-11,13α-dihydrozaluzanin C, lactucopicriside, lactulide A, lactuside A and lactuside B, have been isolated together with known compounds, macrocliniside A, glucozaluzanin C, 11,13α-dihydroglucozaluzanin C, 11β,13-dihydrolactucin and dihydrosantamarin. The structures were established by spectral data and X-ray diffraction analysis.

INTRODUCTION

The sesquiterpene lactone glycosides show considerable biological activity in a survival test [1], but so far they have not been isolated and characterized. In the course of a search for sesquiterpene lactone glycosides in Compositae plants [1–3], we have examined the roots of *Lactuca laciniata* Makino, and isolated three new sesquiterpene glycosides and three new sesquiterpene lactones together with previously known compounds. The identification of these compounds is described in this paper.

RESULTS AND DISCUSSION

9α-Hydroxyzaluzanin C (**1**) showed absorptions at 3440 (hydroxyl) and 1755 cm^{-1} (γ-lactone) in the IR spectrum. In addition to signals of an exocyclic α-methylene-γ-lactone at δ6.24 and 5.58, signals due to two exomethylene groups at 5.44, 5.33 and 5.15 were observed in the ^1H NMR spectrum, and the ^{13}C NMR spectrum exhibited fifteen signals. These data suggested that **1** had a guaianolide-type skeleton. The structure of **1** was finally established by direct comparison with an aglycone of macrocliniside A (**7**) [1].

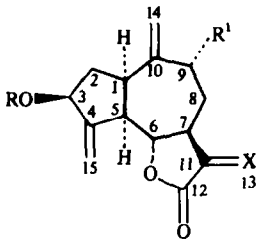
The ^1H NMR spectrum of 9α-hydroxy-11,13α-dihydrozaluzanin C (**2**) was similar to that of **1** except for the absence of the exocyclic methylene proton signals of C-13 and the appearance of a doublet methyl signal at δ1.24. From these data, **2** was assumed to be the reduction product of **1**, and this was supported by ^{13}C NMR data. Finally, **2** was shown to be identical with an aglycone of ixerin H [4] by comparing the ^1H NMR spectra.

Lactucopicriside (**3**) showed absorptions at 3410 (hydroxyl), 1770 (γ-lactone), 1740 (ester) and 1690 cm^{-1} (conjugated carbonyl) in the IR spectrum. Its ^1H NMR spectrum exhibited signals for an exocyclic α-methylene-γ-lactone at δ6.15 and 5.48, an olefinic proton at 6.92, a hydroxymethyl group at 5.18 and 5.00, an anomeric proton at 4.93, a vinylmethyl group at 2.44 (above data were similar to those of picriside A [3]) and an A_2B_2 type at 7.40 and 7.09. Comparing the ^1H NMR signals with those of picriside A, the signals of the α-methylene-γ-lactone were shifted upfield and changed into doublets, suggesting that **3** had an ester group at C-8 [5]. The signals of the ^{13}C NMR spectrum suggested that **3** was a picriside A analogue having a *p*-hydroxyphenylacetate at C-8. The assumption was confirmed by partial hydrolysis of **3** to give picriside A and *p*-hydroxyphenylacetic acid.

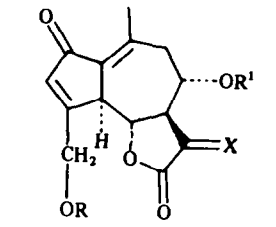
Lactulide A (**4**) showed absorption at 3500 (hydroxyl), 1750 (γ-lactone) and 1650 cm^{-1} (conjugated aldehyde) in the IR spectrum. In the ^{13}C NMR spectrum, fifteen signals were observed and the presence of two double bonds was suggested. We determined the configuration of the double bonds on the basis of NOE experiments. Irradiation of the H-14 aldehydic proton signal (δ9.43) increased the intensity of the H-1 signal (δ6.55) about 11%, and irradiation of the H-15 vinylmethyl signal (δ1.84) produced an about 12% enhancement in the intensity of the H-6 signal (δ4.68). The above results showed that **4** was a melampolide having the 1,10(*E*) and 4,5(*Z*)-configuration. In decoupling experiments, long-range coupling was observed between the carbinol proton signal at δ4.24 and the H-5 signal. This suggested that **4** had a hydroxyl group at C-3. In order to determine the structure of **4**, it was subjected to X-ray analysis. The stereoscopic view of the molecule is shown in Fig. 1. This gives the relative configuration, but in all naturally occurring sesquiterpene lactones the absolute configuration of the C-7 side chain is β.

Lactuside A (**5**) showed a ^1H NMR spectrum which was similar to that of **4**. The ^{13}C NMR spectrum was also similar to that of **4** but six additional signals were observed, which were assigned to a glucopyranosyl residue. Enzymatic hydrolysis of **5** afforded **4** as an aglycone, and acid hydrolysis afforded glucose as a sugar moiety. The glycosidic linkage was deduced to be β from the $J_{C_1\text{-}H_1}$ coupling constant (152 Hz) [6].

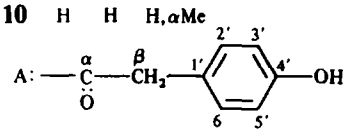
Lactuside B (**6**) showed six signals of a glucopyranosyl residue and fifteen signals assignable to the aglycone moiety in the ^{13}C NMR spectrum. Enzymatic hydrolysis of **6** gave **6a**. Oxidation of **6a** with pyridinium chlorochromate gave **4** [7]. In the ^{13}C NMR spectrum of **6**, the signals showed glycosidation shifts as compared with those of **6a**: C-2 and C-4 (each β-position) at δ32.8 (Δ −2.9 ppm) and 140.6 (Δ −2.8 ppm), respectively, and C-3

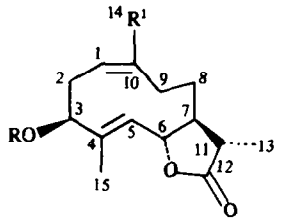


	R	R^1	X
1	H	OH	CH_2
2	H	OH	H,αMe
7	Glc	OH	CH_2
8	Glc	H	CH_2
9	Glc	H	H,αMe

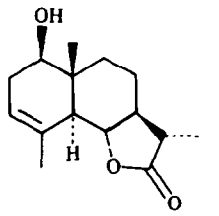


	R	R^1	X
3	Glc	A	CH_2
3a	Glc	H	CH_2
10	H	H	H,αMe





	R	R^1
4	H	CHO
5	Glc	CHO
6	Glc	CH_2OH
6a	H	CH_2OH



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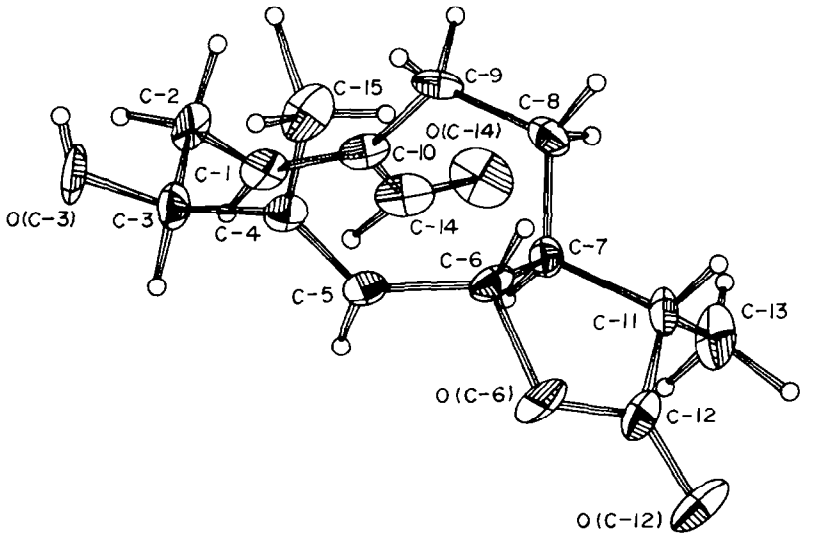

Fig. 1.

Table 1. ^{13}C NMR spectral data for compounds 1–6*

Carbon no.	1	2	3	4	5	6	6a
Aglycone moiety							
1	41.3	41.8	133.4	151.1	150.1	127.4	127.5
2	39.0	39.0	194.2	36.0	32.9	32.8	35.7
3	73.1†	73.3‡	134.8	74.8	79.8	83.3	78.0
4	155.3	155.7	169.0	139.8	136.8	140.6	143.4
5	49.3	49.3	48.5	123.7	127.0	126.7	124.6
6	85.0	84.5	81.1	80.6	80.2	80.8	81.2
7	35.9	35.7	54.3	49.6	49.5	54.5	54.6
8	40.0	41.0	69.8	22.4	22.4	29.0	29.0
9	72.7†	73.0‡	44.0	25.6	25.5	36.8	36.8
10	153.5	153.9	145.3	145.5	145.6	142.1	141.8
11	141.0	45.2	136.9	41.5	41.3	42.3	42.4
12	170.2	178.6	168.2	178.6	178.5	178.4	178.6
13	119.1	13.3	121.4	12.8	12.8	13.3	13.4
14	112.0	111.4	20.9	195.7	195.6	58.6	58.6
15	108.0	107.9	68.5	11.1	11.2	11.6	11.7
Sugar moiety							
1			103.9		101.9	102.5	
2			74.9		74.9	75.0	
3			78.1		78.2	78.2	
4			71.4		71.6	71.7	
5			78.0		78.2	78.1	
6			62.5		62.7	62.8	
p-Hydroxyphenylacetic acid moiety							
α			170.9				
β			40.7				
1			124.3				
2			130.9				
3			116.4				
4			158.0				
5			116.4				
6			130.9				

*Run at 22.5 MHz in pyridine-d_5.
†‡Assignments may be interchanged in each column.

Table 2. ^1H NMR spectral data of compounds 4, 5 (90 MHz) and 6a (400 MHz)

H	4 ($CDCl_3$)	5 (C_5D_5N)	6a (C_5D_5N)
1	6.56 (1H, *br t*, $J=8$ Hz)	6.24 (1H, *br t*, $J=8$ Hz)	5.10 (1H, *dd*, $J=13, 4$ Hz)
3	4.24 (1H, *m*)	3.90–4.90 (*m*)	4.58 (1H, *dd*, $J=10, 7$ Hz)
5	4.97 (1H, *br d*, $J=10$ Hz)	5.04 (1H, *br d*, $J=10$ Hz)	4.94 (1H, *br d*, $J=10$ Hz)
6	4.68 (1H, *t*, $J=10$ Hz)	3.90–4.90 (*m*)	4.86 (1H, *t*, $J=10$ Hz)
13	1.17 (3H, *d*, $J=7$ Hz)	1.18 (3H, *d*, $J=7$ Hz)	1.26 (3H, *d*, $J=7$ Hz)
14	9.43 (1H, *d*, $J=1.5$ Hz)	9.49 (1H, *br s*)	4.19 (1H, *br d*, $J=13$ Hz)
			4.63 (1H, *br d*, $J=13$ Hz)
15	1.89 (3H, *d*, $J=1.5$ Hz)	2.14 (3H, *br s*)	1.90 (3H, *br s*)

(α-position) at δ83.3 (Δ + 5.3 ppm) [8]. Acid hydrolysis of **6** gave glucose and the anomeric structure was determined to be β from the $J_{C_1-H_1}$ value (155 Hz). These results led us to conclude the structure of lactuside B to be **6**.

In addition to new compounds, macrocliniside A, glucozaluzanin C, 11,13α-dihydroglucozaluzanin C, 11β,13-dihydrolactucin and dihydrosantamarin were isolated. The identities of macrocliniside A and glucozaluzanin C, which had been isolated from *Macroclinidium trilobum* Makino [1], were established by direct comparisons with authentic samples. 11,13α-Dihydroglucozaluzanin C was identical with an authentic sample, which had been derived from glucozaluzanin C in our laboratory [9], but was isolated as a natural product for the first time. 11β,13-Dihydrolactucin had been isolated from *Picris hieracioides* L. var. *japonica* Regel in

our laboratory [3]. Dihydrosantamarin was shown to be identical with an authentic sample, which had been derived from naturally occurring santamarin [10], by comparing the ^{13}C NMR data.

EXPERIMENTAL

All mps are uncorr. ^1H and ^{13}C NMR spectra were recorded on JEOL FX-90Q (89.55 and 22.5 MHz respectively) and GX-400 (399.65 MHz). TMS was used as an internal standard.

Plant material. Roots of *Lactuca laciniata* Makino were collected in Shizuoka, Japan in June 1985. Plants were identified by Dr. A. Ueno, and a voucher specimen has been deposited in the Herbarium, Shizuoka College of Pharmacy.

Extraction and isolation. Roots of *L. laciniata* (fr. wt 10 kg) were extracted with H_2O. The crude extract was passed through an Amberlite XAD-2 column, and the eluate with MeOH was concd under red. pres. The residue (33 g) was chromatographed on a polyamide (150 g) column with H_2O as eluant to give Fr. 1 (18 g) and H_2O–MeOH (7:3) to give Fr. 2 (1.4 g). Further elution with MeOH gave Fr. 3 (6.6 g). Fr. 1 was rechromatographed on a silica gel column with C_6H_6–Me_2CO (9:1) as eluant to give 10 mg **1**, 10 mg **2**, 200 mg **4**, 100 mg **10** and 40 mg **11**, and $CHCl_3$–MeOH (9:1) as eluant to give 100 mg **3**, 180 mg **5**, 600 mg **6**, 200 mg **7**, 50 mg **8** and 50 mg **9**.

9α-Hydroxyzaluzanin C (**1**). Colourless crystals from H_2O–MeOH, mp 76–78°, $[\alpha]_D^{25}$ + 13.0° (*c* 0.58; MeOH). $C_{15}H_{18}O_4$ (high resolution MS: 262.1218; calc. 262.1206); IR ν_{max}^{KBr} cm^{-1}: 3440, 1755, 1635. ^1H NMR (90 MHz, $CDCl_3$): δ6.24 (1H, *d*, *J* = 3.6 Hz, H-13a), 5.58 (1H, *d*, *J* = 3.2 Hz, H-13b), 5.44, 5.33 (each 1H, *t*, *J* = 2 Hz, H-15), 5.15 (2H, *s*, H-14), 4.66 (2H, *m*, H-3, H-9), 4.02 (1H, *t*, *J* = 9 Hz, H-6). ^{13}C NMR: Table 1. EIMS (75 eV) *m/z* (rel. int.): 262 $[M]^+$ (4), 244 $[M-H_2O]^+$ (17), 226 $[244-H_2]^+$ (9), 131 (46), 105 (49), 96 (69), 91 (92), 79 (89), 77 (61), 55 (59), 53 (100), 43 (61), 41 (87).

9α-Hydroxy-11,13α-dihydrozaluzanin C (**2**). Amorphous, $[\alpha]_D^{25}$ + 50.7° (*c* 0.75; MeOH). $C_{15}H_{20}O_4$ (high resolution MS: 264.1342; calc. 264.1362); IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1640. ^1H NMR (90 MHz, $CDCl_3$): δ5.36, 5.30 (each 1H, *br s*, H-15), 5.08 (2H, *s*, H-14), 4.60 (2H, *m*, H-3, H-9), 3.98 (1H, *t*, *J* = 9 Hz, H-6), 1.24 (3H, *d*, *J* = 6 Hz, H_3-13). ^{13}C NMR: Table 1. EIMS (75 eV) *m/z* (rel. int.): 264 $[M]^+$ (8), 249 $[M-Me]^+$ (4), 246 $[M-H_2O]^+$ (18), 231 $[249-H_2O]^+$ (5), 228 $[246-H_2O]^+$ (6), 173 (50), 172 (40), 131 (42), 123 (51), 105 (58), 96 (63), 95 (57), 91 (78), 79 (95), 77 (55), 55 (84), 53 (50), 43 (72), 41 (100).

Lactucopicriside (**3**). Amorphous powder. (Found: C, 59.73; H, 5.71. $C_{29}H_{32}O_{12} \cdot 1/2\ H_2O$ requires: C, 59.89; H, 5.72%.) $[\alpha]_D^{25}$ − 15.3° (*c* 1.47; MeOH), IR ν_{max}^{KBr} cm^{-1}: 3410, 1770, 1740, 1690, 1620, 1520. ^1H NMR (90 MHz, C_5D_5N): δ7.40 (2H, *d*, *J* = 9 Hz, H-2',6'), 7.09 (2H, *d*, *J* = 9Hz, H-3',5'), 6.92 (1H, *br s*, H-3), 6.15 (1H, *d*, *J* = 3.1 Hz, H-13a), 5.48 (1H, *d*, *J* = 2.6 Hz, H-13b), 5.18, 5.00 (each 1H, *br d*, *J* = 17 Hz, H-15), 4.93 (1H, *d*, *J* = 7 Hz, anomeric proton), 2.44 (3H, *s*, H_3-14). ^{13}C NMR: Table 1.

Lactulide A (**4**). Colourless prisms from MeOH, mp 191.5–195°. (Found: C, 68.14; H, 7.55. $C_{15}H_{20}O_4$ requires: C, 68.16; H, 7.63%.) $[\alpha]_D^{25}$ − 82.1° (*c* 0.95; C_5H_5N). IR ν_{max}^{KBr} cm^{-1}: 3500, 1750, 1675, 1630. ^{13}C and ^1H NMR: Tables 1 and 2. EIMS (75 eV) *m/z* (rel. int.): 264 $[M]^+$ (4), 246 $[M-H_2O]^+$ (2), 235 $[M-CHO]^+$ (2), 218 (7), 217 (5), 207 (5), 191 (15), 181 (20), 109 (71), 55 (94), 41 (100).

X-Ray analysis. Single crystals of **4** were grown by slow crystallization from MeOH. They were monoclinic, space group $P2_1$, with *a* = 10.364 (3), *b* = 7.059 (2), *c* = 9.734 (3) Å, β = 100.11° (3) and d_{calc} = 1.253 g/cm³ for *Z* = 2 (*M*, 264.32). The intensity data were measured on a Philips PW 1100 four-circle diffractometer (Cu radiation, monochromated, θ-2θ scans). The size of the crystal used for data collection was approximately 0.4 × 0.3 × 0.2 mm³. No absorption correction was necessary (μ = 8.560 cm^{-1}). A total of 1596 reflections was measured for 3° ≤ θ ≤ 78°, of which 1524 reflections were considered to be observed [$I \geq \delta(I)$]. The structure was solved by the direct method using RANTAN program [11] and refined by the block-diagonal least-squares methods assuming the anisotropic temperature factors for non-H atoms and the isotropic ones for H atoms. The final discrepancy indices were *R* = 0.053 using a unit weight for all reflections. The final difference Fourier map was essentially featureless; the highest residual peaks had densities of 0.2 $eÅ^{-3}$. The list of atomic coordinates and thermal parameters, bond distances and angles, comparison of the observed and calculated structure factors and torsion angles have been deposited at the Cambridge Crystallographic Data Centre.

Lactuside A (**5**). Amorphous powder. (Found: C, 59.22; H, 6.84. $C_{21}H_{30}O_9$ requires: C, 57.65; H, 7.60%.) $[\alpha]_D^{25}$ − 73.8° (*c* 0.84; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3410, 1770, 1680, 1630. ^{13}C and ^1H NMR: Tables 1 and 2.

Lactuside B (**6**). Amorphous powder. (Found: C, 57.94; H, 7.44. $C_{21}H_{32}O_9 \cdot 1/2\ H_2O$ requires: C, 57.65; H, 7.60%.) $[\alpha]_D^{22}$ + 21.9° (*c* 0.98; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1760, 1640. ^1H NMR (C_5D_5N): δ1.95 (3H, *br s*, H_3-15), 1.28 (3H, *d*, *J* = 7 Hz, H_3-13). ^{13}C NMR: Table 1.

11,13α-Dihydroglucozaluzanin C (**9**). Amorphous powder, $[\alpha]_D^{22}$ + 13.3° (*c* 0.64; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3420, 1770, 1640. ^1H NMR (90 MHz, C_5D_5N): δ5.90, 5.52 (each 1H, *br s*, H-15), 5.04 (2H, *br s*, H_2-14), 1.18 (3H, *d*, *J* = 7 Hz, H_3-13). ^{13}C NMR (C_5D_5N): δ178.1 (C-12), 149.5 (C-10), 113.2 (C-14), 111.9 (C-15), 104.1 (C-1 of glucose), 83.3 (C-6), 80.4 (C-3), 78.4 (C-3 of glucose), 78.1 (C-5 of glucose), 75.1 (C-2 of glucose), 71.7 (C-4 of glucose), 62.8 (C-6 of glucose), 50.3 (C-5), 49.8 (C-11), 44.0 (C-1), 42.2 (C-7), 37.8 (C-2), 35.9 (C-9), 32.3 (C-8), 13.3 (C-13).

Partial hydrolysis of **3**. Compound **3** (20 mg) was treated with 2 N HCl (1 ml) under a nitrogen atmosphere for 10 hr at 35°. The reaction mixture was passed through an Amberlite XAD-2 column, and the fraction eluted with MeOH was purified by HPLC (MeCN–H_2O, 3:17) to give **3a** (3 mg) and *p*-hydroxyphenylacetic acid (2 mg). *p*-Hydroxyphenylacetic acid was converted into the *p*-bromophenacyl ester and it was shown to be identical with an authentic sample by HPLC, YMC-Pack AM-312 6 mm × 15 cm; MeCN–H_2O (9:11); 1.4 ml/min; UV 220 nm; R_t 6.3 min.

Enzymatic hydrolysis of **5** *and* **6**. Compound **5** (15 mg) was dissolved in H_2O (2 ml) and the soln was treated with crude hesperidinase (10 mg) for 2 hr at 35° with stirring. The soln was passed through an Amberlite XAD-2 column, and the eluate with MeOH was purified on a silica gel column to give **4** (6 mg). Compound **6** (200 mg) was hydrolysed in the same way to give **6a** (80 mg).

Acknowledgements—We thank Professor Y. Iitaka and Dr. A. Itai, Tokyo University, for the X-ray crystallographic analysis of lactulide A. We are also grateful to Professor S. Arihara, Tokushima-Bunri University, for measurement of 400 MHz NMR spectra, and to Mrs. Kitamura of our college for elemental analysis.

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