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MINOR SESQUITERPENE LACTONES FROM *LACTUCA VIROSA*

WANDA KISIEL* and BARBARA BARSZCZ

Department of Phytochemistry, Institute of Pharmacology, Polish Academy of Sciences, Pl-31-343 Kraków, Poland

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Key Word Index—*Lactuca virosa*; Asteraceae; Lactuceae; sesquiterpene lactones; guaianolides; germacranolides; glycosides.

Abstract—One new and six known guaianolides, five known guaianolide glycosides, one new and one known germacrolide glycoside, one known melampolide glycoside, and eugenyl glycoside were isolated from the roots of *Lactuca virosa*. The new compounds were characterized as 11β,13-dihydrolactucin-8-*O*-methacrylate and 3β,14-dihydroxy-11β,13-dihydrocostunolide-3-*O*-β-glucopyranoside by spectral methods. © 1997 Elsevier Science Ltd

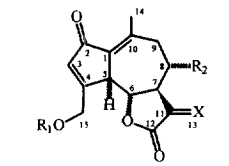
INTRODUCTION

Lactuca virosa L. is a biennial herb producing in the first year a rosette of leaves, and in the second year an erect stem bearing yellow, ligulate flowers. Latex from the flowering parts, when left in the open, dries into the brown gummy product, known as *lactucarium*, which is reputed to be an analgesic, antitussic and a sedative agent due to the presence of the guaiane-type sesquiterpene lactone constituents lactucin and lactucopicrin [1–3]. In a recent pharmacological study freeze-dried latex from the roots of one-year plants reduced the spontaneous locomotor activity in mice at a dose of 2 mg kg^{-1} and showed an analgesic effect at a dose of 15 mg kg^{-1} [4]. The roots, however, were reported to contain no lactucin and lactucopicrin, but the structurally related guaianolides jacquinelin (**2**), 8-desoxylactucin (**1**) and 11β,13-dihydrolactucin (**4**), and the melampolide glucoside lactuside A (**16**) [5, 6].

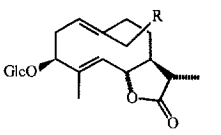
This paper deals with the isolation of further sesquiterpene lactones from this plant which were obtained as minor secondary metabolites from the roots collected in the first year of its vegetative period.

RESULTS AND DISCUSSIONS

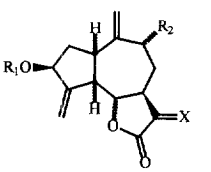
The ethanol extract of the fresh roots, after silica gel column chromatography followed by repetitive preparative TLC and reversed-phase HPLC, yielded 15 sesquiterpene lactone aglycones and glycosides. All the aglycones appeared to be guaianolides derived from lactucin or zaluzanin C. The glycosides were guaianolide-, germacrolide- and melampolide-β-glucopyranosides. Of these, compounds **1**, **2**, **4** and **16** were previously described constituents of *L. virosa* [5, 6], compounds **3**, **5**, **7**, **8**, **12** and **15** were reported from



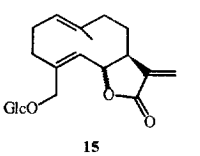
	R_1	R_2	X
1	H	H	CH_2
2	H	H	H,αMe
3	Glc	H	H,αMe
4	H	OH	H,αMe
5	H	OA	H,αMe
6	H	OB	H,αMe
7	Glc	OH	H,αMe



13 R=H
14 R=OH



	R_1	R_2	X
8	H	OH	CH_2
9	H	OH	H,αMe
10	Glc	H	H,αMe
11	Glc	OH	CH_2
12	Glc	OH	H,αMe



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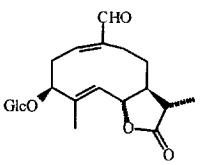

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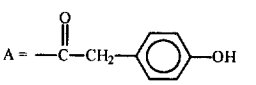

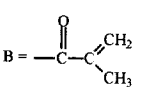

* Author to whom correspondence should be addressed.

other *Lactuca* species [7–11], while compounds **6** and **14** were new plant constituents.

The known compounds were, in order of elution from silica gel: jacquinelin (**2**) [12], 8-desoxylactucin (**1**) [13], 11β,13-dihydrolactucopicrin (**5**) [14], 9α-hydroxyzaluzanin C (**8**) [7], 9α-hydroxy-11β,13-dihydrozaluzanin C (**9**) [7], 11β,13-dihydrolactucin (**4**) [15], picriside B (**15**) [16], 11β,13-dihydroglucozaluzanin C (**10**) [7], crepidiaside B (**3**) [17], lactuside A (**16**) [7], macrocliniside A (**11**) [18], ixerin F (**12**) [19] and cichorioside B (**7**) [20]. They were identified by direct comparison (HPLC, ^{1}H NMR, MS) with compounds in our collection. In addition, eugenyl-β-glucopyranoside was isolated and identified by spectral comparison with reported values [21].

Compound **6** which was isolated in very small amounts from the less polar eluates of the chromatographic column, appeared to be a new lactucin analogue. Its ^{1}H NMR data (Table 1) closely resembled those of 11β,13-dihydrolactucin-8-*O*-acetate, previously isolated from *Lactuca floridana* [22, 23], except for the signals of the esterifying acid.

Table 1. ^{1}H NMR data of compounds **6** and **14** (500.13 MHz, TMS as int. standard)

H	**6** $CDCl_3$	**14** pyridine-d_5
1		4.88 *dd*
2α		2.52 *br ddd*
2β		2.68 *ddd*
3	6.46 *br d*	4.81 *dd*
5	3.68 *br d*	4.90 *br d*
6	3.75 *dd*	4.76 *dd*
7	2.36 *ddd*	1.66 *m*
8α		1.66 *m*
8β	4.94 *ddd*	1.81 *m*
9α	2.77 *dd*	1.81 *m*
9β	2.43 *dd**	3.10 *br dd*
11	2.42 *dq**	2.31 *dq*
13	1.32 *d*	1.23 *d*
14	2.47 *s*	4.47 *br d*
14′		4.10 *br d*
15	4.56 *br d*	1.93 *s*
15′	4.87 *br d*	
	Ester moiety	Glucose moiety
1		4.83 *d*
2		4.08 *dd*
3	1.98 *dd* (CH_3)	4.18–4.27 *m*
4	6.17 *dq* (H-4a)	4.18–4.27 *m*
5	5.69 *dq*(H-4b)	3.91 *m*
6		4.40 *dd*
6′		4.60 *br d*

* Partially overlapped signals.

J (Hz) compound **6**: 5,6 = 6,7 = 10.1; 7,8β = 8β,9α = 10.6; 7,11 = 11.2; 8β,9β = 2; 9α,9β = 13.8; 11,13 = 6.9; 15,15′ = 17.7; ester moiety: 3,4 = 4a,4b = 1.3; compound **14**: 1,2α = 4.0; 1,2β = 12.2; 2α,2β = 12.2; 2α,3 = 5.9; 2β,3 = 10.6; 5,6 = 6,7 = 9.6; 7,11 = 12.4; 11,13 = 6.9; 14,14′ = 12.4; glucose moiety: 1,2 = 7.8; 2,3 = 7.5; 5,6 = 5.3; 6,6′ = 11.7.

Instead of the acetyl methyl singlet in the ^{1}H NMR spectrum, compound **6** exhibited resonances characteristic of a methacrylate. Its EI mass spectrum showed a $[M]^+$ at *m/z* 346 consistent with the molecular formula $C_{19}H_{22}O_6$ and prominent peaks at *m/z* 260 $[M-C_3H_5COOH]^+$, 69 $[C_3H_5CO]^+$ and 41 $[C_3H_5]^+$ which agreed with the structure proposed, i.e. 11β,13-dihydrolactucin-8-*O*-methacrylate.

The second new compound **14** was the most polar of the isolated glycosides from this plant material. The sharp doublet (J = 7.8 Hz) of the anomeric proton at δ 4.83 and remaining signals of the glucose moiety were readily identifiable in its ^{1}H NMR spectrum. Other signals in the spectrum indicated that **14** was a sonchuside A (**13**) (3β-hydroxy-11β,13-dihydrocostunolide-3-*O*-β-glucopyranoside) [24] analogue having a hydroxyl group at C-14. As expected, the negative ion mode LSI mass spectrum showed an $[M-H]^-$ peak at *m/z* 427 consistent with the molecular formula, $C_{21}H_{32}O_9$, containing one oxygen more than that of **13** and a further peak at *m/z* 265 corresponding to the loss of a hexose unit. In the ^{1}H NMR spectrum of **14**, the singlet of the olefinic methyl group at C-10 was replaced by a two-proton AB system (J = 12.4 Hz) at δ 4.47 and 4.10. Consequently, a downfield shift in the resonance of H-9β ($\Delta\delta$ = 0.91 ppm) was observed and the signals of H-2β and H-8β were slightly affected. In all other respects the spectrum was identical with the spectrum of **13** published recently [11]. Based on the above findings, the structure of **14** was 3β,14-dihydroxy-11β,13-dihydrocostunolide-3-*O*-β-glucopyranoside.

It is interesting to note that the examined plant material was rich in lactuside A (**16**), which made up over 50% of the total sesquiterpene lactone constituents.

EXPERIMENTAL

General procedure. CC: silica gel (Merck Art 7733 and 7754); TLC: silica gel (Art 5553); semiprep. and analytical HPLC: Delta-Pak C 18 cartridge column (particle size 15 μm, 25 mm × 100 mm) and μBondapack C 18 column (particle size 10 μm, 2 mm × 300 mm), respectively, using MeOH–H_2O systems as mobile phase and monitoring with a UV photodiode-array detector.

Plant material. Roots of biennial *L. virosa* were collected in October 1995 from plants in the first year of vegetation, growing in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, where a voucher (No 3/96) specimen was deposited. The plant was authenticated by curator of the Garden—E. Binek.

Extraction and isolation of compounds. Fresh roots (5 kg) were cut into pieces and submerged in boiling EtOH (8 l), and left to stand in a cold room. Evapn. of the solvent at red. pres. gave 225 g of residue which was coarsely prefractionated on silica gel using successively hexane, hexane–EtOAc (1:1), EtOAc,

EtOAc–MeOH (1:1) and MeOH. Frs containing lactone constituents, eluted with hexane–EtOAc (1:1) and EtOAc, were combined and the solvent was removed giving 10.49 g of residue which was chromatographed on a silica gel column, using hexane and hexane–EtOAc (up to 100% EtOAc) followed by EtOAc–MeOH (up to 10% MeOH) gradient solvent systems.

Frs from hexane–EtOAc (1:1) were further sepd by prep. TLC ($CHCl_3$–MeOH, 19:1) to give **6** (2.7 mg) and a mixt. of **1** and **2** (81.9 mg). The first frs from EtOAc elution, after prep. TLC ($CHCl_3$–MeOH, 9:1), yielded **5** (3.8 mg), a mixt. of **8** and **9** (4.5 mg) and **4** (24 mg), in that order.

The later EtOAc frs and frs eluted with EtOAc–MeOH (19:1) contained sesquiterpene lactone glycosides. The frs were bulked according to their homogenity, as shown by TLC, and then purified by prep. TLC ($CHCl_3$–MeOH, 17:3 or 4:1) to give a mixt. of less polar glycosides, a mixt. rich in **16** (154 mg), pure compound **16** (122 mg), a mixt. of more polar glycosides and pure compound **14** (7.3 mg), respectively. The former mixt. was processed by semiprep. HPLC (MeOH–H_2O, 1:1, 3 ml min^{-1}) to give eugenol glucoside (R_t = 44.05 min, 3.4 mg) followed by **10** and **15** collected from an apparently single peak (R_t = 73.03 min, 6.6 mg). The latter mixt., after semiprep. HPLC (MeOH–H_2O, 7:13, 3 ml min^{-1}) sepn, furnished **7** (R_t = 20.07 min, 4 mg), **12** (R_t = 47.38 min, 31 mg) and **11** (R_t = 58.54 min, 32 mg). The intermediate mixt., on analytical HPLC (MeOH–H_2O, 7:13, 0.5 ml min^{-1}), was found to consist of **16** (R_t = 5.33 min) and **3** (R_t = 9.50 min) in a ratio *ca* 4:1, respectively. The mixts of **1** and **2** (*ca* 1:1), **8** and **9** (*ca* 1:2), and **10** and **15** (*ca* 1:3) were not sepd further, as the ^1H NMR signals could be assigned to the respective compounds by a careful analysis of the integrals.

11β,13-*Dihydrolactucin*-8-O-*methacrylate* (**6**). Gum. EIMS *m/z* (rel. int.): 346 $[M]^+$ (20.7), 260 $[M-C_3H_5COOH]^+$ (55.6), 231 $[260-CHO]^+$ (33.4), 214 $[260-CO-H_2O]^+$ (29.2), 187 (84.2), 159 (43.4), 86 $[C_3H_5COOH]^+$ (10.0), 69 $[C_3H_5CO]^+$ (90.4), 43 (100), 41 $[C_3H_5]^+$ (99.8); ^1H NMR: Table 1.

3β,14-*Dihydroxy*-11β,13-*dihydrocostunolide*-3-O-β-*glucopyranoside* (**14**). Gum. LSIMS, negative ion mode, *m/z*: 427 $[M-H]^-$, 265 $[M-\text{hexose}-H]^-$; ^1H NMR: Table 1.

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