A Highly Substituted Germacranolide from *Leontodon cichoraceus*

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Z. Naturforsch. **56 c,** 904–908 (2001);
received June 6/July 3, 2001

Asteraceae, Lactuceae, Sesquiterpene Lactones

A re-investigation of subaerial parts of *Leontodon cichoraceus* (Ten.) Sanguin. yielded the new germacrane-type sesquiterpenoid 15-β-D-glucopyranosyl-8-[*p*-(β-D-glucopyranosyloxy)phenylacetyl]-salonitenolide. The structure was established by APCI mass spectrometry and 1D- and 2D-NMR spectroscopy. A survey by HPLC-DAD and HPLC-MS gave no signs of this compound in 23 other taxa of *Leontodon*.

Introduction

The genus *Leontodon* (Lactuceae, Asteraceae) has proven to be a rich source of sesquiterpenoids of the guaiane-type (Pyrek, 1985; Zidorn *et al.*, 1998; Zidorn *et al.*, 2000; Zidorn *et al.*, 2001). A previous investigation of *L. cichoraceus* (Ten.) Sanguin. collected in the Central Italian Marche region yielded glucozaluzanin C (**1**) (Zidorn *et al.*, 2001). In this communication we report about the isolation and structure elucidation of the new natural compound 15-β-D-glucopyranosyl-8-[*p*-(β-D-glucopyranosyloxy)phenylacetyl]-salonitenolide (**2**) from subaerial parts of *L. cichoraceus* collected in the Abruzzo region of Central Italy in May 2000.

Results

The methanol extract (8.8 g) of 79.7 g of subaerial parts from *L. cichoraceus* was chromatographed on silica gel using a gradient of CH_2Cl_2 to MeOH. The fractions containing **2** (5.7 g) were re-chromatographed on silica gel, again using a gradient of CH_2Cl_2 to MeOH. Enriched fractions of **2** (230 mg) were finally purified by Sephadex LH-20 CC using MeOH as eluant to yield 49.5 mg of **2**. The on-line APCI MS spectrum of **2** showed quasi-molecular peaks at *m/z* 740 $[M + NH_4]^+$ and 723 $[M + H]^+$ and major fragment peaks at *m/z* 561 $[M - 162 + H]^+$ and 399 $[M - 2*162 + H]^+$, congruent with a molecular formula of $C_{35}H_{46}O_{16}$. This molecular formula was verified by HRFABMS showing a quasi-molecular peak at 723.287141 $[M + H]^+$ (calculated for $C_{35}H_{47}O_{16}$: 723.286410). ^1H NMR and ^{13}C NMR data (Table I) showed signals assignable to a sesquiterpene moiety, a *p*-hydroxyphenylacetic acid moiety and two glucose moieties. ^1H NMR, ^{13}C NMR, HSQC, HMBC, and GMQCOSY experiments measured in methanol-d_4 and (because of some overlapping signals) in acetone-d_6 revealed that the signals of the sesquiterpene-moiety were assignable to a germacra-1(10),4,11(13)-trienolide derivative, oxygenated in positions 6, 8 and 15. Important HMBC cross-peaks, which revealed the connectivities of the β-

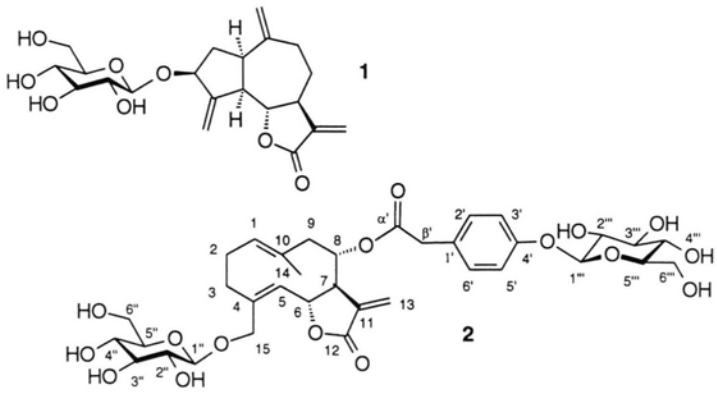

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Table I. NMR data of compound **2**[a].

Position	^{1}H NMR measured in CD_3OD[c] (25 °C)	^{13}C-NMR[b]	^{1}H NMR measured in $(CD_3)_2CO$[d] (45 °C)	^{13}C-NMR
sesquiterpene moiety				
1	5.01 1H, m[e]	129.8 d[e]	5.08 1H, m[e]	130.4
2	2.32 1H, m	26.1 t	2.34 1H, m	26.1
	2.18 1H, m		2.17 1H, m	
3	2.64 1H, m	34.9 t	2.62 1H, ddd (12.0, 5.5, 2.0)	34.8
	2.04 1H, m[e]		2.00 1H, m	
4		141.8 s		142.6
5	4.96 1H, m[e]	129.9 d[e]	5.00 1H, br δ (10.0)	130.6
6	5.19 1H, dd (8.5, 8.5)	78.0 d	5.21 1H, dd (10.0, 8.0)	77.7
7	3.21 1H, dd (8.5, 8.5)	52.9 d	3.24 1H, dd (8.0, 8.0)	53.2
8	5.00 1H, m[e]	73.6 d	5.07 1H, m[e]	73.8
9	2.46 1H, m	48.7[k]	2.49 1H, m	48.8
	2.02 1H, m[e]		2.05 1H, m[e]	
10		132.4 s		133.7
11		136.0 s		137.3[l]
12		171.1 s		170.4
13	6.09 1H, br s	125.1 t	6.09 1H, δ (3.0)	124.6
	5.65 1H, br s		5.70 1H, δ (3.0)	
14	1.54 3H, br s	16.4 q	1.58 3H, s	16.7
15	4.59 1H, δ (12.5)	67.8 t	4.57 1H, dd (13.0, 1.0)	68.3
	4.02 1H, δ (12.5)		4.12 1H, dd (13.0, 1.0)	
p-hydroxyphenylacetic acid moiety				
α'		171.7 s		172.3
β'	3.62 H, δ (10.5)	40.6 t	3.68 2H, m[e]	40.9
	3.48 H, δ (10.5)			
1′		128.0 s		129.4
2′/6′	7.22,[f] 7.20[f] 2H	130.7 d	7.24,[f] 7.22[f] 2H	131.2
3′/5′	7.09,[f] 7.07[f] 2H	117.2 d	7.06,[f] 7.04[f] 2H	117.6
4′		157.5 s		158.7
glucose moiety I				
1″	4.30 1H, δ (8.0)	103.6 d	4.36 1H, δ (7.5)	104.3
2″	3.48 1H, m[e,g]	74.2 d[g]	3.47 1H, m[e,g]	74.9[g]
3″	3.47 1H, m[e,h]	77.3 d[h]	3.51 1H, m[e,h]	77.9[h]
4″	3.31 1H, m[e,i]	70.8 d[i]	3.46 1H, m[e,i]	71.6[i]
5″	3.38 1H, m[e,h]	77.1 d[h]	3.39 1H, m[e,h]	78.2[h]
6″	3.89 1H, dd (12.0, 2.0)[j]	62.0 t[j]	3.89 1H, br δ (12.0)[j]	62.8[j]
	3.70 1H, dd (12.0, 5.5)[j']		3.71 1H, m[e,j']	
glucose moiety II				
1‴	4.90 1H, δ (7.0)	101.6 d	4.95 1H, δ (7.5)	101.6
2‴	3.48 1H, m[e,g]	74.1 d[g]	3.24 1H, m[e,g]	74.9[g]
3‴	3.30 1H, m[e,h]	77.2 d[h]	3.50 1H, m[e,h]	77.9[h]
4‴	3.43 1H, m[e,i]	70.5 d[i]	3.33 1H, m[e,i]	71.9[i]
5‴	3.47 1H, m[e,h]	77.0 d[h]	3.31 1H, m[e,h]	77.6[h]
6‴	3.86 1H, dd (12.0, 2.0)[j]	61.7 t[j]	3.82 1H, br δ (12.0)[j]	63.0[j]
	3.65 1H, dd (12.0, 5.5)[j']		3.66 1H, m[e,j']	

[a] Measured at 500 and 125 MHz, respectively. [b] Multiplicities were derived from DEPT experiments. [c] Referenced to solvent signals at 49.00 ppm (^{13}C NMR) and solvent residual peaks at 3.31 ppm (^{1}H NMR). [d] Referenced to solvent signals at 29.84 ppm (^{13}C NMR) and solvent residual peaks at 2.05 ppm (^{1}H NMR); ^{13}C NMR data were obtained from HSQC and HMBC experiments. [e] Overlapping signals. [f] Most intensive signals of an AA′XX′ spin system. [g,h,i,j,j'] Signals interchangeable in pairs. [k] Signal concealed by the solvent signal, indirectly assigned by HSQC and HMBC experiments. [l] Identified by a very weak HMBC correlation, only.

D-glucose moieties to position 15 of the sesquiterpene and to the *p*-hydroxy position of the *p*-hydroxyphenylacetic acid moiety, are shown in Figure 1. Shift values for H-6 and H-8 indicated that both protons were geminal to an oxygen, which was either part of a lactone or an ester moiety. The

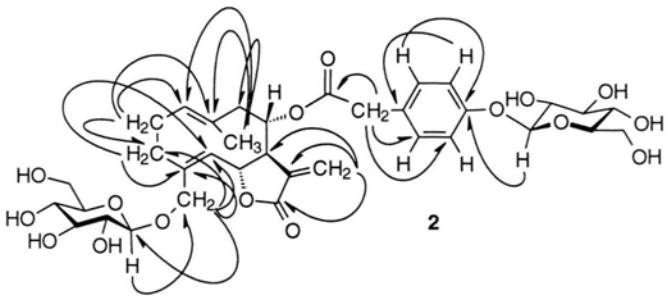

Fig. 1. Important HMBC correlations observed for compound **2** (measured in methanol-d_4).

absence of NOESY correlations between protons in positions 1 and 14 and between protons in positions 5 and 15 revealed that both intra-cyclic double-bonds were *trans*-configured [1(10)*E* and 4*Z*]. ^{1}H NMR coupling patterns of the signals for the protons in positions 6 and 7 and NOESY correlations between signals for H-6 and H-8, revealed that both oxygens were α-configured, when assuming the usual α-configuration of the proton in position 7. Therefore, the signals of the aglycone moiety were assignable either to a 6-acylated artemisiifolin-moiety or an 8-acylated salonitenolide-moiety (Samek *et al.*, 1969; Porter *et al.*, 1970; Yoshioka *et al.*, 1970). No HMBC correlations from H-6 and H-8 to any of the carbonyl carbons (C-12 and C-α') were detectable. However, comparisons of the ^{1}H NMR shift values of the signals for protons H-6, H-7 and H-8 with literature data of other 6α-acyl-15-hydroxygermacra-1(10)*E*,4*Z*,11(13)-trien-12,8-olides (Bohlmann *et al.*, 1981) and 8α-acyl-15-hydroxygermacra-1(10)*E*,4*Z*,11(13)-trien-12,6-olides (Samek *et al.*, 1969; Bruno and Herz, 1988; Miski *et al.*, 1988; Neves *et al.*, 1999) identified the aglycone as salonitenolide. The ^{13}C NMR data (Table I) are in good agreement with those reported for other 8-acyl-15-hydroxysalonitenolide derivatives (Barrero *et al.*, 1989; Neves *et al.*, 1999).

As indicated above, ^{1}H NMR and ^{13}C NMR data showed that the *p*-(glucopyranosyloxy)phenylacetic acid moiety was attached in position 8 and therefore **2** was identified as 15-β-D-glucopyranosyl-8-[*p*-(β-D-glucopyranosyloxy)phenylacetyl]-salonitenolide. Important NOESY correlations of **2** are shown in Figure 2 and suggest that the predominant conformation of **2** in solution is the UU-conformation *sensu* Watson and Kashyap (1986). This conformation was also suggested for the related compound onopordopicrin (Lonergan *et al.*, 1992).

2 is a new natural compound and the first non-guaianolide sesquiterpenoid isolated from a member of the genus *Leontodon*. A chemosystematic survey by HPLC-DAD and HPLC-MS for glucozaluzanin C **1** and 15-β-D-glucopyranosyl-8-[*p*-(β-D-glucopyranosyloxy)phenylacetyl]-salonitenolide **2** showed that both compounds were present in both available samples of *L. cichoraceus* (Abruzzo and Marche). In 23 other *Leontodon* taxa (*L. autumnalis* L., *L. berinii* (Bartl.) Roth, *L. crispus* Vill., *L. croceus* Haenke, *L. duboisii* Sennen ex Widder, *L. helveticus* Mérat emend. Widder, *L. hispidus* L., *L. hyoseroides* Welw. ex Rchb., *L. incanus* (L.) Schrank, *L. longirostris* Talavera in Valdés *et al.*, *L. maroccanus* (Pers.) Ball, *L. montaniformis* Widder, *L. montanus* Lam. subsp. *mela-*

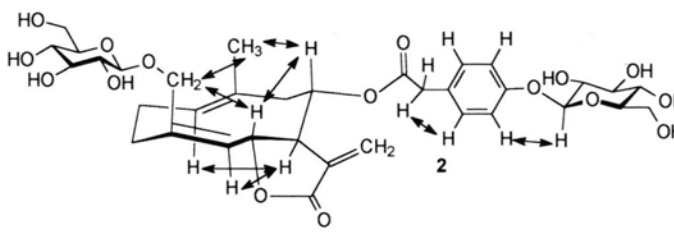

Fig. 2. Important NOESY correlations observed for compound **2** (measured in methanol-d_4).

notrichus (Vierh.) Widder ex Pittoni, *L. montanus* Lam. subsp. *montanus*, *L. muelleri* (Sch.Bip.) Ball, *L. palisae* Izuzquiza, *L. pyrenaicus* Gouan, *L. repens* Schur, *L. rilaensis* Hayek, *L. saxatilis* Lam., *L. scaber* Miel., *L. tenuiflorus* Gaudin, *L. tuberosus* L.), which were analyzed in an earlier study for the occurrence of guaianolides (Zidorn *et al.*, 2000), no sign of these compounds was found. This underlines the isolated position of *L. cichoraceus* within the genus (Zidorn *et al.*, 2001).

Preliminary tests showed no cytotoxic activity of **1** and **2** in a MTT assay on PMNL-cells. These results were not unexpected because other polar sesquiterpene lactone derivatives were also inactive in a previous study (Zidorn *et al.*, 1999).

Experimental

The plant material was collected in May 2000 at Monte Alto between Civita d'Antino and Collelongo/Abruzzo/Italy [altitude: 1450 m (above m.s.l.), N 41°53′, E 13°29′]. A voucher specimen (CZ-2000–05–16–1) is preserved at the Department of Pharmacognosy. Collection data of the other investigated *Leontodon* taxa and details on the analytical extraction procedure are described in Zidorn *et al.* (2000) and collection data of the sample of *L. cichoraceus* collected in the Marche region and details of the isolation of glucozaluzanin C in Zidorn *et al.* (2001).

Silica gel chromatography was carried out with Merck G-60 (230–400 mesh) material.

The melting point was determined on a Kofler hot-stage microscope and is uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian V-500 spectrometer at 500 and 125 MHz, respectively. HPLC-DAD and HPLC-MS analyses were performed as described previously (Zidorn *et al.*, 2000). Retention times of 23.6 min (**1**) and 23.9 min (**2**) were observed, respectively. HRMS analysis was performed on a Finnigan MAT 95 mass spectrometer by FAB ionization (Cs-gun 20 kV, 2 μA).

Preliminary investigations on cytotoxicity were carried out with human polymorphonuclear leukocytes (PMNL) obtained from health volunteer donors. The MTT assay was carried out following Zidorn *et al.* (1999).

15-β-D-glucopyranosyl-8-[*p*-(β-D-glucopyranosyloxy)phenylacetyl]-salonitenolide **(2)**, colorless crystals; mp 125–135° (dec.); FTIR (microspectrometry) ν_{max}^{ZnSe} cm^{-1}: 3400 br, 2925, 1736, 1651, 1613, 1511, 1399, 1366, 1333, 1264, 1233, 1076, 962; NMR data are given in Table I; APCI MS *m/z* 740 (16) $[M + NH_4]^+$, 723 (2) $[M + H]^+$, 561 (42) [M – glucose + H]$^+$, 440 (24) [M – 2*glucose + CH_3CN + H]$^+$, 399 (96) [M – 2*glucose + H]$^+$, 288 (36) [M – *p*-(glucopyranosyloxy)phenylacetyl – glucose – H_2O + CH_3CN+ H]$^+$, 247 (92) [M – *p*-(glucopyranosyloxy)phenylacetyl – glucose – H_2O + H]$^+$, 229 (100) [M – *p*-(glucopyranosyloxy)-phenylacetyl – glucose – 2*H_2O + H]$^+$.

Acknowledgments

The authors wish to thank K.-H. Ongania for HRMS measurements, N. B. Perry for helpful comments on an earlier version of the manuscript, R. Spitaler for helping collect the plant material, S. Sturm for MS measurements, P. Theiner for technical assistance, D. Weigand for IR measurements, and the Austrian Science Fund (FWF, project P12695-BIO) for financial support.

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