A Highly Substituted Germacranolide from *Leontodon cichoraceus*

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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-

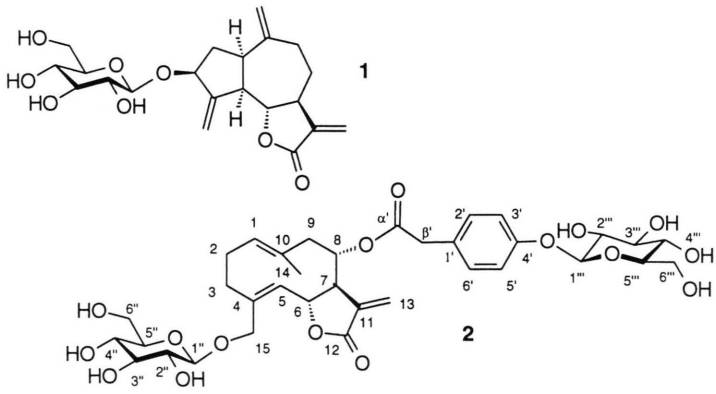

Table I. NMR data of compound **2**[a].

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

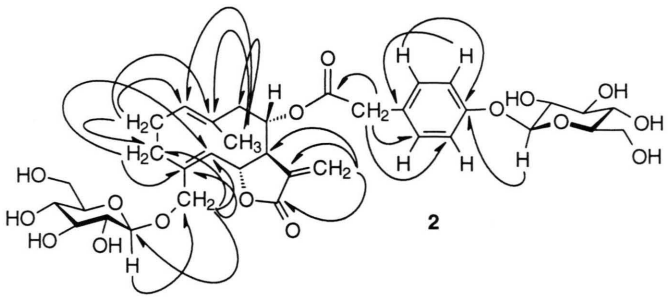

Fig. 1. Important HMBC correlations observed for compound **2** (measured in methanol-d_4).

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-*

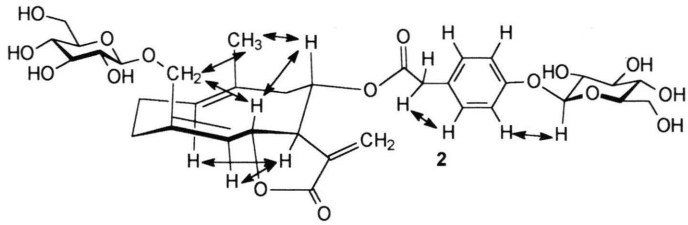

Fig. 2. Important NOESY correlations observed for compound **2** (measured in methanol-d_4).

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]$^+$.

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