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13-Chloro-3-*O*-β-D-glucopyranosylsolstitialin from *Leontodon palisae*: the first genuine chlorinated sesquiterpene lactone glucoside

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Abstract—Apart from a number of known sesquiterpenoids, a novel chlorinated sesquiterpene lactone glucoside—13-chloro-3-*O*-β-D-glucopyranosylsolstitialin—has been isolated from the Southwestern European plant *Leontodon palisae* (Asteraceae, tribe Lactuceae). The structure has been established by high resolution mass spectrometry and 1D as well as 2D NMR spectroscopy. The compound represents the first naturally occurring chlorinated sesquiterpene lactone glucoside. The cytotoxicity of the new compound and related ones was evaluated using the MTT assay.

Sesquiterpene lactones are characteristic constituents of the Asteraceae family.[1] A number of chlorinated sesquiterpene derivatives has been reported from members of the Asteraceae.[2,3] In most cases chlorinated solvents were used in the extraction and isolation procedure of these compounds. Therefore, some of these chlorinated sesquiterpene lactones might actually be artifacts from the isolation process.[2] However, some authors addressed the problem of artifact formation and proved that chlorinated sesquiterpene lactones exist as genuine natural products.[3] Notably, so far no chlorinated sesquiterpene lactone glucosides have been reported.

In the present communication we report about the isolation and structure elucidation of a chlorinated sesquiterpene lactone glucoside from the Southwestern European taxon *Leontodon palisae* Izuzquiza.[4] Our findings corroborate that chlorinated sesquiterpene lactones are indeed biosynthesized by plants and that they might play a vital role in their chemical defense.

An α-methylene-γ-lactone moiety was established to be essential for the cytotoxicity of sesquiterpene lactones.[5] However, the influence of chlorination in position 13 instead of the double bond in position 11(13) of the sesquiterpene skeleton on the cytotoxicity of sesquiterpene lactones has not been investigated previously.

L. palisae was collected in April 2003 in the Alpujarras between Huecija and Benarique/Almeria/Andalucia/Spain [coordinates (WGS84): N 36°58′51″; W 02°36′44″; altitude: 340 m a.m.s.l.; Institut für Pharmazie, Abt. Pharmakognosie voucher code: CZ-20030416A-1].

Phenolics like *p*-hydroxyphenylacetic acid (1.9 mg), apigenin (7.3 mg), and luteolin (19.3 mg) as well as sesquiterpene lactones (Fig. 1) ixerisoside A **1** (4.5 mg), 10α-hydroxy-8-desoxy-10,14-dihydrodesacylcynaropicrin **2** (6.9 mg), 8-deoxylactucin **3** (3.8 mg), 13-chlorosolstitialin **4** (17.2 mg) together with the new 13-chloro-3-*O*-β-D-glucopyranosylsolstitialin **5** (127 mg)[6] were isolated from the EtOAc phase (12.4 g) of the methanolic extract (80.2 g) of air-dried plants (384 g) of *L. palisae* by silica gel 60 (230–400 mesh) column chromatography (CC) using gradients of CH_2Cl_2 and MeOH, Sephadex LH-20 CC using MeOH as an eluant, and successive semi-preparative RP-18 HPLC using gradients of H_2O and MeCN. Known compounds were identified by

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employing mass spectrometry, NMR spectroscopy (^{1}H NMR, ^{13}C NMR, HSQC, HMBC), and by comparison with literature data.

HRMS[6] of compound **5** indicated a molecular formula of $C_{21}H_{29}ClO_9$. ^{1}H NMR data of **5** (Table 1) showed signals assignable to a glucose moiety and signals assignable to a sesquiterpene moiety. The assumption that **5** is a sesquiterpene glucoside was verified by a ^{13}C NMR experiment (Table 1), displaying 21 signals, 15 ascribed to a sesquiterpene moiety and six assignable to a glucose moiety.[3c,7] The ^{1}H NMR and ^{13}C NMR signals for the sesquiterpene part of the molecule were very similar to 13-chlorosolstitialin (**4**).[3c] The published ^{13}C

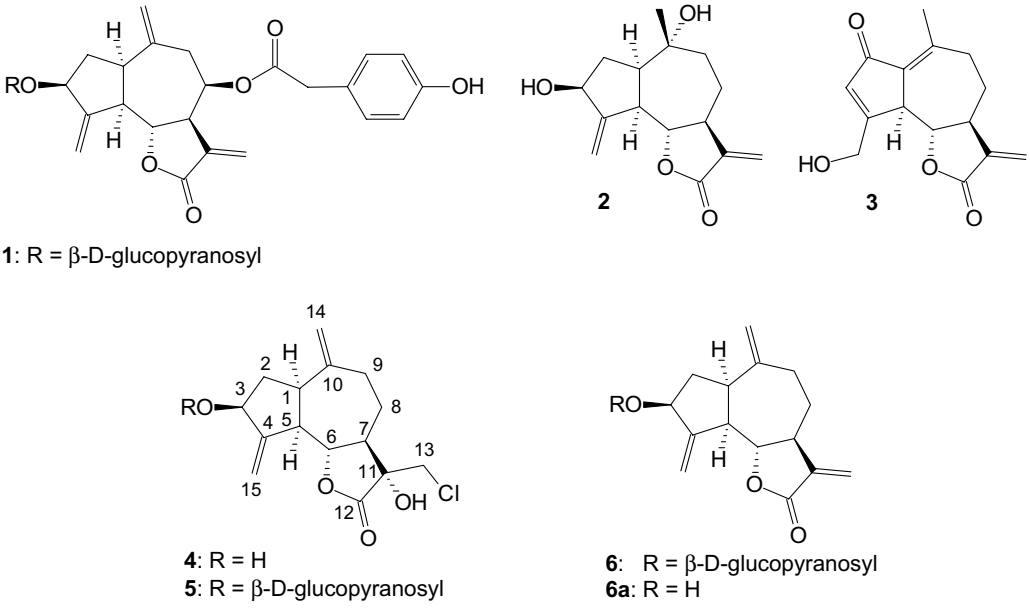

Figure 1. Structures of sesquiterpene lactones isolated from *L. palisae* (**1**–**5**), glucozaluzanin C from *L. cichoraceus* (**6**), and the semi-synthetic derivative zaluzanin C (**6a**).

Table 1. NMR spectral data for compounds **4** and **5**[a]

Position	^{1}H NMR data		^{13}C NMR data	
	4	**5**	**4**	**5**
Sesquiterpene moiety				
1	2.92 1H, m	2.96 1H, m	43.8	45.2
2	2.27 1H, m; 1.67 1H, m	2.36 1H, m; 1.96 1H, m	39.1	38.2
3	4.48 1H, tt (8.0, 2.0)	4.62 1H, ddt (7.5, 6.0, 1.5)	73.6	81.4
4			153.9	150.7
5	2.81 1H, tt (10.0, 2.0)	2.77 1H, tt (10.0, 1.5)	50.9	52.1
6	4.29 1H, t (10.0)	4.34 1H, t (10.0)	83.6	83.2
7	2.45 1H, m	2.42 1H, m	53.6	54.2
8	2.09 1H, m; 1.67 1H, m	2.07 1H, m; 1.66 1H, m	27.2	27.3
9	2.59 1H, m; 2.07 1H, m	2.58 1H, m; 2.05 1H, m	36.4	36.3
10			150.2	150.5
11			78.0	78.2
12			177.9	178.1
13	3.68 H, s; 3.66 H, s	3.68 H, s; 3.66 H, s	44.1	44.4
14	4.97 1H, br s; 4.95 1H, br s	5.02 1H, s; 4.95 1H, s	113.5	114.1
15	5.28 2H, m	5.45 1H, br s; 5.33 1H, br s	110.0	113.7
Glucose moiety				
1′		4.45 1H, d (8.0)		103.3
2′		3.23 1H, m		75.3
3′		3.36 1H, m		78.2
4′		3.28 1H, m		71.8
5′		3.28 1H, m		77.9
6′		3.88 1H, dd (12.0, 1.5) 3.67 1H, dd (12.0, 5.0)		62.9

[a] Measured in CD_3OD (^{1}H at 300 MHz, ^{13}C at 75 MHz) and referenced to solvent residual and solvent signals at 3.31 ppm (^{1}H) and 49.0 ppm (^{13}C), respectively.

Table 2. IC_{50} concentrations (μM) of compounds **1**–**6a** against leukemic cell lines GTB and HL60 analyzed by MTT assays (incubation time 7 days)[a]

	1	2	3	4	5	6	6a
GTB	60.8 ± 2.7	22.8 ± 0.2	4.1 ± 0.4	41.4 ± 3.2	>100[b]	>100[d]	5.2 ± 0.1
HL60	26.8 ± 6.3	19.8 ± 5.2	4.7 ± 0.6	29.4 ± 2.9	>100[c]	>100[e]	6.2 ± 0.3

[a] All assays were carried out in triplicate, tested concentrations: 100, 50, 10, 1, 0.1, and 0.01 μM.
[b] Inhibitory activity at 100 μM: 30.0 ± 1.0%.
[c] Inhibitory activity at 100 μM: 23.6 ± 1.2%.
[d] Inhibitory activity at 100 μM: 19.8 ± 9.8%.
[e] No inhibitory activity at 100 μM.

NMR shift value for C-11 of 13-chlorosolstitialin was obviously misprinted.[3c] Therefore, and because published NMR data for compound **7** were measured in $CDCl_3$, NMR data for this compound measured in deuteromethanol are included in Table 1. The main shift differences between compounds **5** and **4** were observed for H-3 (δ_H 4.62 vs 4.48 ppm) and C-3 (δ_C 81.4 vs 73.6 ppm), implying that the glucopyranosyl moiety is situated at O-3. This assumption was verified by an HMBC experiment, which showed cross peaks between H-3 and C-1′ as well as between H-1′ and C-3. The relative stereochemistry of compound **5** was proven to be identical to that of 13-chlorosolstitialin (**4**)[3c] by means of ^{1}H NMR coupling constants (Table 1) and 1D NOE experiments, which showed correlations from H-3 to H-1 and H-5 as well as from H-5 to H-3 and H-7. Conclusively, compound **5** is 13-chloro-3-*O*-β-D-glucopyranosylsolstitialin, a new natural product, which to the best of our knowledge represents the first chlorinated sesquiterpene lactone glucoside. To rule out that chlorinated sesquiterpenoids **4** and **5** were artifacts from the isolation process, a freshly prepared methanolic extract of *L. palisae* was analyzed by HPLC–MS.[7] The extract displayed signals for all isolated compounds (with compound **5** as one of the main compounds), thus ruling out, that any of the compounds described was an artifact.

Glucozaluzanin C (**6**), which differs from compound **5** only in positions C-11 and C-13, was isolated in a previous investigation from the closely related taxon *Leontodon cichoraceus* (Ten.) Sanguin.[8] Compound **6a** (zaluzanin C) was obtained from **6** via enzymatic hydrolysis.[9] The structure of **6a** was verified by MS and ^{1}H NMR experiments and by comparison with literature data.[10]

Cytotoxicities of the sesquiterpene lactones isolated in this study (**1**–**5**), glucozaluzanin C (**6**) from *L. cichoraceus* and zaluzanin C (**6a**) were assessed using the MTT assay.[11] MTT bioassay results (Table 2) showed pronounced cytotoxic activities (IC_{50} against GTB and HL60 ≈ 5 μM) for compounds **3** and **6a** and lower activities for compounds **2** ($IC_{50,GTB} \approx IC_{50,HL60} \approx$ 20 μM), **4** ($IC_{50,GTB} \approx 40$ μM; $IC_{50,HL60} \approx 30$ μM), and **1** ($IC_{50,GTB} \approx 60$ μM; $IC_{50,HL60} \approx 30$ μM). In contrast, compounds **5** and **6** only slightly inhibited GTB and HL60 cells at the highest concentration tested ($IC_{50} > 100$ μM). In summary, simple sesquiterpene lactones with an α-methylene-γ-lactone moiety, lacking a glucose moiety showed the most pronounced cytotoxic activity. More polar compounds such as **2** (bearing an additional hydroxy group) were less active. The cytotoxic activity was also diminished when the α-methylene-γ-lactone was substituted by an α-hydroxy-α-chloromethyl-γ-lactone moiety as in compound **4**. Glucopyranosyl derivatives such as compounds **5** and **6** show almost no cytotoxic activity, unless the effect of glucosidation is counter-balanced by substitution with apolar moieties as in compound **1**.[11] These findings confirm that an α-methylene-γ-lactone moiety is prerequisite for pronounced cytotoxic activity of sesquiterpene lactones.[5]

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1077, 1029, 903; $[\alpha]_D^{20}$ +29.8 (*c* 1.04, CH_3OH); HRFABMS: $m/z = 483.13673$ $[M+Na]^+$ calcd for $C_{21}H_{29}O_9ClNa$ $m/z = 483.13980$; NMR data are described in Table 1.

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9. Preparation of **6a** from **6**. Compound **6** (20 mg), which was isolated from *L. cichoraceus* in a previous investigation,[8] was dissolved in 0.2 mL EtOH and mixed with 1.8 mL of H_2O containing 20 mg of cellulase (Sigma: C-1184). The mixture was kept at 37 °C for 2 days; then the mixture was extracted with CH_2Cl_2. The organic phase was brought to dryness in vacuo, and compound **6a** (2.0 mg) was purified by silica gel CC using a gradient of CH_2Cl_2 and MeOH.
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11. Zidorn, C.; Stuppner, H.; Tiefenthaler, M.; Konwalinka, G. *J. Nat. Prod.* **1999**, *62*, 984–987, In contrast to our previous investigation, stock solutions in EtOH instead of DMSO were prepared. This resulted in a final EtOH concentration of 0.1% (v/v). In a series of preliminary experiments it was verified that an EtOH concentration of 0.1% (v/v) exhibited no cytotoxicity on the cell lines tested. IC_{50} values for each replicate were calculated using the following formula: $IC_{50} = [(50-LP)/(HP-LP)*(HC-LC)]+LC$. LP = low percentage, that is highest percent inhibition less than 50%; HP = high percentage, that is lowest percent inhibition greater than 50%; HC = high concentration, that is concentration of test substance at the high percentage, LC = low concentration, that is concentration of test substance at the low percentage. All compounds and concentrations were assayed in triplicate and mean values were calculated for each compound and cell line.