A New Eudesmane Derivative from *Leontodon tuberosus*

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Besides the known compounds 2,4,6-trihydroxyacetophenone 4-O-β-D-glucopyranoside and syringaresinol 4'-O-β-D-glucopyranoside, the novel sesquiterpenoid 1,2-dehydro-3-oxocostic acid β-D-glucopyranoside ester was isolated from *Leontodon tuberosus* L. and its structure established by mass spectrometry and 1D- and 2D-NMR spectroscopy. Additionally, a number of fatty and phenolic acids was identified in the crude methanolic extract by HPLC-DAD and HPLC-MS. The chemosystematic impact of the new sesquiterpenoid is discussed briefly.

Key words: Chemosystematics, Lactuceae, Sesquiterpenoids

Introduction

Leontodon tuberosus L. is a perennial herb of up to 35 cm of height, with long slender tubers, which inhabits the Mediterranean region [1 – 2]. The genus *Leontodon* is a rich source of sesquiterpenoids, mainly guaianolide glycosides [3 – 7]. The distribution of the different sesquiterpene types observed so far is in good agreement with Widder's [8] morphologically based classification of the genus *Leontodon* [5 – 7]. Widder [8] divided the genus *Leontodon*, which encompasses approximately 50 species, into two subgenera (Leontodon and Oporinia) and five sections (Asterothrix, Kalbfussia, Leontodon, Oporinia, Thrincia).

In the following communication we report about the first sesquiterpenoid isolated from the section Thrincia.

Results

Compounds **1 – 3** (Fig. 1) were isolated from a methanolic extract (20.0 g) of air-dried subaerial parts (278 g) of *L. tuberosus* by silica gel column chromatography (CC), Sephadex LH-20 CC and reversed phase (RP18) semi-preparative HPLC. ESI mass spectra of **1** measured in the positive mode displayed signals at *m/z* 839 $[2M + Na]^+$ and 431 $[M + Na]^+$; signals in the negative mode included *m/z* 815 $[2M - H]^-$, 653 [2M – glucose – H]$^-$, 407 $[M - H]^-$, and 245 [2M – glucose – H]$^-$, congruent with a molecular mass of 408 and a molecular formula of $C_{21}H_{28}O_8$. FAB HR MS in the positive mode displayed a $[M + H]^+$ signal at *m/z* 409.18668 (calc. for $C_{21}H_{29}O_8$: *m/z* 409.18624) and thus verified the assumed molecular formula.

^{1}H NMR and ^{13}C NMR data (Table 1) displayed signals assignable to a glucose and a sesquiterpene moiety. ^{1}H NMR signals of compound **1** encompassed two olefinic methylene groups [δ_H 6.37 (s, H-13a), 5.84 (s, H-13b); 6.02 (m, H-15a), 5.25 (m, H-15b)], two downfield tertiary protons assignable to a double bond [δ_H 7.00 (d 10.0 Hz, H-1), 5.98 (d 10.0 Hz, H-2)], two further tertiary protons [δ_H 2.69 (m, H-5), 2.66 (m, H-7)], three pairs of endocyclic methylene protons [δ_H 1.92, 1.60; 1.76, 1.69; 1.76, 1.66], a methyl group [δ_H 0.99 (d 3.0 Hz, pos. 14)], and a glucose moiety [δ_H 5.57 (d 8.0 Hz, H-1'), 3.40-3.44 (four protons, H-2', H-3', H-4', H-5'), 3.85 (dd 12.0 Hz, 2.0 Hz, H-6'), 3.69 (dd 12.0 Hz, 5.5 Hz, H-6'*)]. ^{13}C NMR data in combination with DEPT and HSQC experiments showed signals assignable to an carbonic acid moiety (δ_{C-12} 167.0), a ketone (δ_{C-3} 191.3), two olefinic methylene groups (δ_{C-13} 125.7, δ_{C-15} 118.9), three endocyclic methylene groups (δ_C 38.0, 30.1, 28.1), two double bound methine carbons (δ_C 164.0, 127.4), two further methine carbons (δ_C 49.4, 40.5), a methyl group (δ_C 18.1) and a glucose moiety (δ_C 96.1, 78.9, 78.2, 74.0, 71.1, 62.3). HMBC correlations (Fig. 2) established the sesquiterpene moiety as 1,2-dehydro-3-oxocostic acid. The ^{1}H NMR data of the sesquiterpene moiety are in

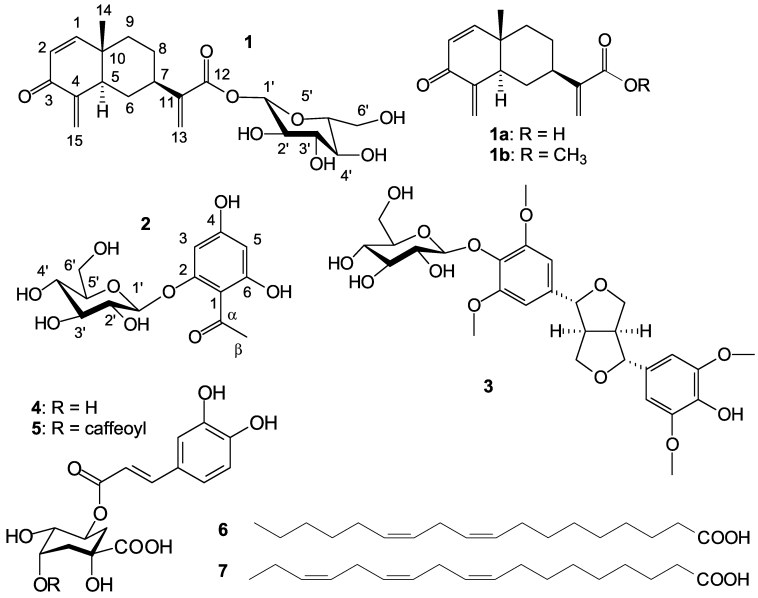

Fig. 1. Structures of *L. tuberosus* constituents (**1** – **7**) and of natural products closely related to compound **1** from different sources (**1a** – **1b**).

Table 1. NMR data 1,2-dehydro-3-oxocostic acid β-D-glucopyranoside ester (**1**).[a]

Pos.	^{1}H NMR	^{13}C NMR	Pos.	^{1}H NMR	^{13}C NMR
Sesquiterpene moiety			*Glucose moiety*		
01	7.00 1H, d (10.0)	164.0	1’	5.57 1H, d (8.0)	96.1
02	5.98 1H, d (10.0)	127.4	2’	3.41 1H, m*	74.0
03	–	191.3	3’	3.40 1H, m*	78.9
04	–	147.5	4’	3.40 1H, m*	71.1
05	2.69 1H, m*	49.4	5’	3.44 1H, m*	78.2
06[b]	1.92 1H, m	30.1	6’	3.85 1H, dd (12.0, 2.0)	62.3

[a] Measured in methanol-d_4 at 500 and 125 MHz, respectively. Spectra are referenced to solvent residual and solvent signals at δ_H = 3.31 ppm and δ_C = 49.0 ppm, respectively; [b] signals might be interchangeable; * overlapping signals.

good agreement with those published for 1,2-dehydro-3-oxocostic acid and its methyl ester [9 – 12]. HMBC crosspeaks from the anomeric proton of the glucose moiety to C-12 of the sesquiterpene moiety established the structure of compound **1** as 1,2-dehydro-3-oxocostic acid β-D-glucopyranoside ester. Compound **1**, for which the same absolute stereochemistry in position C-7 as established for naturally occurring costic acid is assumed [13], is a new natural product and represents the first eudesmane derivative isolated from a member of the genus *Leontodon*.

ESI mass spectra of compound **2** measured in the positive mode displayed signals at *m/z* 683 [2M + Na]$^+$ and 353 [M + Na]$^+$; in the negative mode signals at *m/z* 659 [2M – H]$^-$ and 329 [M – glucose – H]$^-$ were observed; this is congruent with a molecular mass of 330 and a molecular formula of $C_{14}H_{18}O_9$. ^{1}H NMR and ^{13}C NMR data encompassed signals for a glucose moiety and a trihydroxyacetophenone moiety and were in perfect agreement with those reported for 2,4,6-trihydroxyacetophenone 2-O-β-D-glucopyranoside isolated from *Artemisia stolonifera* (Maxim.) V. L. Komarov by Lee *et al.* [14]. However, Lee *et al.* did not take into account that the observed ^{1}H NMR coupling pattern in the aromatic region was also congruent with a 3,4,5-trihydroxyacetophenone derivative. Suksamrarn *et al.* [15], who independently isolated compound **2** from another source (*Curcuma comosa* Roxb.), established the structure arguing with the unusually low IR absorption frequency of the keto moiety, which indicated that the keto function and at least one hydroxy-group were arranged vicinal to each other.

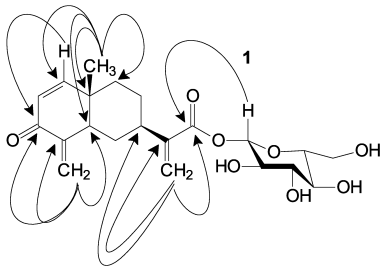

Fig. 2. Important HMBC correlations observed for compound **1**.

We observed that the intensity of the signal assignable to proton H-5 decreased after **2** was dissolved in deuteromethanol for some days, indicating an exchange of proton H-5 against a deuterium atom. This exchange enabled by 1,3-keto-enol-tautomerism – only possible with two free hydroxy-groups in meta-position – is another proof for the correctness of the structure assigned by Lee *et al.* [14] and *Suksamrarn et al.* [15]. The isomeric compound 3,4,5-trihydroxyacetophenone 3-O-β-D-glucopyranoside was reported from *Polygonum cuspidatum* [16]. However, Xiao *et al.* [16] did not report physical data of the compound but – erroneously – cited Lee *et al.* [14], stating that their data were identical to those observed for **2**. Therefore, it remains unclear whether Xiao *et al.* [16] isolated compound **2** or 3,4,5-trihydroxyacetophenone 3-O-β-D-gluco pyranoside. However, compound **2** represents the first acetophenone derivative found in the genus *Leontodon*.

Compound **3** was identified by MS, 1D and 2D NMR and by comparing the obtained data with the literature as syringaresinol 4'-O-β-D-glucopyranoside [17]. Substance **3** represents the first lignan isolated from the genus *Leontodon* and one of very few lignans isolated from the Lactuceae tribe of the Asteraceae family [18 – 19].

Phenolic acids chlorogenic acid (**4**) and 3,5-dicaffeoyl quinic acid (**5**) were detected in the methanol extract of tubers from *L. tuberosus* by HPLC-DAD and HPLC-MS using the reference compounds and analytical systems described by Zidorn and Stuppner [20].

Linolic acid (**7**) and linoleic acid (**8**) were detected in the same extract by HPLC-DAD and HPLC-MS and by comparing the results with reference compounds purchased from Sigma Aldrich (St Louis, USA).

Discussion

Compounds closely related to **1** – 1,2-dehydro-3-oxocostic acid **1a** and 1,2-dehydro-3-oxocostic acid methyl ester **1b** – were isolated from a number of species of the Asteraceae family: *Arctotis revoluta* Jacq., **1a** (subfamily Cichorioideae, tribe Arctoteae [12]), *Centaurea canariensis* Brouss. and *C. arguta* Ness, **1b** (subfamily Cichorioideae, tribe Cardueae [9, 11]), *Cheirolophus sempervirens* (L.) Pomel, **1b** (subfamily Cichorioideae, tribe Cardueae [21]), *Encelia actoni* Elmer and *E. asperifolia* (S. F. Blake) Clark & Kyhos, **1a** (subfamily Asteroideae, tribe Heliantheae [22]), *Montanoa speciosa*, **1a** (subfamily Asteroideae, tribe Heliantheae [10]), respectively. Conclusively, 1,2-dehydro-3-oxocostic acid derivatives seem to be restricted to the Asteraceae family and predominantly occur in the Cichorioideae subfamily. However, these compounds are not restricted to a particular tribe and therefore, their impact as a chemosystematic marker is rather low. The distribution of compound **1** within the genus *Leontodon* might nevertheless yield new data to establish a chemosystematically based infrageneric system of the genus and the investigation of this distribution will be the subject of future studies.

Materials and Methods

Plant material. – *L. tuberosus* was collected on the first of April 2002 W of the height of the pass between Trebisacce and Albidona [province of Cosenza/Calabria region/Italy; altitude: 790 m; coordinates (WGS 84): N 39°53'20"; E 16°29'43"]. A voucher specimen (code: CZ-20020401C-1) is deposited in the herbarium of the Institut für Pharmazie.

Isolation of compounds **1-3**. – Air-dried subaerial parts of *L. tuberosus* were exhaustively extracted with MeOH to give 20.0 g of crude extract. This extract was fractionated by silica gel CC using a gradient of petrol ether, ethylacetate and MeOH. Fraction 11 (572 mg), which eluted with a mixture of ethylacetate/MeOH 3/1 (v/v), was further fractionated by Sephadex LH-20 CC using MeOH as an eluant. Fractions containing **1** (84.6 mg) were combined and **1** (11.7 mg) was isolated by semi-preparative RP-HPLC employing a gradient of H_2O and MeCN. Fractions containing compound **2** were also combined (193 mg) and re-fractionated on Sephadex LH-20 to give an enriched fraction of **2** (36.8 mg). Compound **2** (22.5 mg) was finally purified by semi-preparative HPLC employing a gradient of H_2O and MeCN. Silica gel fraction 12 (230.1 mg), which eluted with a mixture of ethylacetate/MeOH 1/1 (v/v), was fractionated by Sephadex LH-20 CC using MeOH as an eluant to give an enriched fraction of **3** (66.0 mg), which was finally purified by semi-preparative RP-HPLC using a gradient of H_2O and MeCN to give 2.8 mg of compound **3**.

Semi-preparative HPLC. – Column: Waters XTerra Prep MS C18, 7.8 × 100 mm, particle size : 5 μm (Nr.: 186001156); column temperature: 40 °C; guard column: Merck Lichrospher 100 RP-18, particle size 5 μm (Nr.: 50931); HPLC system consisted of: Dionex P580 pump, Dionex ASI-100 autosampler, Dionex

UVD170U UV-detector, and a Gilson 206 fraction collector; detection wavelength: 205 nm; injection volume: 50 μl. The following flow rates, gradients, and collection times were employed: Compound **1**: Flow rate 3.0 ml/min; linear gradient: 0 min 20% MeCN, 10 min 30% MeCN, collection time: 6.1 – 6.9 min. Compound **2**: Flow rate: 2.5 ml/min; linear gradient: 0 min 4.25% MeCN, 20 min 21.25% MeCN, collection time: 5.3 – 9.6 min. Compound **3**: Flow rate: 2.5 ml/min; linear gradient: 0 min 12.75% MeCN, 20 min 21.25% MeCN, collection time: 11.9 – 13.4 min.

NMR spectroscopy. – NMR spectra were recorded on a Varian-Unityplus-500 spectrometer at 500 MHz and 125 MHz, respectively. Spectra were recorded in MeOH-d_4 and referenced to solvent residual signals and solvent signals at $\delta_H = 3.31$ ppm and $\delta_C = 49.0$ ppm, respectively.

ESI mass spectra were recorded in the negative and positive mode on a Finnigan MAT SSQ 7000 mass spectrometer. IR measurements were performed on Bruker IFS25 FTIR micro-spectrometer. Optical rotation was measured on a Perkin Elmer 141 polarimeter.

FAB HR MS was carried out on a Finnigan MAT 95 mass spectrometer in the positive mode and referenced to a matrix signal of $[(\text{glycerol})_4 + H]^+$ at *m/z* 369.19720; Cs-Gun: 20 kV, 3 μA.

1,2-dehydro-3-oxocostic acid β-D-glucopyranoside ester (**1**). – **1** was obtained as a colorless substance decomposing above 201 °C; $[\alpha]_D^{20} - 22.8°$(*c* 0.342, CH_3OH); FTIR (micro spectrometry) ν_{max}^{ZnSe} cm^{-1}: 3400 (br), 2931, 2876, 1721, 1671, 1618, 1599, 1407, 1285, 1232, 1206, 1157, 1077; NMR data are given in Table 1.

HPLC system for the detection of fatty acid derivatives: Instrumentation: Hewlett Packard HP-1100 Liquid Chromatograph employed with a DAD-detector coupled with a Bruker Esquire 3000plus ion trap LC/MS_n. Mobile phase A: H_2O/CH_3COOH 99.9/0.1 (v/v), mobile phase B: MeCN; linear gradient: 0 min: 12% B, 15 min: 15% B, 25 min: 35% B, 30 min: 60% B, 55 min. 95% B; stop time: 60 min; post time: 20 min; flow rate: 1.00 ml/min; oven temperature: 40 °C; column: Zorbax SB-C18 4.6 × 150 mm (particle size 3.5 μm); guard column: Merck LiChroCart 4 × 4 mm packed with LiChrospher RP18 material (5 μm particle size); injection volume: 10 μl; detection wavelength: 205 nm. Retention times (min): 47.9 (**6**), 44.6 (**7**). MS parameters: ESI, alternating negative/positive ionization mode, capillary voltage: 4000 V, end plate offset: 500 V, nebulizer: 2 psi, dry gas (N_2) 4 l/min, dry temperature: 300 °C, scanning range: *m/z* 100 – 1000.

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