

A New Sesquiterpene Lactone Sulfate from *Reichardia gaditana* (Asteraceae)

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The new sesquiterpenoid 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin 3'-sulfate (**1**) was isolated from the methanolic extract of roots of *Reichardia gaditana* L. The compound was isolated by silica gel column chromatography (CC) and repeated Sephadex LH-20 CC. Structure elucidation was accomplished by high-resolution mass spectrometry and by 1D- and 2D-NMR spectroscopy. The chemosystematic significance of the new compound is discussed in the context of sesquiterpenoids from other members of the Lactuceae tribe of the Asteraceae family.

Key words: *Reichardia*, Chemosystematics, NMR Spectroscopy, Sesquiterpenoids, Structure Elucidation

Introduction

Reichardia gaditana (Willk.) Coutinho is an endemic to the West of the Iberian Peninsula; the taxon grows at sandy and rocky places near the sea [1]. So far there are no reports on secondary metabolites from *R. gaditana*. However, other taxa from the genus *Reichardia* yielded lactucin-type sesquiterpene lactones [2–5] as well as caffeic acid derivatives and flavonoids [6].

Results

Isolation and structure elucidation of 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin 3'-sulfate (**1**)

A methanolic extract of air-dried roots of *R. gaditana* collected at the coastline near Tarifa in the Spanish region of Andalusia yielded 54.5 mg of the new natural product **1** (Fig. 1). HRESIMS spectra in the negative mode displayed a signal at $m/z = 425.09136$

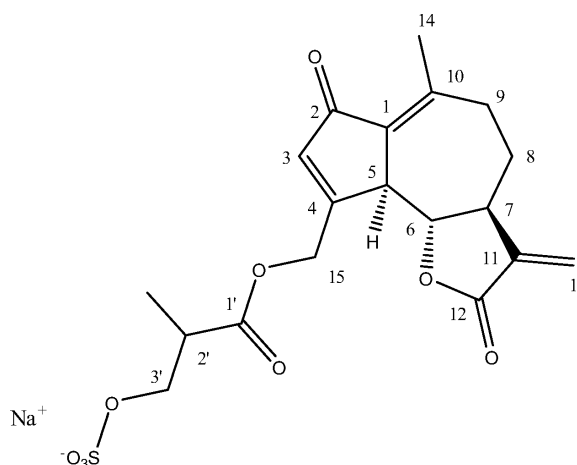


Fig. 1. Structure of sodium 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin 3'-sulfate (**1**).

(calculated for $C_{19}H_{21}O_9S^-$ $m/z = 425.09118$). In combination with results from an elemental analy-

Table 1. NMR data of 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin 3'-sulfate (**1**).^a

Position	¹ H NMR	¹³ C NMR	HMBC
1		131.1	
2		195.9	
3	6.36 1H, q (1.5)	133.7	1, 2, 4, 5, 15
4		167.6	
5	3.91 1H, d (10.0)	50.3	1, 2, 3, 4, 6, 7, 10
6	3.60 1H, t (10.0)	84.9	1, 4, 7, 8, 11
7	3.09 1H, m	52.2	5, 6, 8, 9, 11, 13
8	2.24 1H, m	24.4	6, 7, 9, 10, 11
	1.39 1H, m		6, 7, 9, 10, 11
9	2.65 1H, m	37.3	1, 7, 8, 10, 14
	2.38 1H, m		1, 7, 8, 10, 14
10		155.5	
11		139.5	
12		169.8	
13	6.07 1H, d (5.5)	118.5	6 ^b , 7, 11, 12
	5.53 1H, d (5.5)		6 ^b , 7, 11, 12
14	2.39 3H, br s	21.2	1, 2 ^b , 9, 10
15	5.34 1H, dd (17.0, 1.5)	63.9	2 ^b , 3, 4, 5, 1'
	4.99 1H, ddd (17.0, 1.5, 1.5)		2 ^b , 3, 4, 5, 1'
1'		173.7	
2'	2.94 1H, m	40.2	1', 3', 2'-Me
3'	4.12 2H, m	69.4	1', 2', 2'-Me
2'-Me	1.22 3H, d (7.0)	13.1	1', 2', 3'

^a Measured in [D₄]methanol at 300 and 75 MHz, respectively. Spectra are referenced to solvent residual and solvent peaks of methanol at $\delta_{\text{H}} = 3.31$ ppm and $\delta_{\text{C}} = 49.0$ ppm, respectively. ^b Four-bond correlation.

sis, which suggested a molar ratio of 1:1 for S and Na in compound **1**, and with data from ¹³C NMR experiments, which displayed nineteen signals (Table 1), the molecular formula of **1** was established as C₁₉H₂₁NaO₉S. ¹H NMR and ¹³C NMR data (Table 1) in combination with HSQC and HMBC data revealed that compound **1** was composed of a sesquiterpene moiety, a 2-methyl-3-hydroxypropanoic acid moiety, and a sulfuric acid moiety. Close inspection of ¹H NMR, ¹³C NMR, HSQC, and HMBC data revealed that the sesquiterpene moiety was 8-deoxylactucin [7]. HMBC data furthermore indicated that the hydroxy group in position 15 of the 8-deoxylactucin moiety was acylated with the 2-methyl-3-hydroxypropanoic acid moiety. ¹H NMR and ¹³C NMR shift values of the two protons and the carbon atom in position 3' of the acid moiety indicated that this position was esterified, too. Conclusively, the sulfate moiety had to be connected *via* this position with the rest of the molecule and the compound was established as 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin 3'-sulfate or its sodium salt, respectively. The new compound is a member of the lactucin-type subclass of guaianolides. These compounds are characterized by

two double bonds in positions 1(10) and 3 of the ring system. Lactucin and its derivatives have been reported from various sources within the Lactuceae tribe of the Asteraceae family including chicory, *Cichorium intybus* L. [7–8] and lettuce, *Lactuca sativa* L. [8].

Bioactivity

Cytotoxicity assays by flow cytometry employing Annexin V-PI [9] of compound **1** revealed no effect up to the highest concentration tested (100 μM). The following three multiple myeloma cell lines were tested: LP-1, RPMI-8226, and U266.

Chemosystematic relevance

Terpenoids substituted with sulfate groups are not too rare in marine natural products [10–11]. However, to the best of our knowledge sulfuric acid esters of sesquiterpene lactones in higher plants have up to now only been reported from *Cichorium intybus* and *Lactuca sativa* [8]. As the studies of Sessa *et al.* [8] are quite recent and were performed on two species, which were considered to be well investigated with regards to their secondary metabolite profiles, it is currently hard to assess whether sesquiterpenoid sulfates in higher plants are really very rare or whether they have not been detected formerly because of their instability when applying standard isolation procedures. In any case, currently all reports of sesquiterpenoid sulfates from land plants are from the Lactuceae tribe of the Asteraceae family.

Experimental Section

Materials and methods

Plant material. – *R. gaditana* was collected in Southern Spain near the town of Tarifa Andalusia/Spain [coordinates (WGS84): N 36°00'45"; W 05°36'35"; alt.: 5 m]. Voucher specimens are preserved in the personal herbarium of CZ (CZ-20050401A-1), and in the herbarium of the Institut für Botanik of the University of Innsbruck (IB voucher nr. 26879).

Isolation of compound **1**. – Air-dried, ground roots (305 g) of *R. gaditana* were exhaustively extracted with MeOH by maceration (8 times with 3 liters for one day each time). The resulting solution was dried *in vacuo* to yield 64.3 g of crude extract. This extract was redissolved in a mixture of H₂O and MeOH and successively partitioned with petrol ether, ethyl acetate and butanol. The butanol layer was brought to dryness *in vacuo* (11.4 g) and fractionated by silica gel CC employing a gradient of CH₂Cl₂ and MeOH.

Fractions containing **1** (457 mg) were further subjected to two successive fractionations by Sephadex LH-20 CC using MeOH as a mobile phase yielding 54.5 mg of compound **1**.

NMR spectroscopy. – NMR spectra were recorded on a Bruker-Avance-300 spectrometer at 300 MHz and 75 MHz, respectively. Spectra were recorded in [D₄]MeOH and referenced to solvent residual signals and solvent signals at $\delta_{\text{H}} = 3.31$ ppm and $\delta_{\text{C}} = 49.0$ ppm, respectively.

Mass spectrometry. – High-resolution mass spectra for molecular formula assignment were acquired on a Bruker (Bremen, Germany) APEX Qe Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) equipped with a 12 Tesla superconducting magnet. Dry samples were dissolved in methanol to reach a concentration of 2 mg/mL. For negative electro spray measurements, these sample solutions were diluted 1 : 10 with water/methanol/ammonia (50/50/0.05, v/v/v). For positive electro spray, water/methanol/formic acid (50/50/0.1, v/v/v) was used. Samples were introduced into the micro electro spray source at a flow rate of 120 $\mu\text{L h}^{-1}$, a nebulizer gas pressure of 20 PSI, and a drying gas pressure of 15 PSI (250 °C). Spectra were

externally calibrated on clusters of arginine (10 ppm in methanol).

8-Deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin 3'-sulfate (1)

Compound **1** was obtained as a colorless amorphous solid decomposing above 150 °C. – FTIR (micro spectrometry): $\nu_{\text{max}}^{\text{ZnSe}} = 3471\text{br}, 2941, 1771, 1741, 1686, 1637, 1619, 1508, 1426, 1367, 1333, 1254, 1138, 1074, 997, 870, 815, 759 \text{ cm}^{-1}$. – LRESIMS in the negative mode: $m/z = 473$ [M – H][–], 425 [M – H₂SO₂ + H₂O][–]. – NMR data are given Table 1.

Bioactivity: Cytotoxic activity of compound **1** was assessed employing the Annexin V-PI assay according to Koopman *et al.* [9]. The tested cell lines were purchased from the DSMZ (Braunschweig/Germany).

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