PERGAMON

PHYTOCHEMISTRY

Phytochemistry 50 (1999) 967–972

New sesquiterpene lactones from the Portuguese liverwort *Targionia lorbeeriana*

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Received 4 August 1998

Abstract

Three new sesquiterpene lactones (acetyltrifloculoside, 8,15-acetylsalonitenolide and 8-acetylsalonitenolide) and two known sesquiterpene lactones were isolated from a dichloromethane extract of the Portuguese liverwort *Targionia lorbeeriana*. Their structures were established by spectroscopic methods (EI and D/CI mass spectroscopy, ^{1}H and ^{13}C NMR) and that of acetyltrifloculoside was confirmed by X-ray crystallography. The three isolated guaianolide sesquiterpene lactones presented antifungal activity against *Cladosporium cucumerinum* and larvicidal activity against *Aedes aegypti*. Only one of the isolated lactones presented activity against *Candida albicans*.

Keywords: Liverwort; *T. lorbeeriana*; Targionaceae; Phytochemistry; Bioassay; Terpenoids; Sesquiterpene lactones

1. Introduction

Liverworts produce terpenoids which are of remarkable interest due to their structure and biological activity (Asakawa, 1982).

The genus *Targionia* (Targionaceae) contains three species (Smith, 1991). All that has been reported from this genus is the isolation of two monoterpene acetates from *Targionia hypophylla* which comprise the characteristic fragrance of this plant (Asakawa, Toyota, & Cheminat, 1986).

Targionia lorbeeriana is widely distributed in Portugal (Sim-Sim, 1987) and emits an intense fragrance when it is crushed.

We report here the activity-guided isolation of five sesquiterpene lactones (three of which are new compounds) from the dichloromethane extract of *T. lorbeeriana*, a species which has not been investigated phytochemically.

2. Results and discussion

The dichloromethane extract of *T. lorbeeriana* was first fractionated by flash chromatography on silica gel. Further separations were performed by MPLC on RP-18 (see Section 3) to afford three guaianolide-type sesquiterpene lactones (**1**–**3**) and two germacranolides (**4**–**5**). The structures of the sesquiterpene lactones were determined by ^{1}H and ^{13}C spectroscopy and D/CI-mass spectrometry.

The main sesquiterpene lactone constituent of the *T. lorbeeriana* CH_2Cl_2 extract was the known dehydrocostus lactone (**1**) that was identified by comparison of ^{13}C NMR and ^{1}H NMR data (Tables 1 and 2) with literature values (Mathur, Hiremath, Kulkarni, Kelkar, & Bhattacharyya, 1965; Silva, Garcia, Baker, & Rabi, 1981). This compound was first isolated from *Costus* root (Hikino, Meguro, Kusano, & Takemoto, 1964), but has never been mentioned, to our knowledge, in bryophytes.

The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, but in the case of **2** only two exocyclic double bonds were present, whereas a methyl and an acetyl group (δ_H 1.51 s and 2.04 s) were detected. The presence of a quaternary carbon at δ 86.0 suggested that the methyl and acetyl groups were attached to the same carbon. The C-10 position of these groups was deduced by selective INEPT NMR experiments: irradiation of a methyl group (δ_H 1.51 s) selectively enhanced the signal of the quaternary carbon at δ 86.0 (C-10), of the CH at δ 50.8 (C-1), of the CH_2 at δ 30.6 (C-9) and the signal of the CH_3 at δ 22.35 (acetyl group). Finally, crystals obtained from cloroform–methanol were subjected to X-ray analysis

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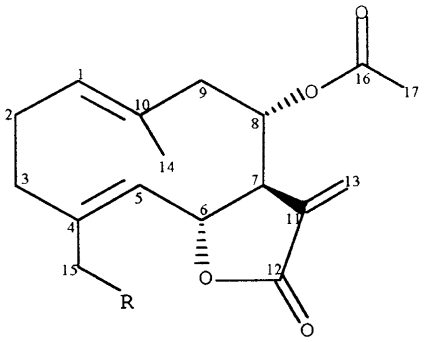
0031-9422/99/$ - see front matter
PII: S0031-9422(98)00648-7



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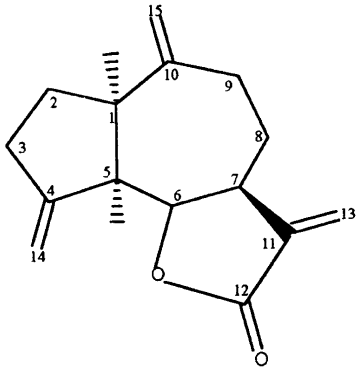
5 R= OH



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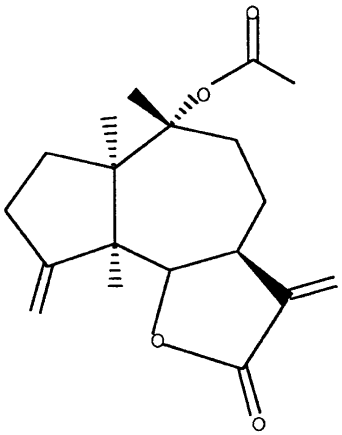

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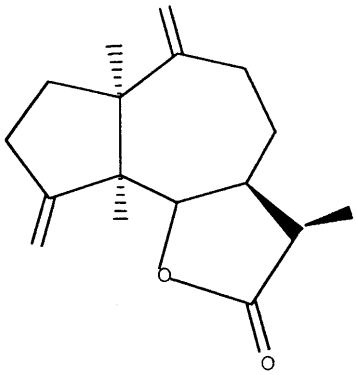

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and the proposed structure was confirmed. Compound **2** was identified as acetyltrifloculoside lactone and is a new compound.

The ^{1}H and ^{13}C NMR spectra of **3** closely resemble those of **1**. However, the H-13 and C-13 methylene signals were replaced by a secondary methyl group signal (δ_H 1.16 d and CH_3 at δ 11.3). The stereochemistry at C-11 was deduced by the $J_{7,11} \cong 7.8$ Hz and the chemical shift of H-13 (Bohlmann & Chen, 1982; Kisiel & Barszcz, 1996). Compound **3** is the H-11 epimer of the known dihydrodehydrocostuslactone (Karve, Deshpande, Kulkarni, & Kelkar, 1983).

The ^{1}H NMR spectrum of compound **4** (Table 3) showed the presence of an olefinic methyl (δ_H 1.51 s), an oxygenated methylene group (δ_H 4.6 s), two acetyls (δ_H 2.3 s), two olefinic protons (δ_H 4.92 and 4.96) and two oxygenated methine groups (δ_H 4.89 and 5.01). As in

Table 1
^{1}H NMR data of compounds **1**, **2** and **3**

^{1}H	**1**	**2**	**3**
1	2.91 m	2.75 m	2.88 m
2α	1.92 m	1.80 m	1.88 m
2β	1.92 m	1.80 m	1.88 m
3α	2.53 m	2.48 m	2.50 m
3β	2.53 m	2.48 m	2.50 m
5	2.89 m	2.80 m	2.81 m
6	3.97 dd	4.01 dd	4.04 dd
7	2.90 m	2.98 m	2.42 m
8α	1.46 m	1.57 m	1.44 m
8β	2.27 m	2.12 m	1.82 m
9α	2.52 m	2.35 m	2.42 m
9β	2.27 m	2.04 m	2.06 m
11			2.66 q
13	6.21 d	6.17 d	1.16 d
13′	5.49 d	5.45 d	1.16 d
14	5.27 d	5.19 dd	5.20 d
14′	5.07 d	5.03 dd	5.05 dd
15	4.90 s		4.87 br s
15′	4.82 s		4.77 s
Me		1.51 s	
CO–Me		2.04 s	

Compound **1** $J_{5,6}=9$ Hz, $J_{6,7}=9$ Hz, $J_{7,13}=3.4$ Hz, $J_{7,13'}=2.9$ Hz, $J_{5,14}=J_{5,14'}=2.2$ Hz.Compound **2** $J_{5,6}=10.2$ Hz, $J_{6,7}=9.5$ Hz, $J_{7,13}=J_{7,13'}=3.4$ Hz, $J_{5,14}=J_{3,14}=2.4$ Hz, $J_{5,14'}=J_{3,14'}=2.2$ Hz.Compound **3** $J_{5,6}=10$ Hz, $J_{6,7}=8.8$ Hz, $J_{13,11}=7.8$ Hz, $J_{7,11} \cong 7.8$ Hz, $J_{14,5}=J_{14',5}=2$ Hz, $J_{3,14'}=1$ Hz.

Table 2
^{13}C NMR chemical shifts of the compounds **1**, **2** and **3**

^{13}C	**1**	**2**	**3**
1	47.5	50.8	46.9
2	32.5	25.6	30.0
3	30.2	29.4	32.4
4	150.9	139.3	150.3
5	51.9	51.9	52.1
6	85.1	80.4	85.1
7	45.0	44.3	44.8
8	30.9	24.2	28.7
9	36.2	30.6	37.4
10	148.8	86.0	151.7
11	139.5	141.2	39.3
12	170.0	170.1	179.9
13	119.9	119.5	11.3
14	109.4	110.6	109.3
15	112.4	26.1	111.7
CO–Me		22.4	
CO–Ac		169.7	

Table 3
^{1}H NMR data of compounds **4** and **5**

^{1}H	**4**	**5**
1	4.96 d	5.05 d
2α	2.24 m	2.21 m
2β	2.24 m	2.21 m
3α	2.06 m	1.99 m
3β	2.52 ddd	2.54 m
5	4.92 br d	4.81 br d
6	4.89 dd	4.98 dd
7	3.04 dddd	2.99 dddd
8	5.01 m	5.07 d
9α	2.44 dd	2.40 dd
9β	2.56 d	2.49 d
13	6.35 d	6.35 dd
13′	5.80 d	5.84 d
14	1.51 s	1.49 s
15	4.60 s	4.30 dd
15′	4.60 s	4.8 d
17	2.10 s	2.09 s
19	2.10 s	

Compound **4** $J_{1,2}=10$ Hz, $J_{5,6}=10$ Hz, $J_{6,7}=8$ Hz, $J_{7,8}=10$ Hz, $J_{7,13}=3.4$ Hz, $J_{7,13'}=2.9$ Hz, $J_{8,9\alpha}\cong 10$ Hz, $J_{9\alpha,9\beta}=13$ Hz.
Compound **5** $J_{1,2}=9$ Hz, $J_{5,6}=10$ Hz, $J_{5,15}=1.4$ Hz $J_{6,7}=10$ Hz, $J_{7,8}=8$ Hz $J_{7,13}=3.2$ Hz, $J_{7,13'}=2.9$ Hz, $J_{8,9\alpha}=10$ Hz, $J_{9\alpha,9\beta}=13$ Hz, $J_{15,15'}=14$ Hz.

the sesquiterpene lactones **1**–**3**, the presence of an α-methylene-γ-lactone was apparent in **4** from the chemical shift of an exocyclic methylene proton at δ_H 6.35 d and δ_H 5.8 d which coupled with an allylic methine at δ_H 3.04. All proton signals shown in the ^{1}H NMR spectrum of **4**, were assigned by analysis of the HMQC spectrum.

The ^{13}C NMR spectrum displayed 19 carbons (Table 4): three carbonyls, six olefinic carbons (one of them an exomethylenic carbon), three methines (two of them oxygenated) an oxygenated methylene, three methyls and three more methylene groups. The D/CI-mass spectrum of **4** showed m/z 366 $[M+NH_4]^+$ and the molecular formula was established as $C_{19}H_{24}O_6$.

The ^{1}H and ^{13}C NMR data gave evidence that two double bounds were present in this compound and were compatible with a structure of a germacrane-type sesquiterpene lactone.

The structure of **4** was elucidated by means of long-range heteronuclear correlation spectroscopy (HMBC). The findings of the HMBC were finally confirmed by the result of COSY experiments, in which couplings between the proton at δ_H 5.01 (H-8) and H-7 and H-9α were found. This indicated that one acetyloxy moiety was attached to C-8. Couplings were also found between the singlet at δ_H 4.60 (H-15) and H-3α and H-5, giving evidence for the second acetyloxy moiety at C-15.

The high field shift of H-1 (δ_H 5.01), as well the values of $J_{7,13}$ and $J_{7,13'}$ (3.4 and 2.9 Hz) and the coupling constants for the protons at the positions 5–7 ($J_{5,6}=10$ Hz, $J_{6,7}=8$ Hz) gave evidence for the *trans* geometries of the double bounds at C-1, C-10 and C-4, C-5 (Bohlmann, Misra, Jakupovic, King, & Robinson, 1985; Zdero, Bohlmann, & Schemeda-Hirschmann, 1987; Hernandez, Catalán, Cerda-García-Rojas, & Joseph-Nathan, 1996). As in costunolide, the shift of the olefinic methyl appears at high field (1.51 ppm), suggesting that **4** presents a conformation as in costunolide with both olefinic groups above the plane (Bohlmann et al., 1985; Doskotch & El-Feraly, 1970). The α-orientation of the acetyl group at C-8 was deduced by the characteristic coupling $J_{7,8}=8$

Table 4
^{13}C NMR chemical shifts of compounds **4** and **5**

^{13}C	**4**	**5**
1	129.3	128.7
2	26.0	26.2
3	34.7	34.6
4	138.9	143.9
5	130.9	129.5
6	76.6	76.7
7	52.6	52.7
8	72.2	72.4
9	48.7	49.0
10	132.3	132.6
11	135.1	135.4
12	169.5	169.7
13	125.2	125.4
14	16.8	16.8
15	61.6	61.5
16	169.4	169.7
17	21.0	21.2
18	170.5	
19	20.8	

Hz, $J_{8,9\alpha}=10$ Hz (Bohlmann & Zdero, 1982; Cardona, Garcia, Navarro, & Pedro, 1994). Compound **4** was identified as 8,15-acetylsalonitenolide and is a new compound.

The ^{13}C NMR spectrum of **5** was very similar to that of **4**, but only two methyl and two carbonyl groups were detected, suggesting that in **5** one acetyl was replaced by a hydroxyl group.

In the ^{1}H NMR spectrum of **5**, a AB system ($J=14$ Hz) is visible at 4.30 and 4.07 corresponding to the methylene group at C-10. The difference in the signal of this group in the compounds **4** and **5**, showed that in **5** the hydroxyl group was attached to the methylene at C-10. Compound **5** was identified as 8-acetylsalonitenolide and is a new compound.

The bryophytes are known to be a rich source of compounds with interesting biological activities (Asakawa, 1989). Therefore, the antifungal activity of the isolated compounds against *Candida albicans* and *Cladosporium cucumerinum* were determined by bioautographic TLC assays and in an agar-dilution assay. Amphotericin B (1 μg) and propiconazole (0.1 μg) were used as reference compounds, respectively for *C. albicans* and *C. cucumerinum*. In the agar dilution assay amphotericin B was used as reference compound at the concentrations of 1 μg/ml and 10 μg/ml, for *C. albicans* and *C. cucumerinum*, respectively. The larvicidal activity against *Aedes aegypti* larvae was also determined using β-asarone (15 ppm) as reference compound. The results are listed in Table 5. Compounds **1**, **2** and **3** presented activity against *C. cucumerinum* and *A. aegypti*, but it was **1** that presented the strongest activity. This compound was the only one with activity against *C. albicans*. Compound **4** only presented activity against *C. cucumerinum* and **5** was inactive in all bioassays.

3. Experimental

3.1. General

^{1}H and ^{13}C spectra were measured in $CDCl_3$ at 200.06 and 50.30 MHz, respectively, except the ^{1}H spectrum of **4**, that was measured at 500 MHz. TMS: int. standard. TLC silica gel 60 F_{254} Al sheets (Merck). CC silica gel (40–63 μm, Merck, 820×50 mm i.d. and 430×40 mm i.d.) MPLC: home packed Lichroprep RP-18 columns (15–25 μm, 460×16 mm i.d.). ^{1}H and ^{13}C NMR: Varian VXR 200, EI-MS and D/CI-MS: Finnigan MAT TSQ-700 Triple-stage quadruple instrument. Purity of compounds was checked by HPLC: Novapak RP-18 column (5 μm, 150×3.9 mm i.d.).

3.2. Plant material

T. lorbeeriana was collected in February 1994 near Águeda, Portugal and identified by Professor Dr R. Mues. Voucher specimen number 5461 was deposited at the Institute of Botany, University of Saarland, Germany.

3.3. Extraction and isolation

Plant material (250 g) was dried on filter paper at room temperature in the dark and extracted at room temperature successively with CH_2Cl_2 and MeOH to afford 11.96 and 12.31 g of extract, respectively.

A portion of CH_2Cl_2 extract (10 g) was subjected to CC on silica gel, using mixts of petrol, EtOAc and MeOH of increasing polarity, giving frs 1–28.

Frs 10 and 11 (2.2 g) were fractionated by CC on silica gel using a petrol–EtOAc–MeOH gradient, giving frs A–

Table 5
Antifungal and larvicidal activities of isolated sesquiterpene lactones

Compound	*Candida albicans*		*Cladosporium cucumerinum*		*Aedes aegypti*
	Bioautography[a]	Agar dil. assay	Bioautography[a]	Agar dil. assay	Larvicidal assay[c]
1	5	40[b]	0.5	20[b]	12.5
2	–	n.d.	10[d]	–	50[d]
3	–	n.d.	3	–	50[d]
4	–	n.d.	5	–	–
5	–	n.d	–	n.d	–

[a] Minimal amount (μg) of compound to inhibit growth on silica gel TLC plate.
[b] Minimal inhibition concentration MIC (μg/ml) of compound.
– means inactive at amount $\leqslant 10$ μg or 50 μg/ml.
n.d. MIC of compound not determined.
LC_{100} (concentration in ppm)
[d] Border line.

O. MPLC of fraction E (650 mg) on RP-18 with MeOH–H_2O (6:4 and 7:3) resulted on the isolation of compound **1** (500 mg).

Compound **2** (42 mg) was isolated from frs 16 and 17 (300 mg) by MPLC with MeOH–H_2O (6:4). Compound **3** (37 mg) was isolated from frs 13 to 15 (400 mg) by MPLC with MeOH–H_2O (6:4). Compound **4** (82 mg) was isolated from frs 18 and 19 (600 mg) by MPLC with MeOH–H_2O (6:4, 5:5 and 4:6). Compound **5** (4.7 mg) was isolated from fr 21 (190 mg) by MPLC with MeOH–H_2O (6:4, 5:5 and 75:25).

3.4. Bioassays

Bioautographic TLC assays with *C. cucumerinum* and *C. albicans* for evaluating biological activity of extract, fractions and pure compounds, were performed as described by Rahalison et al. and by Homans and Fuchs (Homans & Fuchs, 1970; Rahalison, Hamburger, Hostettmann, Monod, & Frenk, 1991; Rahalison, Hamburger, Monod, Frenk, & Hostettmann, 1994). The activity of pure compounds against *C. cucumerinum* and *C. albicans* was further determined according Rahalison et al. (1994). Larvicidal activity against *A. aegypi* of extract and pure compounds was evaluated as described by Cepleanu et al. (1994).

Crystallographic data for compound **2**. $C_{17}H_{22}O_4$, monoclinic, space group $P2_1$, $a=8.527(2)$, $b=9.2006(14)$, $c=10.0373(14)$ Å $\beta=90.48(1)^\circ$, $Z=2$, $T=293$ K, colourless rods, $0.68\times0.30\times0.19$ mm, 3132 reflections measured, 1574 independent reflections ($R_{int}=0.051$), 1314 observed reflections [$I>2\sigma(I)$], final $R1=0.0392$, $Rw2=0.0859$ (observed data), Goodness of fit 1.115, residual density max/min $0.151/-0.121$ eÅ^{-3}. Absorption coefficient $\mu=0.086$ mm^{-1}; no correction for absorption was applied.

Intensity data were collected at room temperature (at -60°C for *INA3*) on a Stoe AED2 4-circle diffractometer using MoKα graphite monochromated radiation ($\lambda=0.71073$ Å) with $\omega/2\Theta$ scans in the 2Θ range 5–51°. The structures were solved by direct methods using the program SHELXS-86 (Sheldrick, 1990). The refinement and all further calculations were carried out using SHELXL-93 (Sheldrick, 1993). The hydroxyl H-atoms were located from difference maps and allowed to refine isotropically. The remainder of the H-atoms were included in calculated positions and allowed to ride on the corresponding C atom. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2.

The bond lengths and angles are normal within experimental error. No attempt was made to determine the absolute configuration of the molecules. Full tables of atomic parameters and bond lengths and angles may be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (UK) on quoting the full journal citation. Further details may be obtained from the author HS-E.

3.5. Dehydrocostus lactone (*1*)

Amorphous white powder, mp 56.0–57.5°C, $[\alpha]_D=-9.7^\circ$ ($CHCl_3$, $c=1.75$). EI/MS m/z (rel. int.): 230 $[M]^+$ (100), 201 (19), 173 (11), 150 (52), 132 (15), 120 (11).

3.6. Acetyltriflocusolide lactone (*2*)

Colourless crystals, mp 153.1–154.5 °C, $[\alpha]_D=-53.1^\circ$ ($CHCl_3$, $c=0.67$). D/CI MS (positive ion mode) m/z (rel. int.): 308 $[M+NH_4]^+$ (28), 248 (100), 231 (11).

3.7. 11-αH-dihydrodehydrocostus lactone (*3*)

Yellow oil, $[\alpha]_D=+94^\circ$ ($CHCl_3$, $c=0.59$). D/CI MS (positive ion mode) m/z (rel. int.): 250 $[M+NH_4]^+$ (100), 233 $[M]^+$ (16), 205 (7), 169 (7), 158 (11).

3.8. 8,15-Acetylsalonitenolide (*4*)

Yellow oil, $[\alpha]_D=+70^\circ$ ($CHCl_3$, $c=0.81$). D/CI MS (positive ion mode) m/z (rel. int.): 366 $[M+NH_4]^+$ (100), 324 (15), 306 (36).

3.9. 8-Acetylsalonitenolide (*5*)

Yellow oil, $[\alpha]_D=+66^\circ$ ($CHCl_3$, $c=0.48$). D/CI MS (positive ion mode) m/z (rel. int.): 324 $[M+NH_4]^+$ (100), 306 $[M]^+$ (19), 282 (24), 266 (20), 264 (96), 248 (11), 246 (23).

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