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Triterpenes with a new 9-epi-cucurbitan skeleton from *Senecio selloi*

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Abstract

From the fresh aerial parts of *Senecio selloi* (Asteraceae), two diastereomeric triterpenes with a hitherto unknown skeleton have been isolated. The structure has been elucidated mainly by advanced NMR experiments, including inverse techniques, HMQC, HMBC, ROESY.

Keywords: Senecio selloi Spreng. De Candolle; Asteraceae; Triterpenes

1. Introduction

Senecio selloi Spreng. De Candolle (Asteraceae) grows as a shrub in southern Brazil. The pyrrolizidine alkaloids retrorsine, senecionine (Habermehl, Martz, Tokarnia, Dobereiner & Mendez, 1988) and l8-hydroxyjaconine (Krebs, Carl & Habermehl, 1996) have been isolated from this plant and are considered to be responsible for its toxicity against cattle, sheep and horses. Recently we reported the isolation of five sesquiterpene peroxides from the aerial parts of *S. selloi*. These compounds show antimalarial activity against *Plasmodium falciparum* (Heinzmann, 1996; Rücker et al., 1996, 1997). We now describe two triterpenes with a hitherto unknown skeleton (Heinzmann, 1996).

2. Results and discussion

From the methylene chloride extract of the fresh aerial parts of *S. selloi*, the compounds **1** and **2** have been isolated by repeated column chromatography on $AgNO_3$-silica gel (1:9). The structure was demonstrated by advanced NMR experiments (Tables 1, 2, 3), including inverse techniques (Kessler et al., 1988), HMQC (C-H-COSY) to coordinate protons and carbons and H-H-COSY and HMBC (C-H-COLOQ) to elucidate the linkage of the atoms.

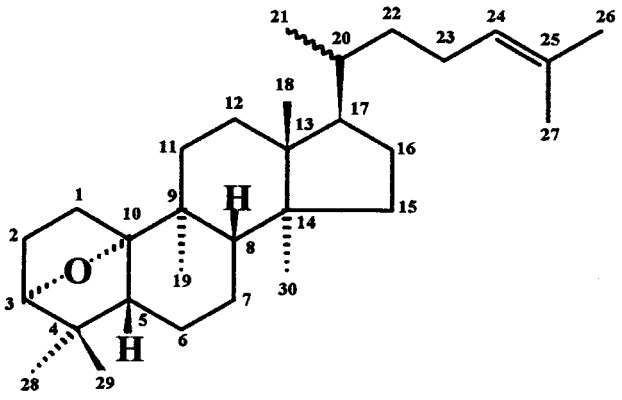

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Table 1
^{1}H NMR spectral data and H-H-COSY of compound **1** (500 MHz, in $CDCl_3$, TMS as reference)

H	**1**		**2**	
	δ (ppm)	H-H-COSY	δ (ppm)	H-H-COSY
1α	1.93	1β-H/2α-H(2β-H/3-H	1.93	1β-H/2β-H/2β-H/5-H
1β	1.45	1α-H/2α-H/2β-H	1.45	1α-H/2α-H/2β-H
2α	1.67	1α-H/lβ-H/2β-H/3-H	1.66	1α-H/1β-H/2β-H
2β	1.92	1α-H/1β-H/2α-H	1.91	1α-H/1β-H/1α-H
3	3.75	1α-H/1β-H/2α-H/5-H/29-Me	3.76	1α-H/1β-H/2α-H/2β-H, 5-H/6α-H/29-Me
5	1.32	6α-H/6β-H	1.30	6α-H/6β-H
6α	1.46	5-H/6β-H/7α-H/7β-H	1.43	5-H/6β-H/7α-H/7β-H
6β	1.37	6α-H/6β-H/7β-H	1.37	5-H/6α-H/7α-H/7β-H
7α	1.72	6α-H/6β-H/7β-H/8-H	1.70	6α-H/6β-H/7β-H/8-H
7β	1.35	6α-H/6β-H/7α-H	1.34	6α-H/6β-H/7α-H/8-H
8	1.64	7α-H/7β-H/30-Me	1.64	7α-H/7β-H
11α	1.18	11β-H/12α-H/12βH	1.18	11β-H/12α-H/12β-H
11β	2.13	11α-H/12α-H/12β-H	2.13	11α-H/12α-H/12β-H/19-Me
12α	1.78	11α-H/11β-H/12β-H/18-Me	1.81	11α-H/11β-H/12β-H/18-Me
12β	1.56	11α-H/11β-H	1.55	11α-H/11β-H/12α-H/18-Me
15α	1.19	15β-H/16α-H/16β-H	1.19	15β-H/16α-H/16β-H
15β	1.09	15α-H/16α-H/16β-H/30-Me	1.08	15α-H/16α-H/16β-H
16α	1.89	15α-H/15β-H/16β-H/17-H	1.86	15α-H/15β-H/16β-H/17-H
16β	1.26	15α-H/15β-H/16α-H/17-H	1.28	15α-H/15β-H/16α-H/17-H
17	1.51	16α-H/16β-H/18-Me/20-H	1.57	16α-H/16β-H/18-Me/20-H
18-Me	0.78	12α-H/17-H	0.78	12α-H/12β-H/30-Me
19-Me	1.14	11β-H	1.14	11β-H
20	1.43	17-H/21-Me/22a-H/22b-H	1.50	17-H/21-Me/22a-H/22b-H
21-Me	0.92	20-H	0.86	20-H
22a	1.04	20-H/22b-H/23a-H/23b-H	1.11	20-H/22b-H/23a-H/23b-H
22b	1.42	20-H/22a-H/23a-H/23b-H	1.60	20-H/22a-H/23a-H/23b-H
23a	1.85	22a-H/22b-H/23b-H, 24-H/26-Me/27-Me	1.88	22a-H/22b-H/23b-H/26-Me
23b	2.04	22a-H/22b-H/23a-H, 24-H/26-Me/27-Me	2.02	22a-H/22b-H/23a-H/26-Me
24	5.09	23a-H/23b-H/26-Me/27-Me	5.08	23a-H/26-Me/27-Me
26-Me	1.68	23a-H/23b-H/24-H/27-Me	1.69	23a-H/23b-H/24-H/27-Me
27-Me	1.60	23a-H/23b-H/24-H/26-Me	1.61	24-H/26-Me
28-Me	0.90	29-Me	0.90	29-Me
29-Me	1.01	3-H/28-Me	1.01	28-Me
30-Me	0.93	15β-H/8-H	0.94	15β-H

The molecular formula of the oily compound **1** ($C_{30}H_{50}O$; HR-EI-MS: required 426.3862, found 426.3870) corresponds to 6 double bond equivalents. NMR data (Tables 1–3) show one double bond suggesting a five ring system, probably a triterpene with 8 CH_3-, 10 CH_2-, 5 CH-groups and 5 quarternary C-atoms. According to the IR spectrum no carbonyl nor OH groups are present but intensive bands for C–O stretching vibrations around 1000 cm^{-1}. The oxygen obviously forms an ether linkage.

Starting with the oxygen bearing methine group 3-H at δ_H 3.75 (J = 6 Hz; δ_C 84.5) (Hadley & Halsall, 1974) a cross signal to δ_H 1.67 (2α-H) is observed in the H,H-COSY experiment (Table 1) as well as long-range couplings with δ_H 1.93/1.45 (1α-H, 1β-H), δ_H 1.32 (5-H) and δ_H 1.01 (29-Me). From the HMBC (Table 2) long-range couplings of C-3 to 1β-H, 28-Me, 29-Me as well as of 3-H to δ_C 32.1 (C-1), δ_C 53.4 (C-5) and δ_C 93.6 (C-10) have been identified. In the same experiment C-28 and C-29 show couplings with 5-H. In the H,H-COSY experiment (Table 1), cross peaks from 5-H only to δ_H 1.46/1.37 (6α,β-H) are observed and from HMBC (Table 2) long-range couplings from C-5 to 1α-H, 1β-H, δ_H 1.72, 1.35 (7α-H, 7β-H) and 28-Me and 29-Me. This led to the conclusion that two quarternary carbons are linked to C-5. The same experiment also showed, that δ_H 1.67 (2α-H) has a long-range coupling to the quarternary C-4 and both 2α-H, 2β-H also to C-1. The oxygen bearing carbon C-10 (δ_C 93.6) is coupling with 1α-H, 3-H and 6α-H,6β-H. These results unambiguously lead to the structure of ring A of **1** with an ether bridge between C-3 and C-10 (Hadley & Halsall, 1977; Li et al., 1993). The structures of rings B, C and D have been deduced in an analogous way (Tables 1 and 2). In the ^{13}C NMR spectrum (Table 2) δ_C 125.3 and δ_C 131.0 (C-24/25) as well as δ_H 5.09 (t, J = 5 Hz) (Table 1) represent a trisubstituted double bond. In the H,H-COSY (Table 1) couplings of the olefinic proton with δ_H 1.85 (23a-H), δ_H 2.04 (23b-H), δ_H 1.68 (26-Me) and 1.60 (27-Me) are

Table 2
^{13}C NMR spectral data, HMQC and HMBC of compounds **1** and **2** (125/500 MHz, in $CDCl_3$, TMS as reference)

C	**1**			**2**	
	δ (ppm)	C-H-COSY	HMBC	δ (ppm)	HMQC
1	32.1	1α-H/1β-H	2α-H/2β-H/3-H/5-H	32.1	1α-H/1β-H
2	24.8	1α-H/1β-H	1α-H/1β-H/3-H	14.8	1α-H/1β-H
3	84.5	3-H	1β-H/2α-H/28-Me/29-Me	84.5	3-H
4	43.5	–	1α-H/2α-H/5-H/28-Me/29-Me	43.5	–
5	53.4	5-H	1α-H/1β-H/3-H/7α-H/7β-H/28-Me/29-Me	53.4	5-H
6	19.7	6α-H/6β-H	5H/7α-H/7β-H	19.7	6α-H/6β-H
7	21.1	7α-H/7β-H	8-H	21.,1	7α-H/7β-H
8	39.4	8-H	7α-H/7β-H/11α-H/19-Me/30-Me	39.4	–
9	37.1	–	5-H/8-H/11α-H/1β-H/12β-H/19-Me	37.,1	–
10	93.6	–	1α-H/3-H/6α-H/6β-H/8-H/11β-H/19-Me	93.6	–
11	31.7	11α-H/11β-H	12α-H/12β-H	31.1	11α-H/11β-H
12	29.9	12α-H/12β-H	11α-H/11β-H/18-Me	30.1	12α-H/12β-H
13	48.4	–	8-H/12α-H/15α-H/16β-H/18-Me/30-Me	48.5	–
14	46.1	–	11α-H/12α-H/12β-H/16α-H/17-H/18-Me/30-Me	46.2	–
15	34.4	15α-H/15β-H	8-H/16α-H/16β-H/30-Me	34.3	15α-H/15β-H
16	28.1	16α-H/16β-H	–	28.2	16α-H/16β-H
17	50.8	l7-H	15α-H/16α-H/20-H/18-Me/21-Me	50.3	17-H
18	15.7	18-Me	12α-H/20-H	15.5	l8-Me
19	20.9	19-Me	5-H/7α-H/7β-H/8-H/11β-H	20.9	19-Me
20	36.,0	20-H	16β-H/17-H/20-H/21-Me/22a-H	35.5	20-H
21	18.7	21-Me	20-H/22a-H	19.1	21-Me
22	36.6	22a-H/22b-H	20-H/21-Me	35.4	22a-H/22b-H
23	25.0	23a-H/23b-H	22a-H	24.8	23a-H/23b-H
24	125.3	24-H	22b-H/26-Me/27-Me	125.2	24-H
25	131.0	–	26-Me/27-Me	131.0	–
26	25.8	26-Me	27-Me	25.8	26-Me
27	17.7	27-Me	26-Me	17.8	27-Me
28	23.1	28-Me	3-H/5-H/29-Me	23.1	28-Me
29	24.4	29-Me	5-H/28-Me	24.5	29-Me
30	18.5	30-Me	8-H/15β-H	18.6	30-Me

observed. HMBC (Table 2) shows long-range couplings from 26-Me and 27-Me to C-24 and C-25. From these results and literature data (Kim et al., 1995) a dimethylallyl side chain on C-17 of **1** is obvious. This was confirmed by EI-MS fragments at *m*/*z* 343 (M-83) and *m*/*z* 314 (M-112).

The spectroscopic data of compound **2** closely resemble those of **1**. Obviously **1** and **2** are epimers (diastereomers). This led to investigations of their relative stereochemistry by ROESY experiments (Table 3). Cross peaks of 1α-H with δ_H 2.13 (11β-H) support an α-position of the C-3/C-10 ether bridge and a trans anellation of rings A/B and B/C. Further cross peaks between δ_H 0.90 (28-Me/6α-H, 28-Me/6β-H); 1β-H/5-H; 6αH/δ 1.14 (19-Me) and δ_H 1.72 (7α-H/19-Me) support the relative configuration. The lack of a NOE between δ_H 1.64 (8-H) and 19-CH_3 confirms the B/C trans linkage. The C/D trans configuration was deduced from a NOE between 8-H and l8-Me and the lack of a cross signal of 8-H and δ_H 0.93 (30-Me). The β-orientation of the side chain was established from a NOE between δ_H 1.51 (17-H) and 30-Me and the lack of a NOE between l8-H and 30-Me. Investigations to elucidate the absolute configuration have not been carried out. Compounds **1** and **2** therefore represent a new 9-epi-cucurbitan skeleton among the triterpenoids.

Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** indicates that they are epimers at C-20. They can be distinguished by interpretation of the chemical shifts of C-21 and C-22. Thus, C-21 is shielded in the 20*R*-isomer relative to that of the 20*S*-isomer, whilst for C-22 the opposite phenomenon is observed (Arriaga-Giner, Rullkötter, Peakman & Wollenweber, 1991). The chemical shifts for C-21 and C-22 in **2** (δ 19.1 and 35.4 ppm) and **1** (δ_C 18.7 and 36.6 ppm) indicate, therefore, a 20*S* configuration of **2** and the 20*R* configuration of **1**.

3. Experimental

3.1. General

$[\alpha]_D$ in $CHCl_3$ at 20°C. IR spectra: Perkin-Elmer 298 in $CHCl_3$. EI-MS: Kratos MS 50 at 70 eV. NMR

Table 3
Nuclear Overhauser Effekt (ROESY experiments) of compounds **1** and **2** (500 MHz, in $CDCl_3$, TMS as reference)

H	1		2	
	δ (ppm)	ROESY-Experiment cross signals with	δ (ppm)	ROESY-Experiment cross signals with
1α	1.93	11β-H	1.93	11β-H
1β	1.45	–	1.45	5-H
2β	1.92	3-H/29-Me	1.91	3-H/29-Me
3	3.75	2β-H/28-Me/29-Me	3.76	2β-H/28-Me
5	1.32	29-Me	1.30	1β-H/29-Me
6α	1.46	19-Me/28-Me	1.43	19-Me/28-Me
6β	1.37	28-Me	1.37	28-Me
7α	1.72	–	1.70	12α-H/19-Me/30-Me
7β	1.35	8-H	1.34	–
8	1.64	7β-H/15β-H/18-Me	1.64	18-Me
11β	2.13	1α-H/18-Me	2.13	1α-H/18-Me
12α	1.78	19-Me/30-Me	1.81	19-Me/30-Me
15α	1.19	30-Me	1.19	–
15β	1.09	8-H	1.08	–
16α	1.89	15α-H/17-H/30-Me	1.86	15α-H
16β	1.26	15β-H/18-Me/20-H	1.28	–
17	1.51	16α-H/21-Me/22a-H/30-Me/23b-H	1.57	21-Me/30-Me
18-Me	0.78	8-H/11β-H/15β-H/16β-H/20-H	0.78	8-H/11β-H/20-H
19-Me	1.14	6α-H/12α-H/30-Me	1.14	6α-H/7α-H/12α-H/30-Me
20	1.43	16β-H/18-Me/21-Me/23b-H	1.50	18-Me
21-Me	0.92	17-H/20-H/23a-H/23b-H	0.86	17-H/23a-H
22a	1.04	17-H/24-H	1.11	–
22b	1.42	24-H	1.60	–
23a	1.85	–	1.88	21-Me
23b	2.04	17-H/20-H/21-Me	2.02	–
24	5.09	22a-H/22b-H	5.08	–
28-Me	0.90	3-H/6α-H/6β-H	0.90	3-H/6α-H/6β-H
29-Me	1.01	2β-H/3-H/5-H	1.01	2β-H/5-H
30-Me	0.93	12α-H/16α-H/17-H/19-Me	0.94	7α-H/12α-H/17-H/19-Me

spectra: Bruker AMX in $CDCl_3$. ^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz.

3.2. Plant material

Fresh aerial parts and leaves of *S. selloi* were collected in 1990 and 1992 in Rio Grande do Sul (Southern Brazil) and were identified by Marcos Sobral (Universidade Federal do Rio Grande do Sul). A voucher specimen is deposited under number 91/8 at the Pharmazeutisches Institut der Universität Bonn, Germany.

3.3. Extraction and isolation

Leaves of *S. selloi* (1006 g) were extracted with CH_2Cl_2 and the extract concentrated in vacuo to yield 93 g residue. The extract was chromatographed on a silica gel column (2.7 kg) using CH_2Cl_2 (10 l) and mixtures of CH_2Cl_2–EtOAc (3.5 l), EtOAc (2.5 l) and MeOH (5 l). It yielded 7 fractions. The 5th fraction (676 mg) was rechromatographed on silica gel with petrol–Et_2O (95:5) and on $AgNO_3$–silica gel (1:9) with petrol–Et_2O (95:5), hexane–Et_2O (95:5 and 97:3) and petrol–EtOAc (95:5) to yield 69 mg of **2** and 14 mg of **1**.

3.4. (20R)-3α,10α-Epoxy-9-epi-cucurbita-24-ene (*1*)

Oil; Rf 0.43 (petrol–Et_2O, 95:5), anisaldehyde: carmine. $[\alpha]_D = +26.4^\circ$ (*c* 3.15). IR ν_{max} cm^{-1} (in mixture with **2**): 2900 (s), 2870 (s), 1720 (m), 1660 (w), 1450 (m), 1380 (m), 1330 (m), 1300 (w), 1255 (m), 1100 (w), 990 (m), 970 (m), 960 (m), 910 (w), 895 (w), 850 (w). MS m/z (rel. int.): 426.3870 [$C_{30}H_{50}O$, requires 426.3862], 412 (32.0), 411 (100), 344 (10.8), 343 (15.9), 313 (20.2), 149 (14.7), 138 (11.3), 137 (68.0), 135 (14.0), 123 (13.9), 121 (16.7), 109 (27.9), 107 (15.3), 95 (30.4), 83 (12.0), 81 (15.2), 69 (35.5). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

3.5. (20S)-3α,10α-Epoxy-9-epi-cucurbita-24-ene(*2*).

Oil; Rf 0.47 (petrol–Et_2O, 95:5), anisaldehyde: carmine. $[\alpha]_D = +77.02^\circ$ (*c* 0.27). IR ν_{max} cm^{-1} see **1**. MS *m/z* (rel. int.): 426.3856 [$C_{30}H_{50}O$, requires 426.3862], 412 (27.5), 411 (100), 343 (11.0), 314 (13.3), 313 (62.3), 149 (14.3), 138 (10.5), 137 (93.8), 135 (15.9), 123

(15.5), 121 (21.4), 109 (33.5), 107 (18.6), 101 (14.8), 95 (40.0), 93 (12.4), 83 (23.9), 82 (37.3), 81 (22.1), 69 (57.3). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

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