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Loasafolioside, a minor iridoid dimer from the leaves of *Loasa acerifolia*[1]

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Abstract

In continuation of our phytochemical studies on *Loasa acerifolia* Dombey, Loasaceae, a further novel dimeric iridoid glucoside named loasafolioside, **1**, was isolated from the leaves. The structure of **1** is similar to the recently isolated iridoid dimer, asaolaside, but owns an hemiacetalic group at position 3 of unit b. Compound **1** exists as an isomeric mixture like the iridoid dimer, laciniatoside I, which has an identical unit b. The structure of **1** was established by 1D and 2D NMR (H H COSY, HMQC, HMBC) and FABMS experiments.

Keywords: Loasa acerifolia; Loasaceae; Iridoid glucosides; Secoiridoid glucosides; Loasafolioside; Asaolaside; Acerifolioside; Tricoloroside methyl ester; Loganin; Loganic acid; Secoxyloganin

1. Introduction

Loasaceae are a nearly exclusively neotropical plant family of less than 300 species. The systematics of this iridoid-containing family (Kooiman, 1974) are under current investigation. The ongoing revisions mainly affect the subfamily Loasoideae with a total of ca 200 species. In this subfamily, besides monomeric iridoids, recently dimeric respectively trimeric compounds were found (Müller & Weigend, 1998; Müller, Kufer, Dietl & Weigend, 1998; Nicoletti, Di Fabio, Serafini, Garbarino, Piovano et al., 1991; Nicoletti, Di Fabio, Pastor de Abram & Urrunaga, 1996). In continuation of our investigations on iridoids in *L. acerifolia*, we isolated a further minor iridoid dimer, **1**, from leaf material. The structure was elucidated primarily by spectroscopic means.

2. Results and discussion

After soxhlet extraction of the powdered material with MeOH, the extract was diluted with H_2O, followed by partitioning between first CH_2Cl_2 and then EtOAc. The EtOAc fraction was separated over Sephadex LH 20. The minor iridoid, **1**, was purified by semipreparative rp HPLC.

UV- and IR-spectra obtained from **1** were characteristic for a compound owning an iridoidic enol ether system conjugated with a carbonyl group (UV 232 nm; IR 1700, 1640 cm^{-1}). Quasi-molecular ion peaks in the positive FAB mass spectrum of **1** at *m/z* 639 $[M + Na]^+$ and *m/z* 623 $[M + Li]^+$, respectively, were consistent with a molecular formula of $C_{28}H_{40}O_{15}$.

The ^{13}C- and ^1H NMR-data of compound **1** showed two sets of major signals, one set similar to secoxyloganin (unit a) and another to a loganetin-like ring system (unit b). A further set of carbon signals with low intensity could be attributed to unit b of a minor isomer of **1**. The signals of **1** corresponding to the secoxyloganin moiety were superimposable on the corresponding resonances of tricoloroside methyl ester,

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Table 1
^{13}C NMR data for iridoid dimers in CD_3OD; **4** in Me_2O–D_6.

C	**1** major	**1** minor	**2**	**3**	**4** major[a]	**4** minor[a]
Unit a						
1	97.6		97.5	97.5	96.9	
3	153.7		153.7	153.7	153.0	
4	109.9		109.9	109.9	110.1	
5	28.8		28.8	28.8	27.4	
6	35.5		35.5	35.6	44.9	
7	173.8		173.8	173.8	201.7	
8	134.5		134.5	134.5	134.8	
9	45.4		45.4	45.6	44.9	
10	120.6		120.8	120.7	120.4	
11	168.7		168.8[c]	168.8	167.0	
MeO–	51.7		51.8	51.7	–	
1′	100.0		99.9[d]	100.0	99.8	
2′	74.6		74.7	74.6	74.3	
3′	78.0		78.0	78.0	77.8	
4′	71.6		71.6	71.6	71.4	
5′	78.4		78.4	78.4	77.8	
6′	62.8		62.8	62.8	62.8	
Unit b						
1	64.8	57.9	97.6	70.8	64.2	57.5
3	97.0	91.8	152.5	172.0	96.6	91.2
4	54.0	–[b]	113.3	53.1	53.4	49.3
5	39.8	32.3	32.6	37.7	39.2	31.9
6	38.3	40.2	40.4	39.0	38.0	39.8
7	78.8	78.7	78.9	80.7	77.7	77.3
8	38.6	38.4	40.9	42.2	38.5	38.2
9	44.2	43.6	47.0	43.3	43.8	43.4
10	12.3	12.5	13.8	13.4	12.5	12.5
11	175.1	173.6	169.3[c]	170.3	174.3	172.5
MeO–	52.3	52.2	51.8	53.1	52.0	51.9
1′			100.2[d]			
2′			74.7			
3′			78.0			
4′			71.6			
5′			78.4			
6′			62.8			

[a] Data from Kocsis et al., 1993.
[b] Signal obscured by solvent signal. Assignments were confirmed by DEPT, ^1H ^1H COSY and HMQC and HMBC methods.
[c,d] Assignments in same vertical column may be interchanged.

2, and asaolaside, **3** (Table 1), two dimeric iridoids which have been recently isolated from *L. acerifolia* (Müller & Weigend, 1998; Müller et al., 1998). The presence and linkage of a single glucose moiety in **1** could be confirmed by ^{13}C- and ^1H NMR and NOE experiments.

In unit b of the molecule, the carbon signals of C-6_b, C-7_b, C-8_b and C-10_b were found to be similar to the values obtained with **2** and **3**. The carbon resonances in the six-membered ring resembled those of **3**, but were lacking the C-3_b carboxy signal. Instead, a carbon signal appeared at δ 97.0. In the H NMR spectrum of **1**, the corresponding signal of H-3_b was found as a doublet at δ 4.72 with a large coupling constant (J = 8.4 Hz, 1H). At δ 2.22, a H-4_b signal could be detected, which correlated in H H COSY with H-3_b, giving evidence for a saturated bond between C-3_b and C-4_b. At δ 64.8, a C-1_b methylene group was indicated by DEPT and HMQC experiments. The corresponding broad proton signal at δ 3.87 gave correlation with H-9_b at δ 1.75 in H H COSY and with C-3_b at δ 97.0 in HMBC. The chemical shift of the C-3_b signal, DEPT and HMBC experiments, proved an hemiacetalic structure at position 3. Two methoxyl signals at δ 3.66 and δ 3.70 could be attributed by their HMBC correlation with the C-11_a and the C-11_b carbon, respectively, to methyl ester groups of the two units. From the downfield shifts of the H-7_b and the C-7_b signals, which were similar to the values obtained with **2** and **3**, we

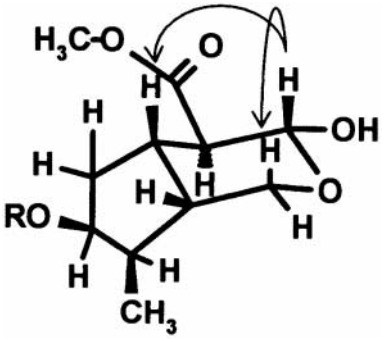

Fig. 1. Unit b of **1** with NOE interactions of proton H-3_b (R = Secoxyloganoyl moiety).

concluded that in **1**, the secoxyloganin moiety is linked via the C-7_a acid function to the C-7_b alcohol.

NOE experiments revealed relevant enhancement between H-3_b and H-5_b, respectively, H-1_b and only weak enhancement between H-3_b and H-4_b (Fig. 1). The large coupling constants between H-3_b and H-4_b ($J_{3,4}$ = 8.4 Hz) and between H-4_b and H-5_b ($J_{4,5}$ = 11.8 Hz) proved an axial position of these protons. The structure of the major isomer of **1** could be elucidated as shown in Fig. 2, with an equatorial α-3-hydroxy group in unit b.

Laciniatoside I **4**, a dimeric iridoid isolated from the aerial parts of *Dipsacus laciniatus* (Kocsis, Szabó & Podányi, 1993), owns the same substructure as now found in **1**. The carbon data reported for unit b of the dominant conformation of the major isomer, **4**, are in good aggreement with the signals obtained for unit b in **1**. On the basis of the spectral data reported for the minor isomer of **4**, the set of minor carbon signals of **1** could be attributed as shown in Table 1. An equilibrium ratio of 5:1 between both isomers in MeOH at room temperature was deduced from the NMR signal intensity of both isomers. Substance **1** is a new natural compound, which was named loasafolioside.

As a result of our previous investigations on the systematics of Loasoideae, several new or rearranged genera have been proposed (Weigend, 1997). Our main interest lies in the newly formed *Nasa* and the rearranged *Loasa s. l.*. The proposed *Loasa s. l.* include the Chilean members of *Loasa* (ca 31 spp. = *Loasa s. str.*), *Caiophora s. str.* (34 spp.) and *Scyphanthus* (1 sp.). The presence of dimeric and trimeric iridoids has now been established as a characteristic feature for *L. acerifolia*. Besides, the iridoid dimer tricoloroside has been isolated from the closely related *L. tricolor* (Nicoletti et al., 1991). In *C. pentlandii*, an iridoid consisting of two secoiridoid units was detected (Kocsis et al., 1993). Preliminary data suggest that these iridoid compounds may be useful systematic markers at the generic or infrageneric level. Tricoloroside methyl ester seems to be restricted to the Chilean members of *Loasa s. str.* Our current screening on Loasaceae aims at providing additional data on the distribution of iridoid oligomers in the other species of Loasoideae.

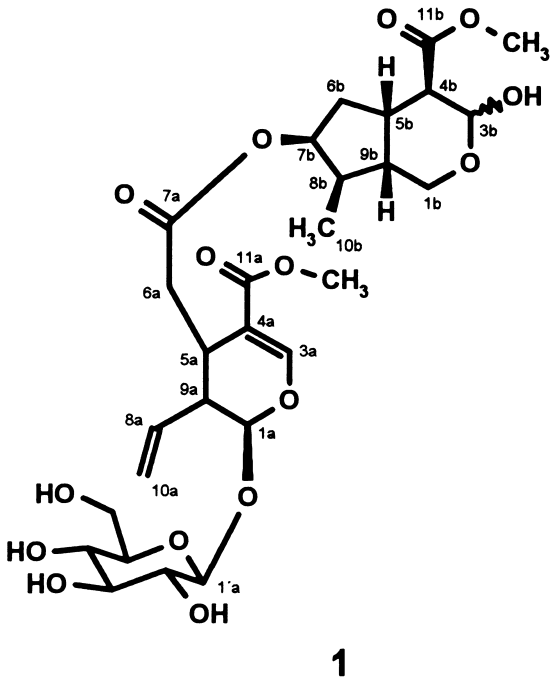

Fig. 2. Structure of **1**.

3. Experimental

Methods and plant material were as published earlier (Müller & Weigend, 1998).

3.1. Extraction and isolation

L. acerifolia leaves (220 g air-dried material) were powdered and extracted in a soxhlet apparatus with 1 l MeOH for 24 hrs. The MeOH extract was concd *in vacuo* to 300 ml and diluted with 600 ml H_2O. The soln was extracted (5×300 ml) subsequently with CH_2Cl_2 and EtOAc. The EtOAc fr. was dried over Na_2SO_4 and concd to dryness. After resuspension in MeOH, the fr. was separated over Sephadex LH 20 material with MeOH (5 ml/h; 10 ml/fr.). Compound **1** was eluted with fr. 19. The purification of **1** was performed with semi-prep HPLC on rp-18 material (column: Knauer, Berlin, Germany; LiChrosorb rp-18 (7 μm) ID 250×16 mm; solvent A: H_2O, solvent B: MeCN; flow: 5 ml/min gradient: 15–50% B in 20 min, 10 min 50% B; det.: UV 210 nm; Rt. **1**: 21 min) and yielded 12 mg **1**.

*3.2. Compound **1**, loasafolioside, 6-[[[3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2h-pyran-4-yl]acetyl]oxy]-octahydro-7-methyl-3-hydroxy-cyclopenta-[c]pyran-4-carboxylic acid methyl ester*

$C_{28}H_{40}O_{15}$: Amorphous white powder; $[\alpha]_{D}^{20}$ −23.5° (*c* 0.00425; MeOH); UV (MeOH) λ_{max} (log ϵ): 232 (3.72) nm; IR (KBr) ν_{max}: 1700, 1640, 1440, 1280, 1070 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 5.48 (1H, d, $J = 4.0$ Hz, H-1_a), 7.48 (1H, d, $J = 1.1$ Hz, H-3_a), 3.3 (1H, m, H-5_a), 2.32 (1H, dd, $J = 8.7$ and 16.5 Hz, H-6_a), 2.89 (1H, dd, $J = 4.9$ and 16.5 Hz, H-6_a), 5.63 (1H, m, H-8_a), 2.76 (1H, m, H-9_a), 5.26 (2H, m, H-10_a), 3.66 (3H, s, MeO-) 4.66 (1H, $J = 7.8$ Hz, H-$1_a'$), 3.21 (1H, m, H-$2_a'$), 3.26–3.40 (H-$3_a'$, H-$4_a'$, H-$5_a'$), 3.65 (1H, m, H-$6_a'$), 3.89 (1H, m, H-$6_a'$), 3.87 (2H, m, H_2 1_b), 4.72 (1H, d, $J = 8.4$ Hz, H-3_b), 2.22 (1H, dd, $J = 8.4$ and 11.8 Hz, H-4_b), 2.47 (1H, m, H-5_b), 1.86 (1H, m, H-6_b), 1.96 (1H, m, H-6_b), 5.23 (1H, m, H-7_b), 2.13 (1H, m, H-8_b), 1.75 (1H, m, H-9_b), 0.97, (3H, d, $J = 6.7$ Hz, H_3-10_b), 3.70 (3H, s, MeO-). Sugar proton signals H-$3_a'$, H-$4_a'$ and H-$5_a'$ overlapped by solvent CD_3OD; for ^{13}C NMR data see Table 1; FABMS (positive ion mode, 3-nitro benzyl alcohole matrix; LiCl) *m*/*z* (rel. int.): 623 *m*/*z* (13) $[M + Li]^+$, 639 (15) $[M + Na]^+$.

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