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IRIDOIDS FROM *LOASA ACERIFOLIA*

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Key Word Index—*Loasa acerifolia*; Loasaceae; iridoid glucosides; secoiridoid glucosides; acerifolioside; tricoloroside methyl ester; loganin; loganic acid.

Abstract—In the course of preliminary chemotaxonomic studies on Loasaceae from South and Central America, 30 species from 6 genera were screened by chromatographic means. From the aerial parts of *Loasa acerifolia* Dombey, the novel trimeric and dimeric iridoid glucosides acerifolioside and tricoloroside methyl ester, consisting of loganin and secoxyloganin moieties, were isolated. The structures were primarily elucidated by NMR spectroscopy. Loganin, loganic acid, rutin, scopoletin and chlorogenic acid were identified by cochromatography with reference compounds. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The family Loasaceae is widespread in the Americas. Many members of the family are used in homeopathy [1] and folk medicine as remedies against allergies, lung diseases, rheumatism [2, 3] and nausea, and for their choleretic and antihelminthic properties [4, 5]. Most of the genera of Loasoideae are urticant. The systematics of Loasaceae are still poorly understood and are being currently investigated [6].

In the first chemotaxonomic investigations on Loasaceae by Kooiman [7], loganin was isolated from the seeds of *Blumenbachia hieronymi* and detected, together with loganic acid, in the leaves of four other species of the family. *Mentzelia* sp. contain loganin, C_9-iridoids, secoiridoids [8], an acyclic C_{13} glucoside [9] and several iridoid chlorohydrins [10, 11]. Iridoids were also isolated from *Schismocarpus matudai* and *Eucnide bartonioides* [12, 13]. The dimeric iridoids tricoloroside and pentlandioside were found in *Loasa tricolor* [14] and *Cajophora pentlandii* [15], respectively.

Our current investigation aims at providing detailed data on the chemotaxonomy of Loasaceae from South America. From *Loasa acerifolia* leaf material, two novel iridoids named acerifolioside and tricoloroside methyl ester were isolated. Their structures were elucidated primarily by spectroscopic means.

RESULTS AND DISCUSSION

Thirty species from six genera of Loasaceae from South and Central America were screened by HPLC and TLC. Methanolic extracts from *L. acerifolia* leaves and seeds contained α,β-unsaturated iridoids, plant acids and flavonoids. By means of HPLC- and TLC-cochromatography with reference compounds, we identified loganin, loganic acid, rutin, scopoletin and chlorogenic acid. Extracts prepared from the aerial parts of *L. acerifolia* contained large amounts of lipophilic iridoids. 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 on Sephadex LH-20. Two iridoids **1** and **2** were purified by semipreparative ODS HPLC.

Both compounds had UV- and IR-spectra typical for an iridoid enol ether system conjugated with a carbonyl group (UV 232 nm; IR 1700, 1640 cm^{-1}). The presence of sugar moieties in both substances was confirmed by ^{13}C and ^{1}H NMR. For an unequivocal assignment of the ^{13}C and ^{1}H NMR resonances and the stereochemistry HMBC, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, NOE and DEPT experiments were performed.

Acid hydrolysis of **1** with diluted HCl provided two aglycone fragments. The positive FAB-mass spectrum of compound **1** gave a mass of *m/z* 776 with quasi-molecular peaks at *m/z* 783 $[M+Li]^+$ and 799 $[M+Na]^+$, respectively, compatible with the molecular formula $C_{34}H_{48}O_{20}$. The 123 NMR data (Table 1)

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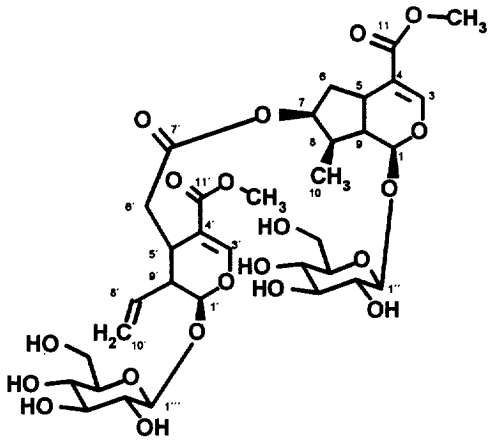
The investigation presented is part of a joint venture between the Institute of Systematic Botany and the Institute of Pharmaceutical Biology, Munich, for the investigation of the biology and systematics of Loasaceae and a screening program for antiinflammatory plant drugs from ethnopharmacology.



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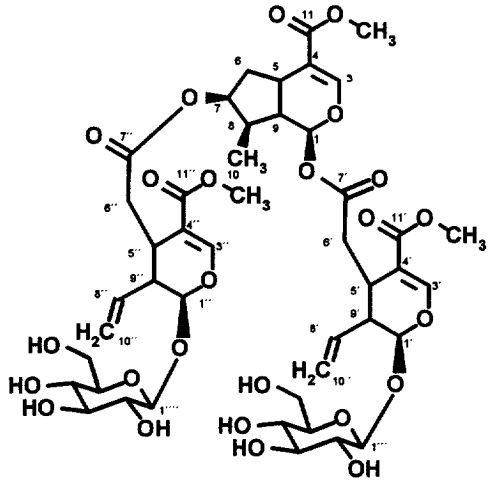

2

of compound **1** showed two sets of signals, one set similar to loganin and another to secoxyloganin [16, 17, data from reference compound], indicating a dimeric structure. From *L. tricolor* Ker., tricoloroside (**3**) an iridoid dimer with a free C-11 carboxy group in the loganin part has been isolated recently [14]. Like **3** (see Table 1), in **1** a downfield shift of H-7 of the loganin moiety at δ 5.13 and the corresponding C-7 at δ 78.9, and an upfield shift of C-6 at δ 40.4 and C-8 at δ 40.9 compared to loganin (**4**), indicated an ester linkage to the C-7 hydroxy group. At δ 173.8 an aliphatic ester carbonyl signal of the secoxyloganin moiety gave evidence for the link to the loganin part via the C-7 carboxy group. This conclusion was confirmed by HMBC experiments, which showed a correlation between the H-7 at δ 5.13 of the loganin part and C-7′ at δ 173.8 of the secoxyloganin part. In **1**, ^{1}H NMR signals at δ 3.68 (*s*, 3H) and 3.69 (*s*, 3H) were characteristic for two methoxyl groups, which correlated in HMBC with the carboxy signals at δ 169.3 and 168.8. Therefore, the two methyl esters were attributed to the C-11 of the loganin and secoxyloganin moieties. The structure of **1** was thus elucidated to be as shown in formula **1**. Compound **1** was named tricoloroside methyl ester. This is the first time this substance has been isolated from plant material. In extracts prepared from *L. tricolor* and other Loasaceae only small amounts of or no tricoloroside methyl ester could be detected by HPLC, while in MeOH and EtOH extracts from *L. acerifolia* leaves and seeds it is a major compound. We therefore conclude that it is not an artifact of the isolation procedures.

The pos. FAB mass spectrum of **2** showed quasi-molecular formula ion peaks at *m/z* 1007 $[M+Li]^+$ and 1023 $[M+Na]^+$, respectively, suggesting the molecular formula to be $C_{45}H_{60}O_{25}$, which was also supported by ^{13}C NMR and DEPT data. In the ^{1}H and ^{13}C NMR spectra of **2**, the signals corresponding to the secoxyloganin and loganetin moieties were superimposable on the corresponding resonances of compound **1**. In comparison with **1**, where the H-1 loganetin signal was found at δ 5.27, the same signal was shifted to δ 6.08 in **2**, showing an ester linkage at this position. By ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments, the carbon at δ 92.4 giving a correlation with the downfield shifted proton at δ 6.08 could be assigned to the C-1 acetal of the loganetin part of **2**. In **1** this carbon signal was at δ 97.6. The upfield shift of $\Delta = -5.2$ ppm in **2** corroborated this conclusion. The two anomeric carbon signals at δ 100.0 and 99.5, which gave correlations with anomeric protons at δ 4.65 (*d*, $J = 8.1$ Hz) and 4.66 (*d*, $J = 8.1$ Hz) were assigned to the anomeric protons of two molecules of β-D-glucopyranose. A nuclear overhauser effect between these anomeric protons and the H-1 acetalic protons of two secoxyloganin moieties at δ 5.46 and δ 5.49 proved the linkage of the sugars.

In **2** an aliphatic ester carbonyl group from the second secoxyloganin moiety was detected at δ 172.6 in addition to the signal at δ 173.7. It was confirmed by HMBC experiments that the three methoxyl groups at δ 3.67, 3.69 and 3.72 were attached to the C-11 carboxy groups of a loganin part at δ 168.8 and two secoxyloganin parts at δ 168.7 (Fig. 1). Therefore, we concluded that the core in compound **2** had the loganetin structure with two secoxyloganin parts linked via their aliphatic C-7 carboxy groups at the 7-hydroxy and 1-hydroxy groups of loganetin respectively. ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, DEPT and NOE experiments resulted in an unequivocal assignment of the other ^{13}C and ^{1}H NMR resonances (see Table 1). The structure of **2**, which was established as shown in formula **2**, is a new natural compound, which we named acerifolioside.

The distribution of iridoids in plants can be regarded as a chemotaxonomically useful marker [18]. Simple iridoids such as loganin, loganic acid and secoxyloganin are found in most genera [7, 8, 12–14] of Loasaceae, whereas C_{10}-deficient iridoids are

Table 1. ^{13}C NMR data for iridoid glucosides in CD_3OD; **3** in D_2O

C	1	3*	4	5	2	C
Loganin moiety						Loganetin moiety
1	97.6	97.4	97.7		92.4	1
3	152.5	152.1	152.1		151.6	3
4	113.3	113.3	114.0		114.0	4
5	32.6	31.1	32.2		31.6	5
6	40.4	39.9	42.7		40.2	6
7	78.9	79.8	75.1		78.8	7
8	40.9	39.1	42.2		40.5	8
9	47.0	46.1	46.5		46.2	9
10	13.8	13.0	13.4		13.2	10
11	169.3‡	170.8	169.5		168.8‡	11
MeO-	51.8		51.6		52.0§	MeO-
1″	100.2§	99.5	100.1			
2″	74.7	73.4	74.7			
3″	78.0	76.4‡	78.0			
4″	71.6	70.4	71.6			
5″	78.4	77.1‡	78.4			
6″	62.8	61.5	62.8			
Secoxyloganin moiety a‖						
1′	97.5	97.7		97.8	97.4	1′
3′	153.7	153.8		153.8	153.8	3′
4′	109.9	109.6		110.0	109.9	4′
5′	28.8	29.6		29.3	29.0	5′
6′	35.5	36.0		35.5	35.5	6′
7′	173.8	175.6		174.9	173.7	7′
8′	134.5	133.3		134.5	134.5	8′
9′	45.4	44.5		45.5	45.5	9′
10′	120.8	121.6		120.5	120.8	10′
11′	168.8‡	170.1		168.2	168.7‡	11′
MeO-	51.8	52.7		52.2/51.9	51.8§	MeO-
1‴	99.9§	99.5		100.1	100.0	1‴
2‴	74.7	73.4		74.7	74.6	2‴
3‴	78.0	76.4‡		78.2	77.9	3‴
4‴	71.6	70.4		71.6	71.5	4‴
5‴	78.4	77.1‡		78.4	78.3	5‴
6‴	62.8	61.5		62.8	62.8	6‴
Secoxyloganin moiety b‖						
					97.4	1″
					153.7	3″
					109.5	4″
					29.0	5″
					35.4	6″
					172.6	7″
					134.4	8″
					45.3	9″
					120.8	10″
					168.7‡	11″
					51.8§	MeO-
					99.5	1‴
					74.6	2‴
					77.9	3‴
					71.5	4‴
					78.3	5‴
					62.8	6‴

* Data from Ref. [14].

† Data of **5** (secologanoside dimethyl ester) from Ref. [17].

Assignments were confirmed by DEPT, ^1H-^1H and ^{13}C-^1H COSY and HMBC methods.

§, ‡ Assignments in same vertical column and ‖ between secoxyloganin moieties in **2** may be interchanged.

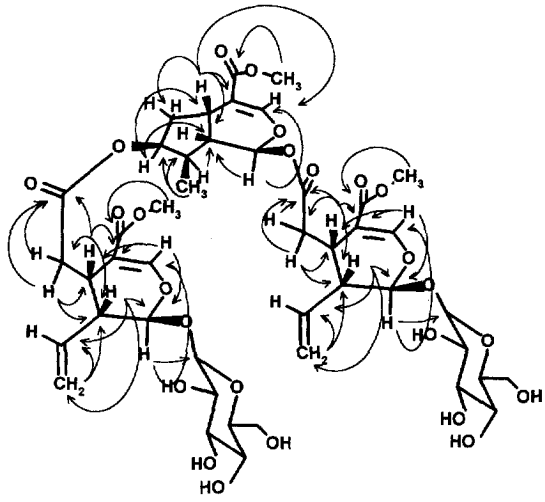

Fig. 1. ^{1}H ^{13}C correlation based on HMBC of acerifolioside (**2**).

characteristic for *Mentzelia* sp. [8, 10, 11]. The presence of oligomeric iridoids in *Cajophora pentlandii* [15], *Loasa tricolor* [14] and *L. acerifolia* confirms the close relationship of *Cajophora* to the Chilean members of *Loasa*. A close relationship is unequivocally suggested by numerous morphological characters and this phytochemical similarity here demonstrated lends additional support to the notion that these two groups share an exclusive common ancestry.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR: MeOH-d_4 at room temp. with TMS or MeOH as int. standards on a Bruker AM-360 (360.13 MHz, 90.6 MHz), Bruker AMX 500 (500.12 MHz, 125.7 MHz) or a Jeol GSX 400 N (399.79 MHz, 100.5 MHz). $J_{C\text{-}H} = 10$ Hz was used for HMBC experiments. NOE measurements were carried out by the 1D difference method. Pos. FAB MS: Kratos MS 80 RFA spectrometer with NBA (7 kV): IR: KBr. HPLC: Hewlett Packard 1090 liquid chromatograph. Separation system 1: column: LiChrospher RP-18, 125-4 (5 μm); precolumn: LiChrospher RP-18 4-4 (5 μm) (Merck, Darmstadt); solvent A: H_2O; solvent B: MeCN; gradient 0–25% B in 30 min 25–80% B in 60 min; flowrate: 1 ml/min; detection: UV-diode array. TLC (separation system 2): silica with PrOH–toluene–HOAc–H_2O (25:20:10:10) (det. UV 254 nm, vis after spraying with anisaldehyde/H_2SO_4). Reference compounds rutin, chlorogenic acid, loganin and loganic acid were purchased from Fa. Roth (Karlsruhe, Germany) and Extrasynthese S.A. (Genay, France).

Plant material

L. acerifolia Dombey seeds and leaves were obtained in autumn 1996 from the Botanical Garden, Munich, Germany, from plants taken into cultivation and identified by Prof. Dr J. Grau (Grau-LOA-89, Chile, Prov. De Valdivia, between Eucó and Rinihue, 110 m, 1990, MSB), Inst. Syst. Bot. Munich. Voucher specimens are deposited at the Herbarium of the Institute for Systematic Botany, Munich (MSB), Germany.

Extraction and isolation

L. acerifolia leaves (85 g air-dried material) were powdered and extracted in a soxhlet apparatus with 1 l MeOH for 24 h. The MeOH extract was concd to 300 ml *in vacuo* and diluted with 600 ml H_2O. The soln was extracted (5 × 300 ml) subsequently with *n*-hexane, CH_2Cl_2, ETOAc and BuOH. The EtOAc fr. was dried over Na_2SO_4 and concd to dryness (600 mg). After resuspension in MeOH, the EtOAc fr. was separated over Sephadex LH-20 with MeOH (5 ml/h). The frs were screened by HPLC (separation system 1) and TLC (separation system 2, det. UV 254 nm, vis after spraying with anisaldehyde/H_2SO_4). Compounds **1** and **2** were eluted with frs 12–17 (90 mg, dried wt). The separation of both compounds was performed by semi-prep. HPLC on ODS (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; R_t. **1**: 19 min; R_t. **2**: 24 min) and yielded 40 mg **1** and 20 mg **2**.

Acid hydrolysis of **1**. 1 mg of compound was dissolved in 1 ml of a 1:1 mixt. of H_2O and conc. HCl and refluxed on the water bath for 30 min The solvent was removed *in vacuo* by repeated addition of MeOH and the residue redissolved in MeOH.

Compound **1** *(tricoloroside methyl ester; [1S-[1α,4aα, 6α,(2R′,3S′,4R′),7α,7aα]]-6-[[[3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxy-carbonyl)-2H-pyran-4-yl]acetyl]oxy]-1-(β-D-glucopyranosyloxy)1, 4a,5,6,7,7a-hexahydro-7-methyl-cyclopenta[c]pyran-4-carboxyclic acid methyl ester).* $C_{34}H_{48}O_{20}$; amorphous powder; $[\alpha]_D^{20}$ −101° (MeOH; *c* 0.078); pos. FAB-MS (3-nitro benzyl alcohol matrix; LiCl): *m/z* 799 $[M+Na]^+$, 783 $[M+Li]^+$; UV: λ_{max}^{MeOH} nm (log ε): 232 (4.28); IR ν_{max} cm^{-1}: 1700, 1640, 1440, 1380, 1280, 1060; ^{1}H NMR (CD_3OD): δ 5.27 (1H, *d*, *J* = 4.7 Hz, H-1), 7.42 (1H, *d*, *J* = 1.28 Hz, H-3), 3.09 (1H, *m*, H-5), 1.71 (1H, *m*, H_a-6), 2.35 (1H, *m*, H_b-6), 5.13 (1H, *m*, H-7), 2.12 (1H, *m*, H-8), 2.07 (1H, *m*, H-9), 1.06, (3H, *d*, *J* = 6.41 Hz, H_3-10), 3.68 (3H, *s*, MeO-), 4.65 (1H, *J* = 7.7 Hz, H-1″), 3.21 (1H, *m*, H-2″), 3.29–3.38 (H-3′, H-4″, H-5″), 3.67 (1H, *m*, H-6″), 3.89 (1H, *dd*, *J* = 12.0 and 1.7 Hz, H-6″), 5.49 (1H, *d*, *J* = 3.9 Hz, H-1′), 7.48 (1H, *d*, *J* = 2.1 Hz, H-3′), 3.38 (1H, *m*, H-5′), 2.37 (1H, *m*, H-6′), 2.98 (1H, *dd*, *J* = 4.7 and 16.7 Hz, H-6′), 5.64 (1H, *m*, H-8′), 2.78 (1H, *m*, H-9′), 5.26 (2H, *m*, H-10′), 3.69 (3H, *s*, MeO-), 4.66 (1H, *d*, *J* = 7.7 Hz, H-1‴), 3.22 (1H, *m*, H-2‴), 3.29–3.38 (H-3″, H-4″, H-5″), 3.68 (1H, *m*, H-6‴), 3.90 (1H, *dd*, *J* = 12.0 and 1.7 Hz, H-6‴). Sugar proton signals

H-3, H-4 and H-5 were overlapped by solvent (CD_3OD); assignment of MeO-, H-1; H-2 and H-6 sugar signals may be interchanged between the two moieties. ^{13}C NMR: Table 1.

Compound 2 acerifolioside; [1S-[1α,4αα,6α(2R′,3S′,4R′),7α,7αα]]-1,6-Di-[[[3-ethenyl-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-pyran-4-yl]acetyl]oxy]-1,4a,5,6,7,7a-hexahydro-7-methylcyclopenta-[c]pyran-4-carboxyclic acid methyl ester). $C_{45}H_{60}O_{25}$; amorphous powder; $[\alpha]_D^{20}$ −150° (MeOH; *c* 0.119); pos. FAB-MS (3-NBA; LiCl) *m/z*: 1023 $[M+Na]^+$, 1007 $[M+Li]^+$; UV: λ_{max}^{MeOH} nm (log ε): 232 (4.50), IR ν_{max} cm^{-1}: 1700, 1640, 1440, 1380, 1280, 1060; ^1H NMR (CD_3OD): δ 6.08 (1H, *d*, *J* = 3.85 Hz, H-1), 7.35 (1H, *d*, *J* = 0.9 Hz, H-3), 3.08 (1H, *m*, H-5), 1.81 (1H, *m*, H_a-6), 2.35 (1H, *m*, H_b-6), 5.13 (1H, *m*, H-7), 2.04 (1H, *m*, H-8), 2.18 (1H, *m*, H-9), 1.07 (3H, *d*, *J* = 6.8 Hz, H_3-10), 3.72 (3H, *s*, MeO-), 5.46 (1H, *d*, *J* = 4.3 Hz, H-1′), 7.47 (1H, *d*, *J* = 1.7 Hz, H-3′), 3.38 (1H, *m*, H-5′), 2.40 (1H, *m*, H-6′), 2.90 (1H, *m*, H-6′), 5.65 (1H, *m*, H-8′), 2.77 (1H, *m*, H-9′), 5.26 (2H, *m*, H_2-10′), 3.67 (3H, *s*, MeO-), 4.65 (1H, *d*, *J* = 8.1 Hz, H-1‴), 3.21 (1H, *m*, H-2‴), 3.29–3.38 (H-3‴, H-4‴, H-5‴), 3.65 (1H, *m*, H-6‴), 3.90 (1H, *dd*, *J* = 12.0 and 1.7 Hz, H-6‴), 5.49 (1H, *d*, *J* = 4.3 Hz, H-1″), 7.48 (1H, *d*, *J* = 1.7 Hz, H-3″), 3.38 (1H, *m*, H-5″), 2.40 (1H, *m*, H-6″), 2.90 (1H, *m*, H-6″), 5.65 (1H, *m*, H-8″), 2.77 (1H, *m*, H-9″), 5.26 (2H, *m*, H_2-10″), 3.68 (3H, *s*, MeO-), 4.66 (1H, *d*, *J* = 8.1 Hz, H-1⁗), 3.22 (1H, *m*, H-2⁗), 3.29–3.38 (H-3⁗, H-4⁗, H-5⁗), 3.66 (1H, *m*, H-6⁗), 3.91 (1H, *dd*, *J* = 12.0 and 1.7 Hz, H-6⁗). Sugar proton signals H-3, H-4 and H-5 overlapped by solvent (CD_3OD); assignments between secoxyloganin moieties may be interchanged. ^{13}C NMR data Table 1.

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