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Iridoid glucosides—chemotaxonomic markers in Loasoideae ☆

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Abstract

As part of a chemotaxonomic screening for iridoids in Loasaceae samples from 74 species, mainly from subfamily Loasoideae, were assayed using HPLC and TLC. The presence of the iridoid dimer tricoloroside methyl ester and other oligomeric iridoids consisting of loganin and secoxyloganin moieties turned out to be characteristic for members of *ser. Macrospermae* and *ser. Floribundae* of the genus *Loasa*. In species of the genus *Caiophora* 10-hydroxyoleoside dimethyl ester and a further not yet identified iridoid serve as chemotaxonomic markers. Other monomeric iridoids (loganin, loganic acid, secoxyloganin, secoxyloganin methyl ester, sweroside, 8-*epi*-kingiside) are widespread throughout the whole family without having systematic value at subfamily or generic level.

Keywords: *Aosa*; *Blumenbachia*; *Caiophora*; *Huidobria*; *Kissenia*; *Klaprothia*; *Loasa*; *Mentzelia*; *Nasa*; *Plakothira*; *Presliophytum*; *Scyphanthus*; *Xylopodia*; Loasaceae; Iridoid glucosides; Secoiridoid glucosides; Asaolaside; Acerifolioside; Loasafolioside; Tricoloroside methyl ester; Loganin; Loganic acid; Secoxyloganin; Secoxyloganin methyl ester; 10-hydroxyoleoside dimethyl ester; 8-*epi*-kingiside; Sweroside

1. Introduction

Loasaceae are a nearly exclusively neotropical plant family of less than 300 species. Most of the species are urticant. In the folk medicine of Latin America and in homeopathy (Hoppe, 1975) members of Loasaceae are used for the treatment of lung diseases, rheuma, allergies and various impairments of the digestive organs (Gusinde, 1936; Soucoup, 1970; De Mösbach, 1992; De Feo, 1992) (Weigend, personal observation). The systematics of Loasaceae are under current investigation. The ongoing revisions mainly affect the subfamily of Loasoideae with a total of *ca.* 200 species. (Grau, 1988, 1996, 1997; Grau & Bayer, 1994; Weigend, 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1998, in prep, in press) These studies aim at replacing the sometimes artificial classification of the family by Urban and Gilg (1900) with more natural entities.

As a result of our studies a number of new or rearranged groups have been proposed in the subfamily Loasoideae. The subfamily is now subdivided into only two instead of three tribes, Klaprothieae and Loaseae (Kissenieae have been included in Loaseae).

In the small first tribe Klaprothieae (six species) a new monotypic genus *Xylopodia* (Weigend 1997) has been described in addition to the known two, *Klaprothia* Kunth and *Plakothira* Florence. The second and much larger tribe Loaseae contains the remaining *ca.* 190 species of the subfamily. There are a number of small genera with a total of 10 species which have variously retained primitive characters (the so-called "Lower Loaseae"). These are *Huidobria*, (Grau, 1997) *Presliophytum* (Urb. and Gilg) Weigend, *Chichicaste* Weigend, *Loasa* Adans. *ser. Malesherbioideae*

☆ The investigation here presented is part of a joint venture between the Institute of Systematic Botany and the Institute of Pharmacy, Department of Pharmaceutical Biology, Munich, for the investigation of the biology and systematics of Loasaceae and a screening program for anti-inflammatory plant drugs from ethnopharmacology.

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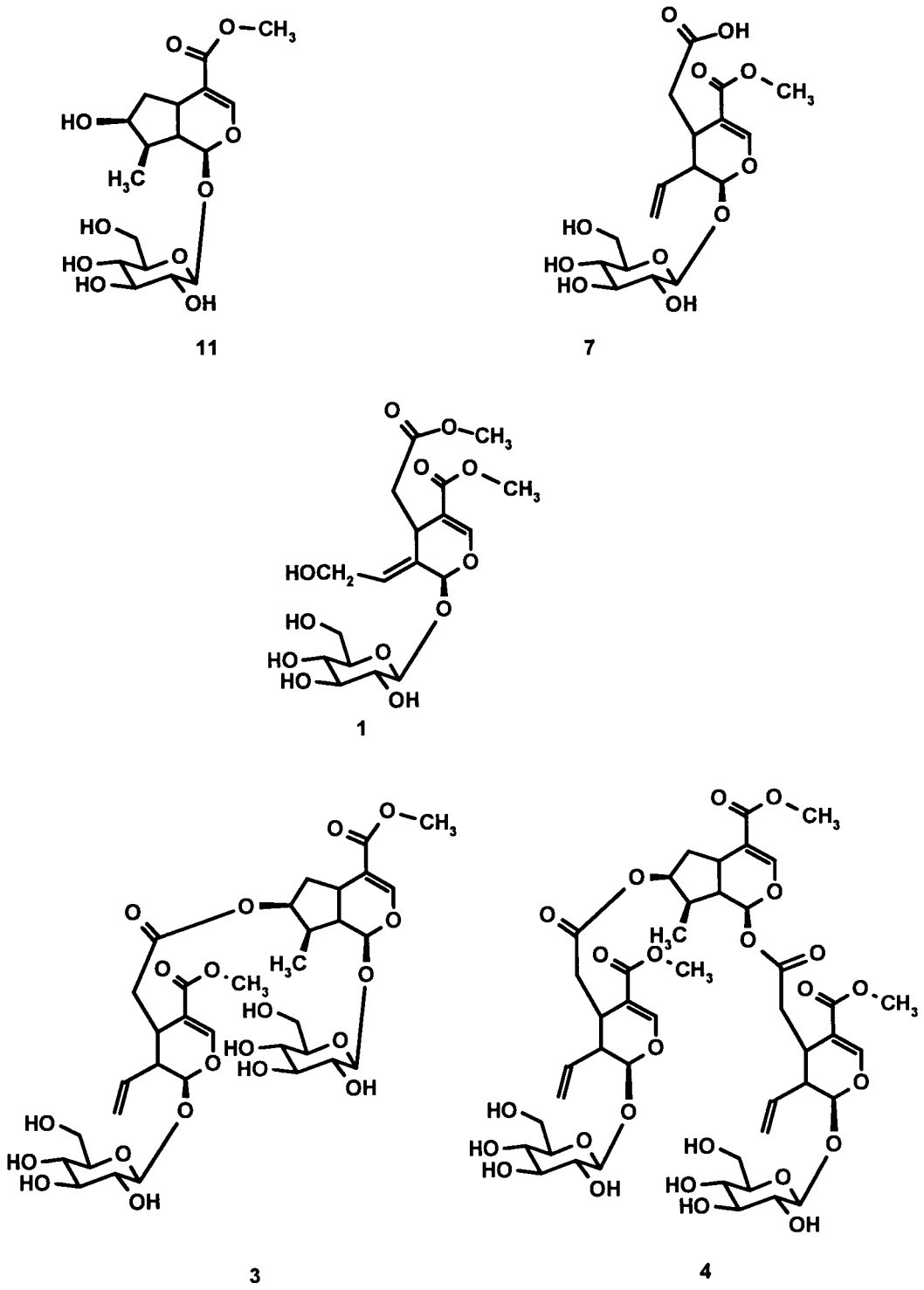

Scheme 1

(Weigend, 1997) and *Kissenia* Endl (Weigend, 1997). The "Higher Loasaeae" on the other hand share various derived characters and contain all the large groups of the tribe. The core genera used to be *Caiophora* K. Presl. and *Loasa* Adans., but these have been dramatically redefined: from *Loasa* the genera *Aosa* Weigend (6 spp., Brazil) and *Nasa* Weigend (largest genus with *ca.* 100 and largely restricted to the

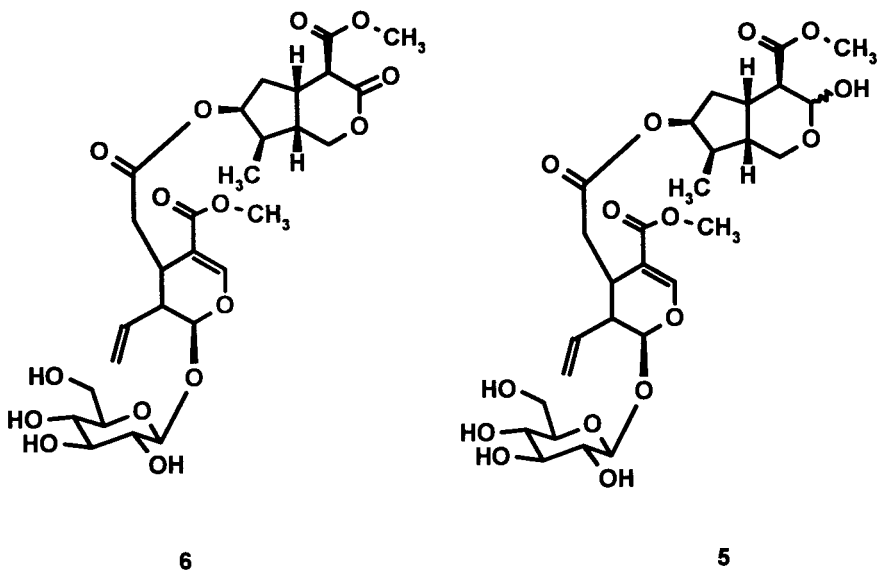

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Fig. 1 (*continued*)

Central and Northern Andes) have been segregated. The redefined genus *Loasa* is now exclusively South Andean (*ca.* 33 spp., largely confined to Chile). The limits of the species *Caiophora* have also been redrawn: two sections (*Angulatae* and *Bialatae*) of Urban and Gilg's *Caiophora* have been removed to the southeastern South American genus *Blumenbachia* Schrad. The remaining members of *Caiophora* and *Loasa* and the tiny Chilean genus *Scyphanthus* D. Don appear to be an exclusive monophyletic group and very closely related to each other. Our phytochemical studies therefore concentrated on these central climax groups in the subfamily to provide chemotaxonomic data to supplement the morphological investigations, while at the same time producing an overview over generalized distribution patterns of iridoids in the subfamily.

Loasaceae belong to the iridoid containing families. Loganin was first isolated from the seeds of *Blumenbachia hieronymi* Urb. by Kooiman. He also discovered this compound together with loganic acid chromatographically in the leaves of *B. hieronymi* Urb., *Caiophora lateritia* Klotsch, *Loasa vulcanica* Andr. (=*Nasa triphylla* Weigend) and *Mentzelia lindleyi* Torr. and Gray (Kooiman, 1974). Most of the phytochemical investigations in Loasaceae concentrated on members of the subfamily Mentzelioideae. The genus *Mentzelia* can be characterized by the presence of C_9-iridoids (Danielson, Hawes & Bliss, 1973a, 1973b; Jensen, Mikkelsen & Nielsen, 1981) and iridoid chlorohydrins (El Naggar, Beal & Doskotch, 1982; Catalano, Flamini, Bilia, Morelli, & Nicoletti, 1995). Monomeric iridoids, respectively, secoiridoids were also isolated from *Eucnide bartonioides* Zucc (Rodriguez, Schripsema & Jensen, 1997) and *Schismocarpus matudai* Steyerm (Damtoft, Jensen & Neilsen, 1993). In Loasoideae, oligomeric compounds could be isolated in addition to iridoid monomers (Kooiman, 1974; Di Fabio, Nicoletti, Piovano, Chamy & Garbarino, 1995). A bis-secoiridoid consisting of secologanol and 10-hydroxyoleoside-11-methyl ester was found in *Caiophora pentlandii* (Nicoletti, Di Fabio, Pastor de Abram & Urrunaga, 1996). Mixed type dimers and a trimer, consisting of secoxyloganin and loganin units, could be detected in *L. tricolor* and *L. acerifolia* (Nicoletti, Di Fabio, Serafini, Garbarino, Piovano & Chamy, 1991; Müller & Weigend, 1998, 1999; Müller, Kufer, Dietl & Weigend, 1999). The presence of iridoid dimers in *L. acerifolia*, *L. tricolor* and *C. pentlandii* raises the question whether iridoid dimers may serve as chemotaxonomic markers for Loasoideae or some of their subgroups. A close relationship between *Loasa* and *Caiophora* is indicated by numerous morphological characters.

2. Results and discussion

Seventy-four species of Loasaceae (Table 1), most of them from Loasoideae, were screened for iridoids using HPLC and TLC methods. Plant material was obtained from wild collected plants as well as from plants taken into cultivation. Identification of compounds was performed by isolation and structure elucidation with spectroscopic means (1D H–NMR, C–

Table 1
Samples of Loasaceae and voucher no. (sample number) .
W = wild collected plants; C = cultivated plants, seeds from; H = herbarium material

Aosa gilgiana (Urb.) Weigend = *Loasa gilgiana* Urb.	H, dos Santos i Mayo 298, Brasil
Aosa rupestris (Gard.) Weigend = *Loasa rupestris* Gard.	C, Bot. Garden Vienna, Brasil
Blumenbachia hieronymi Urb.	C, Bot. Garden Munich, Argentina
Blumenbachia insignis Schrad.	C, Harvard, Argentinia
Blumenbachia prietea Gay = *Caiophora prietea* (Gay) Urb. and Gilg	H, Grau 2844, Chile
Blumenbachia silvestris Poepp. = *Caiophora silvestris* (Poepp.) Urb. and Gilg	H, Grau, 24.4.68, s.n., Chile
Caiophora andina Urb. and Gilg	C, R. Kraus, Bolivia
Caiophora buraeavii Urb. and Gilg	C, R. Kraus, Bolivia
Caiophora cf. contorta 1 (Desr.) K. Presl	C, Weigend, Dostert and Drießle 97/417, Peru
Caiophora chuquitensis (Meyen) Urb. and Gilg	C, R. Kraus, Bolivia
Caiophora cirsiifolia K. Presl	W, Weigend and Dostert 97/14, Peru
Caiophora contorta (Desr.) K. Presl	W, Weigend and Horn 3801, Ecuador
Caiophora cf. contorta 2 (Desr.) K. Presl	W, Weigend and Dostert 97/31, Peru
Caiophora coronata (Arn.) Hook. and Arn.	C, Bot. Garden Edinburgh, Chile
Caiophora pentlandii (Graham) Loudon	W, Weigend and Förther 97/783, Peru
Caiophora pterosperma (Ruiz and Pavón ex G. Don) Urb. and Gilg	W, Weigend and Dostert 97/29, Peru
Caiophora sepiaria (Ruiz and Pavón ex G. Don) Macbr.	C, Kraus 4, cult. Nr. LOA–120, Chile
	W, Weigend, Dostert and Drießle 97/246, Peru
	C, Weigend, Dostert and Drießle 97/314, Peru
	C, Weigend, Dostert and Drießle 97/314, Peru (seeds)
	C, Weigend, Dostert and Drießle 97/465, Peru
	W, Weigend and Förther 97/797, Peru
	W, Weigend and Dostert 97/177, Peru
Caiophora superba R.A. Phil	W, Weigend and Förther 97/795, Peru
Caiophora superba x sepiaria	W, Weigend and Förther 97/796, Peru
Caiophora tenuis Killip	C, Weigend and Dostert 97/84, Peru
Huidobria fruticosa Phil.	H, Bayer, Grau, Marticorena and Rodríguez, Nr. BY 4887, Chile
Kissenia capensis Endl.	H, Weigend s.n., South Africa, 1992
Klaprothia fasciculata (K. Presl) Poston	W, Weigend and Dostert 98/164, Peru
Klaprothia mentzelioides Kunth	W, Weigend and Dostert 98/235, Peru
Loasa acanthifolia Desr.	H, Grau 15.6.95 s.n., Chile
Loasa acerifolia Domb. ex A.L. Juss.	C, Bayer, BY 4494, kult. no. LOA–89, Chile
Loasa elongata Hook. and Arn.	W, Ehrhart and Grau, 97/1145, Chile
Loasa heterophylla Hook. and Arn.	W, Ehrhart and Grau, 97/406, Chile
Loasa illapelina Phil.	W, Ehrhart and Grau, 97/1169, Chile
Loasa insons Poepp.	H, Grau LOA–128, Chile
Loasa malesherbioides Phil.	W, Ehrhart and Grau 97/1337, Chile
Loasa nitida Desr.	W, Weigend and Förther 97/561, Peru
	C, Grau LOA–113, Chile
Loasa pallida Gill. ex Arn.	H, Grau 3106, Chile
Loasa pinnatifida Gill.	H, Grau 2885, Chile
Loasa placei Lindl.	C, Grau, LOA–90, Chile
Loasa prostrata Gill. ex Arn.	C, Grau and Ehrhart, 94/406, Chile
Loasa sclareifolia Juss.	H, Looser 8.XII.R7, Chile
Loasa tricolor Ker Gawl.	C, Kraus 44, kult. no. LOA–126
Loasa triloba Domb. ex A.L. Juss	H, Grau, LOA–62, Chile
Loasa volubilis Poepp. ex Urb. and Gilg	H, Grau 3055, Chile
Mentzelia spec. nov.	W, Weigend, Dostert and Drießle 97/459, Peru
Mentzelia arborescens Urb. and Gilg	C, Bot. Garden Munich since 1930
Mentzelia chilensis Gay	W, Weigend, Dostert and Drießle 97/432, Peru
	W, Weigend and Dostert 97/144b, Peru
Mentzelia heterosepala Weigend and Rodriguez	W, Weigend, Dostert and Drießle 97/388, Peru
Nasa bicornuta (Weigend) Weigend = *Loasa bicornuta* Weigend	W, Weigend and Dostert 98/182, Peru
Nasa cf. cymbopetala Urb. and Gilg	W, Weigend and Dostert 97/135, Peru
Nasa chenopodiifolia (Desr.) Weigend = *Loasa chenopodiifolia* (Desr.)	W, Weigend and Dostert 97/176, Peru

Nasa cymbopetala (Urb. and Gilg) Weigend = *Loasa cymbopetala* Urb. and Gilg	W, Weigend and Dostert 97/146, Peru
Nasa spec. nov. 1	C, Weigend, Dostert and Drießle 97/377, Peru
Nasa hornii (Weigend) Weigend = *Loasa hornii* Weigend	C, Weigend and Horn 3815, Ecuador
Nasa jungifolia (Weigend) Weigend = *Loasa jungifolia* Weigend	C, Weigend and Horn 3838, Ecuador
Nasa lehmanniana (Urb. and Gilg) Weigend = *Loasa lehmanniana* Urb. and Gilg	W, Weigend 3635, Columbia, (seeds)
Nasa loxensis (Kunth) Weigend = *Loasa loxensis* Kunth	C, Weigend and Horn 3830, Ecuador
Nasa macrantha (Urb. and Gilg) Weigend = *Loasa macrantha* Urb. and Gilg	W, Weigend and Förther 97/617, Peru
Nasa macrothyrsa (Urb. and Gilg) Weigend = *Loasa macrothyrsa* Urb. and Gilg	C, Weigend, Dostert and Drießle 97/428, Peru
Nasa magnifica (Urb. and Gilg) Weigend = *Loasa magnifica* Urb. and Gilg	W, Weigend and Dostert 97/11, Peru W, Weigend and Dostert 97/134, Peru W, Weigend, Dostert and Drießle 97/468, Peru (seeds)
Nasa olmosiana (Gilg ex Macbr.) Weigend = *Loasa olmosiana* Gilg ex Macbr.	W, Weigend and Dostert 98/163, Peru
Nasa ramirezii (Weigend) Weigend = *Loasa ramirezii* Weigend	W, Weigend and Ramirez 3523, Columbia
Nasa ranunculifolia (Kunth) Weigend = *Loasa ranunculifolia* Kunth	C, Weigend, Dostert and Drießle 97/238, Peru
Nasa rubrastra (Weigend) Weigend = *Loasa rubrastra* Weigend	C, Schwerdtfeger 22207
Nasa stuebelii (Urb. and Gilg) Weigend = *Loasa stuebelii* (Urb. and Gilg)	W, Weigend et al. 98/383, Peru
Nasa spec. nov. 2	W, Weigend and Dostert 98/282, Peru
Nasa tingomariensis (Macbr.) Weigend = *Loasa tingomariensis* Macbr.	C, Weigend and Dostert 97/100, Peru
Nasa trianae (Urb. and Gilg) Weigend = *Loasa trianae* Urb. and Gilg	C, Weigend et al. 3610, Columbia
Nasa triphylla (Juss.) Weigend ssp. *triphylla* = *Loasa triphylla* Juss. ssp. *triphylla*	C, Botanic. Gard. Munich, Ecuador
Nasa triphylla Juss. Ssp. *papaverifolia* (Kunth) Weigend = *Loasa papaverifolia* Kunth	C, Weigend and Horn 3802, Ecuador
Nasa cf. triphylla (Juss.) Weigend	W, C, Weigend, Dostert and Drießle 97/307, Peru C, Weigend, Dostert and Drießle 97/307, Peru (seeds)
Nasa urens (Jacq.) Weigend = *Loasa urens* Jacq.	W, Weigend and Förther 97/542, Peru
Nasa weberbaueri (Urb. and Gilg) Weigend = *Loasa weberbaueri* Urb. and Gilg	W, Weigend and Dostert 98/196, Peru
Plakothira frutescens Florence	W, SP15,026, Marquesas Islands
Plakothira parviflora Florence	C, Nat. Trop. Bot. Gardens Hawaii, Marquesas Islands
Presliophytum arequipense Weigend	W, Weigend and Förther 97/642, Peru
Presliophytum heucheraefolium (Killip) Weigend = *L. heucheraefolium* Killip	W, Weigend and Dostert 97/120, Peru
Presliophytum incanum (Graham) Weigend = *L. incanum* Graham	W, Weigend and Dostert 97/12 Peru
Scyphanthus elegans D. Don	H, Ehrhart and Grau, LOA–135, Chile
Xylopodia klaprothioides Weigend	C, Weigend, Dostert and Drießle 450, Peru

NMR, H–H COSY, HMQC, HMBC, MS, UV-online, IR) or by cochromatography with authentic reference compounds.

2.1. 10-Hydroxyoleoside dimethyl ester—a chemotaxonomic marker for Caiophora s. str.

Caiophora s. str. includes 34 species which are mainly distributed in the high Andes from Central Peru to Northern Argentina and Chile. From *Caiophora sepiaria* a monomeric secoiridoid was isolated and structurally elucidated by spectroscopic means. The compound could be identified as 10-hydroxyoleoside dimethyl ester **1** (see Scheme 1) by comparing the spectroscopic data with reference values of model compounds from literature (see Section 3). Compound **1** was accompanied by an unknown iridoid **2** (UV spectrum online HPLC λ_{max}: 238 nm). Compound **1** was present in 12 out of 14 taxa from various *Caiophora* species, compound **2** in 10 out of 14

Table 2
Iridoides in Loasaceae.

(M) = *ser. Macrospermae*; (A) = *ser. Acanthifoliae*; (P) = *ser. Pinnatae*; (D) = *ser. Deserticolae*; (F) = *ser. Floribundae*; n.i.d = no iridoids detected; a = oligomeric iridoids; b = hydrophilic iridoids; c = large variety of iridoids; d = 11-methyl- and 11-hydroxymethyl-derivatives not included

		10-Hydroxy-oleoside dimethyl ester **1**	Compound **2**	Tricoloroside methyl ester **3**	Aceri-folioside **4**	Loasa-folioside **5**	Asaola-side **6**	Secoxy-loganin **7**	8-epi-Kingiside **8**	Sweroside **9**	Secoxyloganin-methylester **10**	Loganin **11**	Loganic acid **12**	Unknown iridoid (rt. 19 min)	Comments
Loasoideae – Loaseae (higher)	*Loasa acerifolia* Domb. ex A.L. Juss. (M)			x	x	x	x	x				x	x	x	a
	L. tricolor Ker Gawl.(M)			x	x	x		x							
	L. heterophylla Hook. and Arn. (M)			x				x				x			a
	L. insons Poepp. (M)			x				x				x			
	L. prostrata Gill. ex Arn.(M)			x								x		x	
	L. nitida Desr. (M)			x	x	x	x	x				x		x	
	L. placei Lindl. (M)			x										x	
	L. triloba Domb. ex A.L. Juss (M)			x				x				x			a
	L. illapelina Phil. (F)			x				x	x	x					
	L. pallida Gill. ex Arn. (F?)							x							
	L. acanthifolia Desr. (A)							x					x		
	L. sclareifolia Juss. (A)														
	L. pinnatifida Gill. (P)								x	x			x		
	L. volubilis Poepp. ex Urb. and Gilg (P)								x	x					
	L. elongata Hook. and Arn. (D)														
	Caiophora andina Urb. and Gilg	x	x									x			
	C. buraeavii Urb. and Gilg														n.i.d.
	C. chuquitensis (Meyen) Urb. and Gilg	x	x												
	C. cirsiifolia K. Presl	x													
	C. contorta (Desr.) K. Presl	x	x									x			b
	C. cf. contorta 1	x	x									x			
	C. coronata (Arn.) Hook. and Arn.	x	x					x		x				x	
	C. pentlandii (Graham) Loudon		x												b
	C. cf. contorta 2	x													b
	C. pterosperma (Ruiz and Pavon ex G. Don) Urb. and Gilg	x	x												
	C. sepiaria (Ruiz and Pavón ex G. Don) Macbr.	x	x										x		b
	C. superba x sepiaria	x	x									x			
	C. superba R.A. Phil	x						x				x			
	C. tenuis Killip	x	x										x		b
	Scyphanthus elegans D. Don							x							
	Blumenbachia hieronymi Urb.							x	x			x	x		
	B. insignis Schrad.								x			x			
	B. prietea Gay (= *Caiophora prietea* (Gay) Urb. and Gilg)														n.i.d.
	B. silvestris Poepp. (= *C. silvestris* (Poepp.) Urb. and Gilg)											x			

Table 2 (*continued*)

	10-Hydroxy-oleoside dimethyl ester **1**	Compound **2**	Tricoloroside methyl ester **3**	Aceri-folioside **4**	Loasa-folioside **5**	Asaola-side **6**	Secoxy-loganin **7**	8-epi-Kingiside **8**	Sweroside **9**	Secoxyloganin-methylester **10**	Loganin **11**	Loganic acid **12**	Unknown iridoid (rt. 19 min)	Comments
Nasa bicornuta (Weigend) Weigend (= *Loasa bicornuta* Weigend)														n.i.d.
N. chenopodiifolia (Desr.) Weigend (= *L. chenopodiifolia* Desr.)							x					x	x	
N. cymbopetala (Urb. and Gilg) Weigend (= *L. cymbopetala* Urb. and Gilg)												x		
N. spec. nov. 1														n.i.d.
N. hornii (Weigend) Weigend (= *L. hornii* Weigend)														n.i.d.
N. jungifolia (Weigend) Weigend (= *L. jungifolia* Weigend)											x			
N. loxensis (Kunth) Weigend (= *L. loxensis* Kunth)														n.i.d.
N. macrantha (Urb. and Gilg) Weigend (= *L. macrantha* Urb. and Gilg)														n.i.d.
N. macrothyrsa (Urb. and Gilg) Weigend (= *L. macrothyrsa* Urb. and Gilg)														
N. magnifica (Urb. and Gilg) Weigend (= *L. magnifica* Urb. and Gilg)								x						
N. olmosiana (Gilg ex Macbr.) Weigend (= *L. olmosiana* Gilg ex Macbr.)														n.i.d.
N. cf. triphylla (Juss.) Weigend							x			x	x		x	
N. ramirezii (Weigend) Weigend (= *L. ramirezii* Weigend)														
N. ranunculifolia (Kunth) Weigend (= *L. ranunculifolia* Kunth)									x			x	x	
N. rubrastra (Weigend) Weigend (= *L. rubrastra* Weigend)								x	x			x	x	
N. sp. cf. cymbopetala												x		
N. spec. nov. 2														n.i.d.
N. stuebelii (Urb. and Gilg) Weigend (= *L. stuebelii* Urb. and Gilg)														n.i.d.
N. tingomariensis (Macbr.) Weigend														

(continued on next page)

Table 2 (*continued*)

		10-Hydroxy-oleoside dimethyl ester **1**	Compound **2**	Tricoloroside methyl ester **3**	Aceri-folioside **4**	Loasa-folioside **5**	Asaola-side **6**	Secoxy-loganin **7**	8-epi-Kingiside **8**	Sweroside **9**	Secoxyloganin-methylester **10**	Loganin **11**	Loganic acid **12**	Unknown iridoid (rt. 19 min)	Comments
	(= *L. tingomariensis* Macbr.) *N. trianae* (Urb. and Gilg) Weigend												x		
	(= *L. trianae* Urb. and Gilg) *N. triphylla* (Juss.) Weigend *ssp.triphylla*								x	x				x	
	(= *L. triphylla* Juss. *ssp. triphylla*) *N. triphylla* Juss. ssp. papaverifolia							x				x		x	
	(Kunth) Weigend (= *L. papaverifolia* Kunth) *N. urens* (Jacq.) Weigend														
	(= *L. urens* Jacq.) *N. weberbaueri* (Urb. and Gilg) Weigend							x			x				
	(= *L. weberbaueri* Urb. and Gilg)							x				x	x		
	Aosa gilgiana (Urb.) Weigend														
	(= *L. gilgiana* Urb.) *A. rupestris* (Gard.) Weigend											x			
	(= *L. rupestris* Gard.)														
Loasoideae – Loaseae (lower)	*Huidobria fruticosa* Phil.								x		x	x			c
	Kissenia capensis Endl.								x	x	x		x	x	c
	Loasa malesherbioides Phil.											x			
	Presliophytum arequipense Weigend							x	x	x					
	P. heucheraefolium (Killip) Weigend														
	(= *L. heucheraefolium* Killip) *P. incanum* (Graham) Weigend							x		x				x	
	(= *L. incanum* Graham)								x	x					
Loasoideae – Klaprothieae	*Klaprothia fasciculata* (K. Presl) Poston														n.i.d.
	K. mentzelioides Kunth							x							
	Plakothira frutescens Florence														
	P. parviflora Florence							x				x		x	
	Xylopodia klaprothioides Weigend									x			x		
Mentzelioideae	*Mentzelia arborescens* Urb. and Gilg														d
	M. spec. nov.														d; n.i.d.
	M. chilensis Gay														d; n.i.d.
	M. heterosepala Weigend and Rodriguez												x		d

Table 3
Concentration of tricoloroside methyl ester in aerial parts of *Macrospermae* and *Floribundae*. .
$n = 3$

Species	Tricoloroside methyl ester [% of dry weight ± SD]
L. acerifolia	4.19 ± 0.250
L. placei	0.10 ± 0.007
L. prostrata	0.62 ± 0.003
L. nitida	1.12 ± 0.037
L. heterophylla	2.82 ± 0.049
L. tricolor	0.46 ± 0.009
L. illapelina	0.06 ± 0.005

taxa (see Table 2). In samples from other genera of Loasaceae neither of the two compounds was detectable. From the results above, we conclude that 10-hydroxyoleoside dimethyl ester can be regarded as a chemotaxonomic marker for *Caiophora spp*. Further hydrophilic iridoids with unknown structures were detected in 5 *Caiophora spp*. Table 2.

On the basis of morphological data *Caiophora sect. Bialatae* (= *Gripidea*) and *Caiophora sect. Angulatae* have recently been removed from *Caiophora* and included in *Blumenbachia* (Weigend, 1997). During our investigations on two species of the *sect. Angulatae* [*Blumenbachia silvestris* Poepp. (= *Caiophora silvestris* (Poepp.) Urb. and Gilg) and *Blumenbachia prietea* Gay (= *Caiophora prietea* (Gay) Urb. and Gilg.) neither 10-hydroxyoleoside dimethyl ester **1** nor compound **2** were detected. Therefore the phytochemical data give no evidence for a relationship of *Angulatae* Urb. and Gilg. to *Caiophora s.str.* but rather support the proposed link to *Blumenbachia*, where 10-hydroxyoleoside dimethyl ester **1** is also missing.

2.2. Tricoloroside methyl ester and iridoid oligomers in ser. Macrospermae and Floribundae

The revised genus of *Loasa* Adans. *s. str.*, (Weigend, 1997) which is almost completely restricted to Chile, can be divided into seven series following the classification of Urban and Gilg, (1900). Three of them, *ser. Acanthifoliae* (2 sp.), *ser. Macrospermae* (9 sp.) and *ser. Floribundae* (3 sp.) are closely related and can be separated from the other series, *ser. Pinnatae* (9–10 sp.), *ser. Volubiles* (4 sp.), *ser. Acaules* (2 sp.) and *ser. Deserticolae* (1–2 sp.), on the basis of the number and size of seeds and other morphological characters. Morphological reinvestigations of *ser. Macrospermae* and *ser. Floribundae* indicate a very close relationship. However, *L. pallida* should be removed from this group due to morphological features. Another pair of very closely related series are *ser. Pinnatae* and *ser. Volubiles* (Grau, unpublished results).

From *L. acerifolia*, *ser. Macrospermae*, we could isolate four novel dimeric and trimeric iridoids respectively (Müller & Weigend, 1998, 1999; Müller et al., 1999;). The major iridoid dimer is tricoloroside methyl ester **3** which consists of a loganin and a secoxyloganin moiety (see Scheme. 1). Our investigations on samples of other Chilean members of *Loasa* revealed that **3** is present in all species screened of *ser. Macrospermae* and in *L. illapelina* (*ser. Floribundae*). The minor iridoid oligomers **4** and **5** (see Scheme. 1) from *L. acerifolia* were present in another 2 species of Macrospermae (*L. tricolor, L. nitida*). In the Chilean Andes *L. tricolor*, *L. prostrata*, *L. insons* and *L. heterophylla* form a closely related group (Grau, 1996). While **3** was detected in all members of the *Loasa tricolor* group, the minor iridoid acerifolioside **4** was only present in *L. tricolor*.

The concentrations of **3** in leaf material from *Macrospermae* and *Floribundae* ranged from 0.06% (*L. illapelina*) to 4.19% (*L. acerifolia*) of dry weight (see Table 3). The highest amount of **3** in various parts of the plant was found in the seeds (*L. acerifolia*: 12.4%) (see Table 4). An accumulation of **3** was also detected in seeds of *L. nitida*. However, **3** was not found in *L. pallida* and in samples of other species from Loasaceae.

From these results we conclude that mainly the major iridoid dimer **3** can be regarded as a chemotaxonomic marker for the two core series of *Loasa s. str.* namely the *ser. Macrospermae* and *Floribundae* p.p. The universal presence of this compound indicates a close relationship within this group and underscores its likely monophyly. The finding of **3** in *L. illapelina* but not in *L. pallida* supports the delimitation as proposed by Grau (i.e., unification of the series *Macrospermae* and *Floribundae* and exclusion of *L. pallida*).

2.3. Distribution of monomeric iridoids

Other simple monomeric iridoids (loganin, loganic acid, secoxyloganin, secoxyloganin methyl ester, sweroside, 8-epi-kingiside) were found throughout Loasaceae. In Loasoideae monomeric iridoids and

Table 4
Concentration of tricoloroside methyl ester in various parts of *L. acerifolia*, *ser. Macrospermae*

Part	Tricoloroside methyl ester [% dry weight ± SD]
Leaves	4.19 ± 0.250
Flowers	4.21 ± 0.223
Seeds	12.38 ± 0.109
Stem	0.96 ± 0.049

secoiridoids were detected in both tribes Klaprothieae and Loaseae.

The Higher Loaseae appear as a derived and probably monophyletic assemblage of species on the basis of morphological data as opposed to the paraphyletic Lower Loaseae. Yet both groups are equally heterogeneous with regards to phytochemistry and no evolutionary progression or clearly derived character states can be found which characterize the Higher Loaseae as such. Of the Lower Loaseae the African genus *Kissenia* is especially noteworthy for its large number of iridoid compounds.

The large and morphologically highly diversified genus *Nasa* generally has low iridoid contents, is not very diverse with regards to the compounds found and no obvious phytochemical groups within the genus could be so far detected. This sets *Nasa* apart from the other two large genera of Loasoideae, *Loasa* and *Caiophora*.

Our studies on the distribution of iridoids indicate that they are good taxonomic markers in the two genera *Loasa* and *Caiophora*, both at generic and infrageneric level. The documentation of a dimeric iridoid from *Caiophora pentlandii* (Nicoletti et al., 1996) initially indicated the possibility that dimeric iridoids might be common to the—morphologically very natural—group *Caiophora s.str.—Loasa s.str.—Scyphanthus* and exclusive to it. However, our own investigations failed to find evidence of dimeric compounds in *Scyphanthus* or *Caiophora* (including samples of *C. pentlandii* from the type locality). Thus phytochemistry does not provide evidence on the relationships between these groups.

3. Materials and methods

3.1.1. General

The ^{1}H and ^{13}C NMR spectra were measured in MeOH-d_4 at room temperature with TMS or MeOH as internal standards on a Bruker AM–360 (360.13 MHz, 90.6 MHz). FAB mass spectrometry was performed on a Kratos MS 80 RFA spectrometer with NBA (7 kV). UV spectra were recorded on a Perkin-Elmer 550S spectrophotometer or recorded online HPLC. IR spectra were obtained from KBr disks on a Beckmann Acculab 1.

HPLC analysis was performed on a 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 + 33 μl H_3PO_4 85%/l; solvent B: MeCN + 33 μl H_3PO_4 85%/l; gradient 0% B – 25% B in 30 min., 25% – 80% B in 60 min; flowrate: 1 ml/min; detection: UV-diode array.

TLC was performed on silica with separation system 2 (EtOH–MeOH–H_2O 77:15:8) or separation system 3 (PrOH–toluene–HOAc–H_2O 25:20:10:10); det.: UV 254 nm, vis after spraying with vanillin/H_2SO_4.

3.1.2. Plant material

Plant material was obtained from wild collected plants during 1996, 1997 and 1998, from plants taken in cultivation and from herbarian material from MSB. Identification follows the latest publications on Loasoideae (Grau, 1988, 1996, 1997; Grau & Bayer, 1994; Weigend, 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1998, in prep, in press). Some of the plants screened have been recently collected in Peru and belong to new species which have not yet been published. In the list of samples these are quoted as "cf." followed by their nearest known relative or as "spec. nov. 1", "spec. nov. 2" etc. For a detailed listing and voucher numbers see Table 1.

3.2. Preparation of HPLC and TLC samples

One gram of powdered plant material was extracted with 5 ml MeOH on a water bath. After filtration the volume was refilled to 5.0 ml.

3.3. Isolation and identification of reference compounds

10-Hydroxyoleoside dimethyl ester **1** was isolated from *Caiophora sepiaria* (Ruiz and Pavón ex G. Don) Macbr. (Voucher No.: Weigend, Dostert and Drießle 97/314). *C. sepiaria* aerial parts (10 g air-dried material) were powdered and extracted in a soxhlet apparatus with 150 ml MeOH for 5 h. The MeOH extract was concd to 50 ml in vacuo and diluted with 150 ml H_2O. The soln was extracted (3 × 150 ml) subsequently with CH_2Cl_2 and EtOAc. The aequous/methanolic residue was lyophilized (3 g). After resuspension in MeOH the residue was separated over Sephadex LH 20 material with MeOH (5 ml/hr; 2.5 ml/fr.). The frs. were screened by HPLC (separation system 1) and TLC (separation system 2, det. UV 254 nm, vis after spraying with vanillin/H_2SO_4). Compound **1** was eluted with frs. 22–25. The separation of **1** was performed with prep. TLC on silica material (separation system 3) and yielded 15 mg. Identification of **1** was performed on the basis of the spectroscopic data and comparison with reference values of model compounds from literature (Shen, Lin & Chen, 1990). **1** was also present in extracts prepared with acetone or EtOH and therefore is no artifact of isolation procedures.

10-Hydroxyoleoside dimethyl ester **1**. Amorphous powder, $C_{18}H_{26}O_{12}$, pos. FAB–MS (positive ion mode, 3-nitro benzyl alcohole matrix; LiCl) m/z (rel. int): 457 (20) $[M+Na]^+$, 441 (15) $[M+Li]^+$; uv (online HPLC: λ_{max}, nm): 238; ^{1}H NMR (CD_3OD): δ 5.97 (1H, bs,

H–1), 7.54 (1H, s, H–3), 3.97 (1H, dd, J = 9.7 and 4.1 Hz, H–5), 2.51 (1H, dd, J = 15.0 and 9.7 Hz, H–6) 2.78 (1H, dd, J = 15.0 and 4.1 Hz, H–6), 6.17 (1H, bt, J = 6.3 Hz H–8), 4.22 (1H, ddd, J = 13.7, 5.7 and 1.6 Hz, H–10), 4.32 (1H, dd, J = 13.7 and 7.6 Hz, H–10), 4.83 (1H, d, J = 7.7 Hz, H–1′), 3.65 (1H, m, H–6′), 3.90 (1H, dd, J = 12.0 and 1.5 Hz, H–6′) 3.18–3.47 (H–2′, H–3′, H–4′, H–5′), 3.64 (OCH_3), 3.72 (OCH_3); ^{13}C NMR (CD_3OD): δ 94.6 (C–1), 155.0 (C–3), 109.2 (C–4), 32.3 (C–5), 41.0 (C–6), 173.5 (C–7), 129.4 (C–8), 130.9 (C–9), 59.2 (C–10), 168.4 (C–11), 52.3 (OCH_3), 52.0 (OCH_3), 100.9 (C–1′), 74.7 (C–2′), 77.9 (C–3′), 71.4 (C–4′), 78.4 (C–5′), 62.7 (C–6′).

Compound **2** was characterized chromatographically (HPLC: separation system 1: Rt = 12.2 min.; online UV-spectrum: $\lambda_{max} = 238$ nm; TLC: separation system 3: Rf. 0.44; det.: blueish in vis after spraying with vanillin/H_2SO_4). The UV-spectrum of **2** is typical for an iridoidic enol ether system conjugated with a carbonyl group and the behaviour in TLC after detection with vanillin/H_2SO_4 is similar to **1**.

Tricoloroside methyl ester **3**, *acerifolioside* **4**, *loasafolioside* **5**, *asaolaside* **6** and *secoxyloganin* **7** were isolated and identified as described elsewhere (Müller & Weigend, 1998, 1999; Müller et al., 1999;).

8-epi-kingiside **8** and *sweroside* **9** were isolated from *Presliophytum arequipense* Weigend (Voucher No.: Weigend and Förther 97/642,W). *P. arequipense* aerial parts (10 g air-dried material) were powdered and extracted in a soxhlet apparatus with 150 ml MeOH for 5 h. The MeOH extract was concd to 50 ml in vacuo and diluted with 150 ml H_2O. The soln was extracted (3 × 150 ml) subsequently with CH_2Cl_2 and EtOAc. After resuspension in MeOH the EtOAc extract was separated over Sephadex LH 20 material with MeOH (5 ml/hr, 2.5 ml/fr.). The frs. were screened by TLC (separation system 2, det. UV 254 nm, vis after spraying with vanillin/H_2SO_4). Compounds **8** and **9** were eluted with frs. 25–29. The separation of both compounds was performed with prep. TLC on silica material (separation system 3) and yielded 10 mg **8** and 12 mg **9**. The identification of which was done on the basis of the spectroscopic data and comparison with reference values of model compounds from literature (Kuwajima, Matsuuchi, Takaishi, Inoue, Fukita & Inouye, 1989; Van de Sluis & Labadie, 1980).

Secoxyloganin methyl ester **10** was obtained by methanolysis of acerifolioside. Ten milligrams of acerifolioside were dissolved in 10.0 ml MeOH and kept in the dark at room temperature for 4 weeks. After concentrating the solution in vacuo it was separated over Sephadex LH 20 material with MeOH (5 ml/hr). The frs. (2 ml) were screened by TLC (separation system 2, det. UV 254 nm, vis after spraying with anisaldehyde/H_2SO_4). Compound **10** was eluted with frs. 18–20. Identification of **10** was performed on the basis of the spectroscopic data and comparison with reference values from literature (Boros & Stermitz, 1991).

The loganin and loganic acid references were purchased from Fa. Roth (Karlsruhe, Germany) and Extrasynthese S.A. (Genay, France).

3.4. Quantitation of tricoloroside methyl ester in plant extracts

One gram of powdered plant material was extracted in a soxhlet apparatus with 20 ml MeOH for 5.5 h. After filtration the solution was adjusted to 25.0 ml with MeOH. Chromatographical separation of the extracts was performed using HPLC separation system 1 (see General Procedures). Quantitation of tricoloroside methyl ester in extracts ($n = 3$) was performed by external calibration (Area [AU] = 13,5 + tricoloroside methyl ester [μg] × 193.3 [AU/μg]; $n = 11$, residual standard deviation 11.0 AU; CV = 2.1%). Linearity was checked according to Mandel (Funk, Dammann & Donnevert, 1992) between 0.045 μg and 4.96 μg tricoloroside methyl ester per injection. Recovery rate: 99.5%.

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