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Ipangulines and minalobines, chemotaxonomic markers of the infrageneric *Ipomoea* taxon subgenus Quamoclit, section Mina ☆

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Abstract

A comprehensive GC–MS analysis of 8 *Ipomoea* species belonging to the subgenus Quamoclit, section Mina revealed that the members of this taxon form combinations of two necine bases with rare necic acids resulting in unique pyrrolizidine alkaloids. The occurrence and diversity of these metabolites show remarkable variations: Some species, especially *Ipomoea hederifolia* and *Ipomoea lobata*, are able to synthesize a large number of alkaloids whereas others, especially *Ipomoea coccinea* and *Ipomoea quamoclit*, are poor synthesizers with only a few compounds. However, these metabolites are apparently chemotaxonomic markers of this infrageneric taxon in general. They represent either esters of (−)-platynecine (altogether 48 ipangulines and 4 further esters including results of a previous study) or esters of (−)-trachelanthamidine, an additional novel structural type called minalobines (altogether 21 alkaloids). Both types are characterized by section-specific rare necic acids, e.g., ipangulinic/isoipangulinic acid, phenylacetic acid. The alkaloids of *Ipomoea cholulensis*, *I. coccinea*, *I. hederifolia*, *Ipomoea neei*, and *Ipomoea quamoclit* were mono and diesters of platynecine. Minalobines turned out to be metabolites of *I. lobata* (Cerv.) Thell. (syn.: *Mina lobata* Cerv.) lacking ipangulines. The major alkaloid of this species, minalobine R, has been isolated and identified as 9-*O*-(*threo*-2-hydroxy-2-methyl-3-phenylacetoxy-butyryl)-(−)-trachelanthamidine on the basis of spectral data. Apparently only two of the species included in this study, *Ipomoea cristulata* and *Ipomoea sloteri*, are able to synthesize both, ipangulines as well as minalobines. Minalobine O could be isolated as a major alkaloid of *I. cristulata*, its structure has been established as 9-*O*-(*erythro*-2-hydroxy-2-methyl-3-tigloyloxy-butyryl)-(−)-trachelanthamidine on the basis of spectral data.

Keywords: *Ipomoea*; Subgenus Quamoclit; Section Mina; Convolvulaceae; Pyrrolizidine alkaloids; Platynecine esters; Trachelanthamidine esters; Ipangulines; Minalobines

1. Introduction

Attempts to delimit species-groups of the largest convolvulaceous genus *Ipomoea* comprising at least 600 spp. (Austin and Huáman, 1996; Judd et al., 1999), however probably much more, have been made since shortly after

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the time of Linnaeus' Species Plantarum (1753). Some of the more recent arrangements were begun by Verdcourt (1957, 1963), and continued by Austin and Huáman (1996); Austin (1998); and Austin et al. (2001). The latest morphological classification of infrageneric taxa recognizes the three subgenera Eriospermum, Ipomoea, and Quamoclit, (Austin, 1980, 1997). O'Donell (1959) made a systematic study of what is now called subgenus Quamoclit (Moench) Clarke section Mina (Cervantes) Grisebach. He recognized 13 species within the section later being considered to comprise 18 species (Austin and Huáman, 1996). They are exclusively native to the New World; most of them are Mexican, however they range from the United States to Argentina. In contrast to all other *Ipomoea* spp. they are characterized *inter alia* by salverformed corollas, red and/or yellow, rarely with purple patches, the stamens exserted.

Although some authors segregate the group as the separate genus *Quamoclit,* the species closest affinities are clearly within *Ipomoea*. Usually, people who maintain *Quamoclit* as a genus are familiar only with *Ipomoea hederifolia* and *Ipomoea quamoclit*, and not with the morphological range within this section. Because those two species were introduced into the Old World in the early 1500s, people there have a mistaken view of them. As O'Donell (1953) and Wilkin (1999) have pointed out, *Ipomoea decemcornuta* O'Donell is one species that bridges the morphological gap with *Ipomoea*.

In two previous papers we reported on the discovery of ipangulines, a novel, unique type of pyrrolizidine alkaloids as constituents of *I. hederifolia* L. (syn.: *Ipomoea angulata* Lam.) (Jenett-Siems et al., 1993, 1998). These compounds are characterized by the novel combination of the saturated necine base (−)-platynecine with aliphatic and/or an aromatic moiety containing carboxylic acids. In the report of 1998 we already noted the presence of these metabolites in the related species *Ipomoea neei* (Spreng.) O'Donell and *Ipomoea sloteri* (House) van Ooststr. In this former report the latter species was called *I.* x *perigrenium* cv. Cardinal. However, this is only one of different horticultural terms for the ornamental Cardinal Climber which is not itself a hybrid but the allotetraploid species *Ipomoea sloteri* derived from the hybrids between *Ipomoea coccinea* L. and *I. quamoclit* L. (Eckenwalder, 1986). We were able to harvest seeds in our greenhouse which unambiguously belong to *I. sloteri*.

The present communication describes the results of a comprehensive GC–MS analysis of five additional members of the section Mina in comparison with the three species previously checked. The aim of this study has been to figure out if the ipangulines are chemotaxonomic markers of this section in general. Moreover, this study led to the discovery of an additional novel type of pyrrolizidine alkaloids structurally related to the ipangulines for which we propose the term minalobines since the first compound of this new type, minalobine R, has been isolated from *I. lobata* (Cerv.) Thell. (syn.: *Mina lobata* Cerv.). The necine base of the latter type is represented by (−)-trachelanthamidine instead of (−)-platynecine; however, the necic acids are identical or closely related to those of the ipangulines although only esterification at O-9 of the necine base is possible due to the lack of a second hydroxyl (at C-7). Furthermore, the isolation and characterisation of one further minalobine from *Ipomoea cristulata* Hall. f. are reported.

2. Results

2.1. GC–MS analysis

The study revealed that pyrrolizidine alkaloids with a saturated necine base are a common feature of all species investigated. They were detected in all plant organs with a large variety in roots and shoots and a smaller range also in the seeds. Therefore, only the results for the vegetative plant parts are given in Table 1. In contrast to species from other pyrrolizidine alkaloid containing families, e.g., Asteraceae or Boraginaceae, no reduction of N-oxides was necessary prior to GC–MS analysis, since pyrrolizidines are present as free bases in the Convolvulaceae. Altogether 52 platynecine esters (48 ipangulines and 4 further esters, Table 1) and 21 trachelanthamidine esters (minalobines, Table 1) were identified. The ipangulines are divided up according to the classification already chosen previously (Jenett-Siems et al., 1998; Fig. 1): ipangulines A (diesters containing one phenylacetyl moiety), ipangulines B (diesters with one salicyloyl moiety), ipangulines C (diesters with two aliphatic acyl moieties), ipangulines D (either 7- or 9-monoesters), and ipangulines X (ambiguous acyl moieties). However, a classification of the minalobines seems to be of no use; therefore their individual terms are just chosen alphabetically (Table 1; Fig. 1). At least 11 diesters out of 21 minalobines could be detected; without exception they show *erythro*- or *threo*-2,3-dihydroxy-2-methylbutyric acid (ipangulinic/isoipangulinic acid; Roeder, 1995), respectively, as the acyl component directly conjugated with the necine base. The second, individual ester moiety is formed by the free 3-hydroxyl group of the first acyl residue and differential acids. At least six minalobines represent monoesters. The acyl component of four compounds could not be determined.

Anhydroplatynecine (**1**), a compound detected in all eight species, may be an artefact formed by the GC–MS procedure from platynecine (**3**) and/ or its esters, respectively. Compounds **5**, **10**, **12**, and **16** are not classified as ipangulines though belonging to group D since they are already known from plants out of the family Convolvulaceae (see Section 3).

Table 1
GC–MS analysis of the pyrrolizidine alkaloids (relative abundance in %) from eight species of the section Mina (r, roots; s, shoots; t, trace; *e*-HMBA, *erythro*-2,3-dihydroxy-2-methylbutyryl moiety; *t*-HMBA, *threo*-2,3-dihydroxy-2-methylbutyryl; Ac-HMBA, 3-acetoxy-2-hydroxy-2-methylbutyryl; DiAc-HMBA, 2,3-diacetoxy-2-methylbutyryl; MeBu-HMBA, 2-hydroxy-3-(2-methylbutyryloxy)-2-methylbutyryl; MeBu, 2-methylbutyryl; PAA, phenylacetyl; Sal, salicyloyl; Tigl, tigloyl)

	Alkaloid	R^1 (at O^7)	R^2 (at O^9)	RI	$[M]^+$, *m/z*	*I. hederifolia*[a]	*I. cholulensis*		*I. coccinea*	*I. cristulata*		*I. lobata*		*I. neei*		*I. quamoclit*		*I. sloteri*	
						s	r	s	s	r	s	r	s	r	s	r	s	r	s
1	Anhydroplatynecine[b]	–	–	1130	139	t	t	t	t	t	t	t		t	t	t		t	t
2	Trachelanthamidine	–	H	1252	141							t							
3	Platynecine	H	H	1442	157										t				
4	Minalobine A	–	COC_2H_5O	1540	213							t	t						
5	9-Acetylplatynecine	H	Ac	1557	199										t				
6	Ipanguline D_1	H	COC_3H_7	1703	227	t													
7	Minalobine B	–	Tigl	1705	223							t							
8	Minalobine C	–	COC_4H_7O	1720	239							t							
9	Minalobine D	–	COC_4H_9O	1760	241							t	t						
10	7-(2-Methylbutyryl)-platynecine	MeBu	H	1778	241	t		t							t				
11	Minalobine E	–	COC_4H_9O	1795	241													t	
12	9-(2-Methylbutyryl)-platynecine	H	MeBu	1803	241	t		t							t				
13	Minalobine F	–	?	1825	313							t	t						
14	Minalobine G	–	COC_4H_9O	1847	241							t							
15	Ipanguline D_2	H	COC_4H_9O	1886	257	t													
16	9-Tigloylplatynecine	H	Tigl	1898	239	t													
17	Minalobine H	–	$COC_6H_{11}O_3$	1922	299							t							
18	Isoipanguline D_3	H	*t*-HMBA	2022	273	t													
19	Ipanguline D_3	H	*e*-HMBA	2037	273	t									t				
20	Minalobine I	–	COC_3H_7-HMBA	2049	327							t							
21	Ipanguline D_4	H	$COC_6H_{11}O_2$	2085	299	t													
22	Minalobine J	–	COC_3H_7-HMBA	2095	327							t							
23	Ipanguline X_6[c]	?	?	2100	315										30				
24	Isoipanguline D_5	H	Ac-*t*-HMBA	2108	315	t		t											
25	Ipanguline D_{12}	Ac-HMBA	H	2125	315										t				
26	Minalobine K	–	MeBu-HMBA	2142	341							25	t						
27	Ipanguline D_5	H	Ac-*e*-HMBA	2157	315	1		t						t	3				
28	Ipanguline D_6	PAA	H	2168	275	t													
29	Minalobine L	–	MeBu-HMBA	2183	341					t		t							
30	Minalobine M	–	Tigl-*t*-HMBA	2200	339					1		t							
31	Ipanguline D_7	H	PAA	2212	275	t		t											
32	Ipanguline X_7[c]	?	?	2217	315			t						t	12				
33	Minalobine N	–	COC_4H_9O-HMBA	2223	357							t							
34	Ipanguline D_8	H	$COC_8H_{15}O_3$	2226	343	t													
35	Minalobine O	–	Tigl-*e*-HMBA	2236	339					10	92								t
36	Isoipanguline D_9	H	DiAc-*t*-HMBA	2246	357	5	t	t						t	8				
37	Ipanguline D_9	H	DiAc-*e*-HMBA	2283	357		t												
38	Minalobine P	–	COC_4H_9O-HMBA	2290	357							t							
39	Ipanguline X_1[c]	?	?	2302	385	t													
40	Isoipanguline C_1	MeBu	*t*-HMBA	2308	357	8								50	40			90	97
41	Ipanguline C_1	MeBu	*e*-HMBA	2311	357	t	30	20						t	t			t	t
42	Minalobine Q	–	COC_4H_9O-HMBA	2312	357					t									
43	Ipanguline C_6	Tigl	HMBA	2318	355					t									
44	Isoipanguline D_{10}[d]	H	MeBu-HMBA	2327	357	1			t							t			
45	Ipanguline D_{10}	H	MeBu-HMBA	2349	357					5									
46	Ipanguline D_{13}	Tigl-HMBA	H	2358	355													t	
47	Ipanguline D_{14}	H	Tigl-HMBA	2375	355													4	
48	Ipanguline X_2[c]	?	?	2376	355	t	28	t								t			
49	Ipanguline D_{15}	H	Tigl-HMBA	2387	355					87	t								3
50	Isoipanguline C_2	MeBu	Ac-*t*-HMBA	2391	399	5	t							24					

(*continued on next page*)

Table 1 (*continued*)

	Alkaloid	R^1 (at O^7)	R^2 (at O^9)	RI	$[M]^+$, *m/z*	*I. hederifolia*[a]	*I. cholulensis*		*I. coccinea*	*I. cristulata*		*I. lobata*		*I. neei*		*I. quamoclit*		*I. sloteri*	
						s	r	s	₃	r	s	r	s	r	s	r	s	r	s
51	Ipanguline D_6	Tigl-HMBA	H	2395	355													t	
52	Ipanguline C_2	MeBu	Ac-*e*-HMBA	2426	399	1		t						23	4				
53	Ipanguline D_{18}	H	Tigl-HMBA	2480	355					t									
54	Ipanguline C_7	Tigl	HMBA	2494	355					t									
55	Ipanguline C_3	MeBu	DiAc-HMBA	2506	441	5													
56	Minalobine R	–	PAA-HMBA	2526	375							72	97						
57	Ipanguline C_4	MeBu	MeBu-HMBA	2606	441	1													
58	Minalobine S	–	?	2630	375							t							
59	Minalobine T	–	?	2634	375							t							
60	Minalobine U	–	?	2645	391							t							
61	Ipanguline C_5	MeBu	Tigl-HMBA	2645	439		t												t
62	Ipanguline X_3[c]	?	?	2667	439	t													
63	Isoipanguline A_1	PAA	*t*-HMBA	2702	391	12	7	70											
64	Ipanguline A_1	PAA	*e*-HMBA	2707	391	2	3	6											
65	Isoipanguline B_1	Sal	*t*-HMBA	2770	393	18	t	t											
66	Ipanguline B_1	Sal	e-HMBA	2772	393	3	29	t											
67	Isoipanguline A_2	PAA	Ac-*t*-HMBA	2782	433	8													
68	Ipanguline A_2	PAA	Ac-*e*-HMBA	2810	433	1		t											
69	Isoipanguline B_2	Sal	Ac-*t*-HMBA	2837	435	t													
70	Ipanguline B_2	Sal	Ac-*e*-HMBA	2873	435	t													
71	Isoipanguline A_3	PAA	DiAc-*t*-HMBA	2886	475	13													
72	Ipanguline A_3	PAA	DiAc-*e*-HMBA	2891	475	2													
73	Ipanguline B_3	Sal	DiAc-HMBA	2927	477	4													
74	Ipanguline A_4	PAA	MeBu-HMBA	2985	475	5													
75	Ipanguline X_4[c]	?	?	3055	497	t													
76	Ipanguline X_5[c]	?	?	3083	499	1													

[a] Data of the shoots of *I. hederifolia* given for comparison, already published in Jenett-Siems et al. (1998).

[b] Anhydroplatynecine might be an artefact due to GC–MS analysis.

[c] According to GC–MS a platynecine ester but identity of necic acids uncertain.

[d] This compound was formerly called ipanguline D_{10} (Jenett-Siems et al., 1998) but detection of **45** in *I. cristulata* unambiguously proved that **44** had to be the iso-derivative.

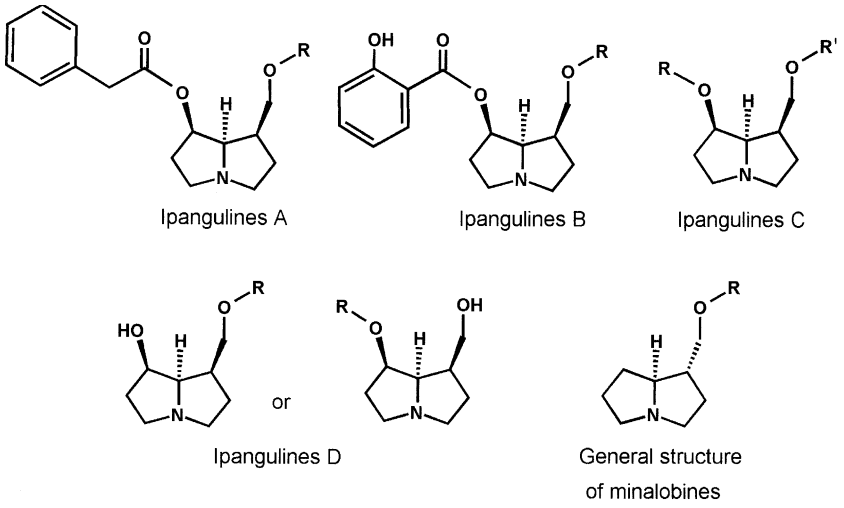


Fig. 1. The four structural types of ipangulines (necine base: platynecine) and general structure of minalobines (necine base: trachelanthamidine); R,R′ = aliphatic necic acid.

2.1.1. I. cholulensis Kunth.

The main alkaloids in shoots and roots were isoipanguline/ipanguline A_1 (**63/64**) and ipanguline C_1 (**41**). Several further congeners, including isoipanguline/ipanguline B_1 (**65/66**), were detected, altogether 19 compounds, but no trachelanthamidine esters.

2.1.2. I. coccinea L.

The shoots contained only small amounts of anhydroplatynecine (**1**) but no esters, while traces of ipanguline D_{13} (**46**) and D_{14} (**47**) were present in the seeds. Unfortunately, roots could not be analyzed, due to lack of plant material.

2.1.3. I. cristulata Hallier f.

Main PAs were minalobine O (**35**), minalobine M (**30**), and ipanguline D_{10} (**45**) in the leaves and again **35** and ipanguline D_{15} (**49**) in the roots. Additionally, minalobines L (**29**) and Q (**42**) and the ipangulines D_{18} (**53**), C_6 (**43**), and C_7 (**54**) were detected as minor constituents.

2.1.4. I. lobata (Cerv.) Thell.

Besides anhydroplatynecine (**1**) only trachelanthamidine derivatives were present in *I. Iobata,* altogether 19 alkaloids. The dominating PA in leaves and roots was minalobine R (**56**), with minalobine K (**26**) occurring also in higher amounts only in the roots.

2.1.5. I. neei (Spreng.) O'Donell

Altogether 15 ipangulines were detected in different parts of *I. neei*. Major alkaloids in the leaves were ipanguline X_6 (**23**) and isoipanguline C_1 (**40**), whereas again **40** and isoipanguline/ipanguline C_2 (**50/52**) were dominating in the roots.

2.1.6. I. quamoclit L.

In the shoots and seeds of *I. quamoclit* no alkaloids were detected. Only the roots contained small amounts of PAs, namely anhydroplatynecine (**1**), ipanguline D_{10} (**44**) and ipanguline X_2 (**48**).

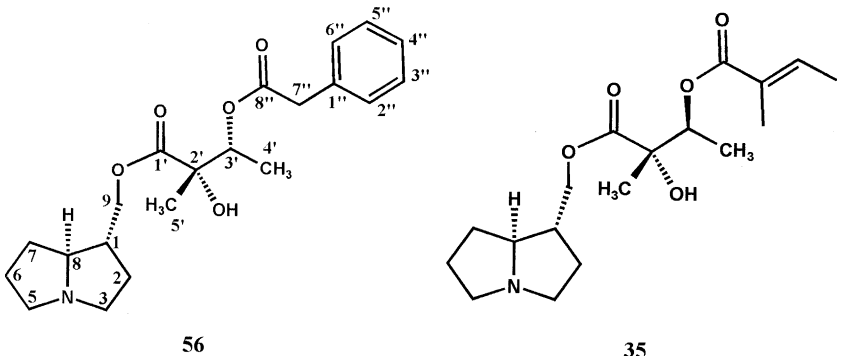
2.1.7. I. sloteri (House) van Ooststr. cv. Cardinal

The shoots contained isoipanguline C_1 (**40**) as main constituent. In the roots, again **40**, ipanguline D_{14} (**47**) and D_{15} (**49**) represented the major alkaloids. Besides further minor ipangulines, the trachelanthamidine ester minalobine O (**35**) was detected.

2.2. Isolation and structure elucidation of novel pyrrolizidine alkaloids

2.2.1. Minalobine R

The crude alkaloid fraction of the epigeal parts of *I. lobata* yielded one compound giving a positive reaction with Dragendorff's reagent. From the HRMS a molecular formula of $C_{21}H_{29}NO_5$ could be deduced. Characteristic fragments at *m/z* 124 (base peak), 96 and 83 in the EIMS suggested the compound to be a derivative of a saturated 1-hydroxymethyl-pyrrolizidine. The ester residue could be identified by comparison of the ^{1}H NMR with the corresponding isoipanguline A_1 (Jenett-Siems et al., 1993). The NMR spectrum displayed signals for a *threo*-2,3-dihydroxy-2-methylbutyric acid moiety as well as a phenylacetic acid residue. The downfield shift of H-3 of the *threo*-2,3-dihydroxy-2-methylbutyric acid moiety at δ 5.05 (1H, *q*, J = 6.5 Hz) revealed the attachment of phenylacetic acid at this position. After hydrolysis of the alkaloid, GC–MS comparison of the necine base with authentic isoretronecanol and trachelanthamidine together with determination of the optical rotation



56 35

Fig. 2. Structures of minalobine R (**56**) from *I. lobata* and minalobine O (**35**) from *I. cristulata* (2,3-dihydroxy-2-methylbutyric acid residue: rel. config.).

suggested the isolated compound to be a derivative of (−)-trachelanthamidine (Robins and Sakdarat, 1981). We named this new alkaloid minalobine R (**56**, Fig. 2).

2.2.2. Minalobine O

The crude alkaloid fraction of the epigeal parts of *I. cristulata* again yielded one compound giving a positive reaction with Dragendorff's reagent. From the HRMS a molecular formula of $C_{18}H_{29}NO_5$ could be deduced. The same characteristic fragments at *m/z* 124 (base peak), 96 and 83 in the EIMS again hinted to a derivative of a saturated 1-hydroxymethyl-pyrrolizidine. The ^{1}H NMR spectrum displayed signals for a *erythro*-2,3-dihydroxy-2-methylbutyric acid (Jenett-Siems et al., 1993) moiety as well as typical resonances for a tiglate residue (Logie et al., 1994). The downfield shift of H-3 of the *erythro*-2,3-dihydroxy-2-methylbutyric acid moiety at δ 5.09 (1H, *q*, $J = 6.3$ Hz) revealed the attachment of tiglic acid at this position. Thus, the new alkaloid had to be 9-*O*-(*erythro*-2-hydroxy-2-methyl-3-tigloyloxy-butyryl)-(−)-trachelanthamidine, which we named minalobine O (**35**, Fig. 2).

3. Discussion

The occurrence of three different structural types of pyrrolizidine alkaloids in the family Convolvulaceae has been documented in previous studies: (1) loline alkaloids (1-aminopyrrolizidines) as constituents of *Argyreia mollis* (Burm. f.) Choisy (Tofern et al., 1999), (2) retronecine esters of the lycopsamine type (Mann et al., 1996; Mann, 1997) as constituents of *Merremia quinquefolia* (L.) Hall. f. and *M. cissoides* (Lam.) Hall. f., and (3) platynecine esters of the ipanguline type (Jenett-Siems et al., 1993, 1998). Whereas the first two types are also present in other angiosperm families the latter is unique in the plant kingdom. The present report includes another novel structural type of pyrrolizidine alkaloids which has been named minalobines. They share with the ipangulines a saturated necine base and identical or similar necic acids. However, (−)-trachelanthamidine (**2**) instead of (−)-platynecine (**3**) enables only an esterification at O-9.

Thus, the structural comparison of minalobine R (**56**) with isoipanguline A_1 (**63**) shows that just the same two necic acids are included in different combinations: both alkaloids have in common isoipangulinic acid (*threo*-2,3-dihydroxy-2-methylbutyric acid; Roeder, 1995) as the acid component at C-1; however, the second necic acid, phenylacetic acid, is conjugated with (1) the C-3 hydroxyl of isoipangulinic acid in the case of **56** forming one diester center and (2) the C-7 hydroxyl of platynecine like in **63** forming a diester with two monoester centers, respectively. Both acids are very rare as necic acids: Isoipangulinic acid has been found only in one retronecine type pyrrolizidine alkaloid of two *Cryptantha* spp. (Boraginaceae); furthermore, this acid or its *erythro*-isomer is conjugated with another retronecine type compound of *Senecio caudatus* (Asteraceae) (Hartmann and Witte, 1995). On the other hand, the necine base platynecine is also rare: The occurrence of its esters outside the genus *Ipomoea* is restricted to certain *Senecio* spp. (Asteraceae). However, trachelanthamidine esters are more frequent, e.g., in different genera of the Orchidaceae which are able to synthesize alkaloids of the phalaenopsine type.

Five of the Mina species investigated in this study, *I. cholulensis*, *I. coccinea*, *I. hederifolia*, *I. neei*, and *I. quamoclit*, showed platynecine mono- and diesters (one or two aliphatic necic acyl moieties; ipangulines C and D). Only two of them, *I. cholulensis* and *I. hederifolia*, are characterized by the additional occurrence of esters with one aliphatic acyl moiety and one phenylacetyl (ipangulines A) or one salicyloyl residue (ipangulines B). These two species represent the members with the most complex alkaloid spectrum from the qualitative point of view as well as the species with the highest total alkaloid content. This is of special interest since *I. cholulensis* is considered as a neotropical plant of the highlands with *I. hederifolia* as its corresponding tropical lowland relative. On the other hand *I. coccinea*, which

is considered to be the corresponding temperate relative of *I. hederifolia*, and also *I. quamoclit* show only very few ipangulines in low concentrations. Furthermore, it is interesting to notice that *I. neei* is a fairly good producer of these metabolites since it is considered to be perhaps a primitive member of the section (Austin, 1975).

Only, *I. lobata* turned out to synthesize 18 minalobines but no ipangulines (left aside anhydroplatynecine (**1**)). The two remaining species, *I. cristulata* and *I. sloteri* are characterized by the occurrence of both, minalobines and ipangulines. The latter species only contains minalobine O (**35**) as a minor component together with several ipangulines already known from *I. hederifolia*. Thus, surprisingly the horticultural hybrid *I. sloteri* is a much more efficient alkaloid producer than its parents (*I. quamoclit* and *I. coccinea*). *I. cristulata* is the only species which revealed both alkaloid types side by side as major constituents.

From the biosynthetic point of view, the co-occurrence of platynecine derivatives and trachelanthamidine derivatives is conspicuous. The difference between these two necine bases is not only caused by the presence or absence of an additional hydroxyl at C-7 but also by the stereochemistry at C-1. It is unknown if the formation of platynecine (**3**) in plants is based on isoretronecanol, the lβ-epimer of trachelanthamidine (**2**), or on a configurative reversal at C-l of trachelanthamidine (hydroxylation at C-7 left aside). Such a reversal might take place by the formation of a 1,9-dehydro intermediate. In case of the section Mina the analyses of certain members revealed both the presence of minalobines, the potential precursors, and ipangulines, their potential final products, e.g., the common occurrence of ipanguline D_{15} (**49**) and its 7-deoxy derivative minalobine O (**35**) in the roots of *I. sloteri*.

Anyhow, the occurrence of unique pyrrolizidine alkaloids formed by the combination of a saturated necine base with section-specific rare necic acids can be considered as a common chemotaxonomic marker of Mina. We could neither find this type of alkaloids in any other *Ipomoea* species beyond this section nor in any other convolvulaceous species of numerous genera (total number of investigated species: >120).

4. Experimental

4.1. General

Preparative high performance liquid chromatography (HPLC) was performed on a Pharmacia LKB instrument on a Superpac Pep-S C-2/C-18 (5 μm) reversed-phase column. Optical rotations were measured with a Perkin–Elmer 241 MC. EIMS and HR-EIMS were recorded on a Varian MAT 711 (80 eV). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AMX 400 MHz spectrometer (TMS as int. standard).

4.2. Plant material

Seeds of *I. quamoclit* were collected near San Lorenzo, Panama, and of *I. neei* near Boquete, Panama. Seeds of *I. cristulata* were kindly supplied by the Conservation and Science Department, Arizona Sonora Desert Museum, Tucson, AZ, and of *I. cholulensis* by J.L.T. Muñoz, Centro de Investigation Cientifica de Yucatan A. C. (CICY), Mérida, Yucatan, Mexico. Seeds of *I. coccinea* were kindly supplied by the Jardin Botanique, Museum National d'Histoire Naturelle, Paris. Seeds of *I. sloteri* cv. Cardinal and young plants of *I. lobata* were purchased from Gartencenter Pluta, Berlin-Dahlem. Plants were grown from seeds in the greenhouse and provided roots and shoots for alkaloid extraction. Voucher specimens are deposited at the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin.

4.3. Extraction and isolation

Ground epigeal parts of *I. lobata* (450 g) were extracted with 10 l of MeOH (80%). After evaporation the extract was acidified and partitioned between water and organic solvents. The aqueous layer was then alkalinized and extracted with $CHCl_3$. The crude alkaloid fraction was reduced with Zn/HCl and purified by preparative HPLC (Rp-18, H_2O (+0.5% H_3PO_4)/MeOH 60:40) to give minalobine R (30 mg).

Ground epigeal parts of *I. cristulata* (50 g) were extracted with 0.5 l of MeOH. After evaporation the extract was acidified and partitioned between water and organic solvents. The aqueous layer was then alkalinized and extracted with $CHCl_3$. The crude alkaloid fraction consisted of one major compound, minalobine O (5 mg).

4.4. Minalobine R (*56*)

Yellow oil. $[\alpha]_D^{20}$ −35° (MeOH; *c* 0.15); ^{1}H NMR (400 MHz, $CDCl_3$): 1.26(3H, *d*, *J* = 6.5 Hz, H-4′); 1.29 (3 H, *s*, H-5′); 1.36–1.47 (2 H, *m*, H-2a, H-6a); 1.63–1.89 (5 H, *m*, H-1, H-2b, H-6b, H-7); 2.43 (1H, *ddd*, *J* = 6.0, 10.0, 10.0 Hz, H-3a/H-5a); 2.49 (1H, *ddd*, *J* = 6.0, 6.0, 12.0 Hz, H-3a/H-5a); 2.90 (1H, *ddd*, *J* = 6.0, 12.0, 12.0 Hz, H-3b/H-5b); 3.07 (2H, *m*, H-3b/H-5b, H-8); 3.51 (2H, *s*, H-7″); 3.64 (1H, *dd*, *J* = 6.5, 11.0 Hz, H-9a); 3.86 (1 H, *dd*, *J* = 7.0, 11.0, H-9b); 3.94 (1H, *br s*, OH); 5.05 (1H, *q*, *J* = 6.5 Hz, H-3′); 7.19–7.31 (5H, *m*, H-2″-H-6″); ^{13}C NMR (100 MHz, $CDCl_3$): 13.2 (*q*, C-4′), 21.9 (*q*, C-5′), 25.8 (*t*, C-7), 30.4 (*t*, C-2/C-6), 31.6 (*t*, C-2/C-6), 41.4 (*t*, C-7″), 44.6 (*d*, C-1), 54.2 (*t*, C-3/C-5), 54.7 (*t*, C-3/C-5), 67.8 (*d*, C-8), 68.2 (*t*, C-9), 74.5 (*d*, C-3′), 75.8 (*s*, C-2′), 127.0 (*d*, C-4″), 128.5 (*d*, C-3″/C-5″), 129.2 (*d*, C-2″/C-6″), 133.7 (*s*, C-1″), 170.2 (*s*,

Table 2
GC–MS (70 eV) fragmentation patterns of pyrrolizidine alkaloids newly described in this study, for all other compounds compare Jenett-Siems et al. (1998)

Compound	*m/z* (rel. int. %)
Trachelanthamidine (**2**)	141 $[M]^+$ (37); 124 (12); 110 (10); 83 (100); 82 (52)
Minalobine A (**4**)	213 $[M]^+$ (20); 140 (2); 124 (100); 110 (7); 95 (15); 83 (71); 82 (24)
Minalobine B (**7**)	223 $[M]^+$ (10); 124 (100); 110 (5); 95 (10); 83 (45); 82 (18); 55 (29)
Minalobine C (**8**)	239 $[M]^+$ (11); 168 (2); 124 (100); 95 (5); 83 (28); 82 (18)
Minalobine D (**9**)	241 $[M]^+$ (5); 197(3); 140(12); 124(100); 110(5); 95 (8); 83 (31); 82 (17)
Minalobine E (**11**)	241 $[M]^+$ (5); 197 (2); 140 (7); 124 (85); 110 (5); 95 (8); 83 (32); 82 (20)
Minalobine F (**13**)	313 $[M]^+$ (2); 239 (7); 145 (12); 142 (19); 124 (100); 110 (4); 96 (7); 83 (20); 82 (14)
Minalobine G (**14**)	241 $[M]^+$ (2); 157 (5); 140 (7); 124 (85); 110 (5); 96 (7); 83 (67); 82 (38)
Minalobine H (**17**)	299 $[M]^+$ (7); 212 (10); 239 (2); 142 (12); 124 (100); 110 (4); 95 (7); 83 (18); 82 (11)
Minalobine I (**20**)	327 $[M]^+$ (8); 256 (1); 212 (15); 142(11); 124 (100); 110 (3); 96 (7); 83 (20); 82 (10); 71 (20)
Minalobine J (**22**)	327 $[M]^+$ (6); 256 (5); 212 (13); 142 (17); 124 (100); 110 (4); 96 (6); 83 (18); 82 (10); 71 (22)
Minalobine K (**26**)	341 $[M]^+$ (9); 256(3); 212 (11); 142 (13); 124 (100); 110 (3); 96 (5); 83 (18); 82 (9); 57 (22)
Minalobine L (**29**)	341 $[M]^+$ (5); 256 (3); 212 (8); 142 (8); 124 (100); 110 (3); 96 (4); 83 (16); 82 (9); 57 (22)
Minalobine M (**30**)	339 $[M]^+$ (4); 256 (1); 212 (10); 142 (8); 124 (100); 110 (3); 96 (8); 83 (52); 82 (9); 55 (38)
Minalobine N (**33**)	357 $[M]^+$ (2); 212 (2); 142 (10); 124 (100); 110 (3); 96 (5); 83 (18); 82 (9)
Minalobine P (**38**)	357 $[M]^+$ (2); 256 (12); 212 (8); 142 (19); 124 (100); 110 (3); 96 (21); 83 (20); 82 (27)
Minalobine Q (**42**)	357 $[M]^+$ (1); 256 (2); 212 (5); 140 (5); 124 (100); 110 (5); 96 (8); 83 (81)
Ipanguline C_6 (**43**)	355 $[M]^+$ (2); 242 (8); 224 (7); 196 (8); 140 (29); 138 (31); 122 (30); 95 (33); 83 (78); 82 (100)
Ipanguline D_{17} (**45**)	357 $[M]^+$ (5); 242 (12); 228 (14); 140 (34); 122 (19); 96 (72); 95 (53); 82 (100); 57 (48)
Ipanguline D_{18} (**53**)	355 $[M]^+$ (3); 296 (1); 240 (5); 228 (10); 196 (23); 140 (17); 139 (22); 122 (12); 96 (32); 95 (38); 83 (64); 82 (100)
Ipanguline C_7 (**54**)	355 $[M]^+$ (2); 254 (21); 240 (8); 222 (14); 196 (12); 140 (17); 138 (54); 122 (48); 95 (51); 83 (62); 82 (100)
Minalobine S (**58**)	375 $[M]^+$ (1); 242 (50); 140 (10); 124 (100); 110 (3); 96 (5); 83 (25); 82 (11)
Minalobine T (**59**)	375 $[M]^+$ (1); 242 (48); 140 (4); 124 (100); 110 (3); 96 (5); 83 (23); 82(10)
Minalobine U (**60**)	391 $[M]^+$ (12); 258 (45); 212 (2); 142 (8); 124 (100); 110 (3); 96 (5); 83 (19); 82 (10)

C-8″), 174.7 (*s*, C-1′); EIMS (80 eV) *m/z* (rel. int.): 375 $[M]^+$ (14); 256 (3); 213 (4); 142 (8); 140 (3); 124 (100); 96 (7); 91 (24); 83 (24); HREIMS *m/z*: 375.2044 ($C_{21}H_{29}NO_5^+$, calc. 375.2046); 256.1549 ($C_{13}H_{22}NO_4^+$, calc. 256.1549); 124.1126 ($C_8H_{14}N^+$, calc. 124.1126).

4.5. Hydrolysis of minalobine R

10 mg of minalobine R (**56**) were refluxed with 200 mg $Ba(OH)_2$ in water for 2 h. Extraction of the aqueous layer with $CHCl_3$/isopropanol 3:1 yielded 2 mg of (−)-trachelanthamidine. $[\alpha]_D^{20} = -3$ (*c* 0.1, EtOH); lit.: −13.5 (*c* 2, EtOH) (Robins and Sakdarat, 1981).

4.6. Minalobine O (*35*)

Yellow oil. ^{1}H NMR (400 MHz, acetone-d_6): 1.25 (3 H, *d*, *J* = 6.3 Hz, H-4′); 1.20–1.28 (2H, *m*, H-2a, H-6a); 1.38 (3H, *s*, H-5′); 1.36–1.44 (5H, *m*, H-1, H-2b, H-6b, H-7); 1.77 (3H, *d*, *J* = 5.5 Hz, H-4″); 1.78 (3H, *s*, H-5″); 2.20 (2 H, *m*, H-3a, H-5a); 2.49 (1H, *m*, H-3b/H-5b); 2.80–2.90 (2H, *m*, H-3b/H-5b, H-8); 4.21 (1H, *dd*, *J* = 9.0 Hz, 11.4 Hz, H-9a); 4.35 (1 H, *dd*, *J* = 5.6, 11.4 Hz, H-9b); 5.09 (1 H, *q*, *J* = 6.3 Hz, H-3′); 8.87 (1H, *q*, *J* = 5.5 Hz, H-3″); ^{13}C NMR (100 MHz, acetone-d_6): 12.2 (*q*, C-4″), 13.9 (*q*, C-4′), 14.4 (*q*, C-5″), 22.6 (*q*, C-5′), 25.8 (*t*, C-7), 30.9 (*t*, C-2/C-6), 31.4 (*t*, C-2/

C-6), 40.4 (*d*, C-1), 52.0 (*t*, C-3/C-5), 53.1 (*t*, C-3/C-5), 65.3 (*d*, C-8), 67.9 (*t*, C-9), 75.2 (*d*, C-3′), 77.1 (*s*, C-2′), 129.4 (*d*, C-2″), 138.3 (*d*, C-3″), 169.2 (*s*, C-1″), 174.7 (*s*, C-1′); EIMS (80 eV) *m/z* (rel. int.): 339 $[M]^+$ (6); 256 (2); 212 (7); 196 (6); 140 (10); 124 (100); 96 (18); 83 (75); 55 (65); HREIMS *m/z*: 339.2044 ($C_{18}H_{29}NO_5^+$, calc. 339.2046); 256.1548 ($C_{13}H_{22}NO_4^+$ calc. 256.1549); 212.1286 ($C_{11}H_{18}NO_3^+$, calc. 212.1287); 124.1127 ($C_8H_{14}N^+$, calc. 124.1126).

4.7. GC–MS-analysis

Ground plant parts (50 g) were extracted three times with 500 ml MeOH (80%). After evaporation the extract was acidified and partitioned between water and organic solvents. The aqueous layer was then alkalinized and extracted with $CHCl_3$. The crude alkaloids were subjected to GC–MS analysis. The GC–MS system consisted of a Carlo Erba 5160/Fisons 8060 GC equipped with a 30 m × 0.32 mm fused silica capillary column coated with the methyl silicone stationary phase DB 1 (J&W Scientific, California). Helium was used as carrier gas. Conditions during split injection: injector 250 °C, split 1:20, temperature program 70–300 °C, 6 °C/min. The capillary column was directly coupled to the quadrupole mass spectrometer Finnigan MAT 4515/MD800. Retention indices (RI): Kovats indices (Kovats, 1958; Hartmann and Witte, 1995) were calculated in respect to a set of co-injected hydrocarbons. For EIMS fragmentation patterns of pyrrolizidine alkaloids newly described in this study see Table 2.

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