Pergamon

PII: S0031-9422(97)01082-0

Phytochemistry, Vol. 47, No. 8, pp. 1551–1560, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PYRROLIZIDINE ALKALOIDS OF *IPOMOEA HEDERIFOLIA* AND RELATED SPECIES*

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(*Received* 22 *September* 1997)

Key Word Index—*Ipomoea hederifolia*; *I. neei*; *I.* × *perigrenium*; *I. quamoclit*; Convolvulaceae; pyrrolizidine alkaloids; platynecine derivatives; ipangulines.

Abstract—The common necine base of the novel pyrrolizidine alkaloids (ipangulines) isolated recently from *Ipomoea hederifolia* is not turneforcidine but its 1β-epimer, platynecine. Three novel ipangulines were isolated from the aerial parts of *I. hederifolia* and identified as 9-*O*-[2-hydroxy-3-(2-acetoxy)-2-methylbutyryl]-7-*O*-salicyloylplatynecine (ipanguline B_2), 9-*O*-[2-hydroxy-3-(2-methylbutyryloxy)-2-methylbutyryl]-platynecine (ipanguline D_{10}) and 9-*O*-salicyloylplatynecine (ipanguline D_{11}). Comprehensive GC-mass spectrometric analysis also revealed a great variety of >38 platynecine monoesters and diesters. They are classified as ipangulines A (diesters with one phenylacetic acid moiety), ipangulines B (diesters with one salicylic acid moiety), ipangulines C (diesters with two aliphatic moieties) and ipangulines D (either 7- or 9-monoesters). In shoot tips and young leaves, total ipangulines concentrations are up to 0.45% (on dry wt basis). The ipangulines are stored as tertiary alkaloids and not as *N*-oxides. Seeds of different provenance showed similar alkaloid patterns. Taxonomically related species, such as *I. neei* and *I.* × *perigrenium*, also contain platynecine esters in their vegetative tissues.

INTRODUCTION

The Convolvulaceae is a large family with almost 2000 species, which are distributed predominantly in the tropical areas of the world. They are known to produce a wide variety of alkaloids, such as ergolines [1, 2], pyrrolidines (e.g., hygrine and cuscohygrine) and the closely related tropanes [3–5]. Some *Ipomoea* species contain unique indolizidine alkaloids of the ipalbidine-type [6] and serotonin-hydroxycinnamic acid conjugates of the ipobscurine-type [7]. Recently, 1-hydroxymethylpyrrolizidines (pyrrolizidine alkaloids, PAs) and 1-aminopyrrolizidines (loline alkaloids) were detected in the tropical bindweed, *Ipomoea hederifolia* [8], and *Argyreia mollis* [9], respectively. These were the first reports of PAs and lolines within the Convolvulaceae.

The PAs found in *I. hederifolia* so far [8], represent a unique type of PAs, i.e. diesters of a necine base esterified to one aliphatic acid and one arylalkylic or arylic acid; they were named ipangulines. Among some 360 naturally occurring PAs, aromatic necic acids are only known from the orchid alkaloids (phalaenopsine-type) [10] but these are exclusive monoesters. To characterise this new type of PA in more detail further alkaloids were isolated from *I. hederifolia* and a comprehensive GC-mass spectrometric analysis was performed to detect the whole variety of structures and minor components.

RESULTS AND DISCUSSION

Identification of platynecine and isolation of novel ipangulines

Analysis of the necine base obtained after hydrolysis of the total PA fraction from *I. hederifolia* revealed a single necine base with an *RI* of 1450, identical with that of authentic platynecine. Since turneforcidine, the 1α-epimer of platynecine, was originally described as

*Part 4 in the series "Phytochemistry and Chemotaxonomy of the Convolvulaceae". For Part 3 see Ref. [1]. Presented in part at the IOCD/CYTED International Joint Symposium, Panamá, Panama, 1997.

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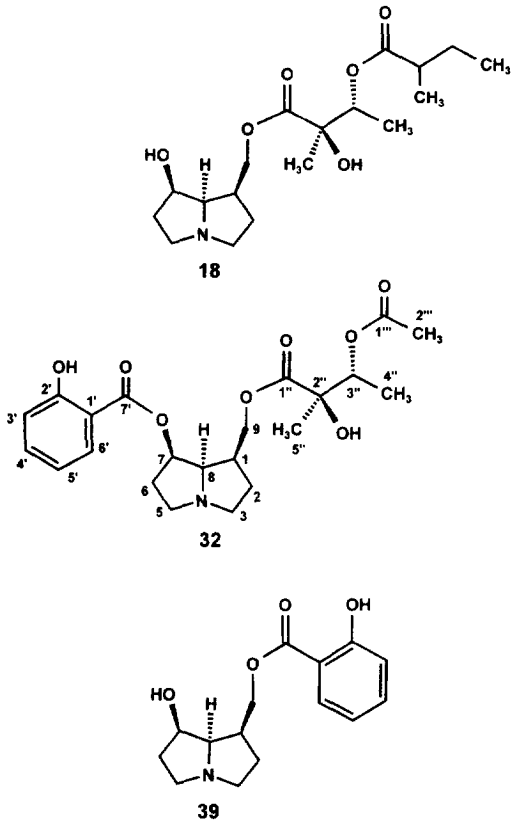

Fig. 1. Structures of novel alkaloids isolated from *Ipomoea hederifolia*.

the necine base of ipangulines [8], the identity of the necine base was reinvestigated. Platynecine was synthesised by catalytic hydrogenation of retronecine [11]. Comparison of its spectroscopic properties with those of the necine moiety of the ipangulines showed that both compounds were identical.

From dried epigeal part of *I. hederifolia*, three novel PAs could be isolated and their structures elucidated by mass, ^{1}H NMR and ^{1}H, ^{1}H COSY spectroscopy. Two of these alkaloids, ipanguline B_2 (**32**) and ipanguline D_{10} (**18**), were also detected by GC-mass spectrometry. The third one, ipanguline D_{11} (**39**), which is the first platynecine monoester with an aryl residue (i.e. salicylic acid) esterified to the C-9 hydroxyl group (Fig. 1), could not be detected by GC-mass spectrometry because we used material from different development stages for isolation.

The EI mass spectrum of **32** showed a $[M]^+$ at *m/z* 435, corresponding to a molecular formula of $C_{22}H_{29}NO_8$. Compared with the values of ipanguline B_1 (**28**) this suggested an additional acetyl moiety. The ^{1}H NMR data (Table 1) showed a downfield shift of H-3″ (δ 5.09, 1H, *q*, 6.5 Hz), suggesting that the acetyl moiety is esterified with the hydroxyl at C-3″ of the 2,3-dihydroxy-2-methylbutyric acid. The chemical shifts of H-4″ and H-5″ were in agreement with an *erythro*-orientation of the hydroxyl groups, as in ipanguline B_1 [8, 12]. Thus, **32** is the 3″-acetyl derivative of ipanguline B_1. The other isolated fraction turned out to be a 3:1 mixture of two closely related alkaloids. Ipanguline D_{11} (**39**) showed a $[M]^+$ at *m/z* 277, corresponding to a molecular formula of $C_{15}H_{19}NO_4$. The high intensity of the fragment at *m/z* 95, together with peaks at *m/z* 139 and 82, suggested the compound to be a 9-monoester of a saturated 7-hydroxy-1-hydroxymethylpyrrolizidine [13]. This was confirmed by the ^{1}H NMR data (Table 1) showing an upfield shift of H-7 in comparison to the other ipangulines. In the aromatic region, the typical pattern for a salicylic acid moiety could be observed, whereas there were no signals corresponding to a 2,3-dihydroxy-2-methylbutyric acid. Thus, **39** is 9-*O*-salicyloylplatynecine. Ipanguline D_{10} (**18**) exhibited similar NMR data (Table 1) for the necine moiety. Instead of salicylic acid, a 2,3-dihydroxy-2-methylbutyric acid moiety could be deduced from the ^{1}H NMR. Because the *q* for H-3′ (δ 5.16, 1H, 6.5 Hz) showed a downfield shift in comparison to ipanguline A_1, esterification of the C-3′ hydroxyl is indicated. In addition, typical signals for a 2-methylbutyric acid could be observed: δ 0.9 (3H, *t*, 7.0 Hz, H-4″), δ 1.12 (3H, *d*, 7.0 Hz, H-5″), δ 1.65 (2H, *m*, 2 × H-3″) and δ 2.30 (1H, *m*, H-2″). This is in agreement with the mass spectral data. Ipanguline D_{10} is characterised by a $[M]^+$ at *m/z* 357, corresponding to a molecular formula of $C_{18}H_{31}NO_6$, which is in accordance with 9-*O*-[2-hydroxy-3-(2-methylbutyryloxy)2-methylbutyryl]-platynecine (**18**).

Analysis of alkaloids in shoots

Shoots of *I. hederifolia* grown in the greenhouse were separated into stems, shoot tips, including very young leaves, and mature leaves. The three samples were subjected to a rigorous analysis by GC-mass spectrometry [14]. This revealed a great diversity of alkaloids, all derivatives of platynecine (Table 2). Only platynecine derivatives which on EI or CI mass spectrometry show a clear $[M]^+$ or $[M+H]^+$ are included. i.e. a total of 38 compounds from *I. hederifolia* and an additional eight alkaloids (i.e., **40**–**47**) from *I. neei* and *I.* × *perigrenium cv.* Cardinal, respectively. The great variety of structures is brought about by esterification of platynecine to various aliphatic or aromatic necic acids and the formation of various combinations of monoesters or diesters. Since the PAs originally isolated from *I. hederifolia* were named ipangulines [8], we enlarged this classification to distinguish between the various types of compounds (Fig. 2). Ipangulines A comprise diesters esterified with phenylacetic acid at the C-7 hydroxyl and an aliphatic acid at the C-9 hydroxyl; ipangulines B are the respective diesters containing a salicyloyl residue at the C-7 hydroxyl; the ipangulines C are diesters with two aliphatic necic acids and the ipangulines D are either 7- or 9-monoesters. Structures with still ambiguous necic acid moieties were classified as ipanguline X. To identify the structures of the most prominent necic

Table 1. ^{1}H NMR data of platynecine and compounds **18**, **32** and **39** [*J* (Hz) in parentheses]

H	Platynecine (CD_3OD)	**18** ($CDCl_3$)	**32** ($CDCl_3$)	**39** ($CDCl_3$)
1	2.62 *tt* (6.0, 12.0)	2.65 *m*	2.60–2.92 *m**	2.81–2.96 *m**
2a	2.05 *m*	1.78–2.10 *m**	2.00–2.40 *m**	1.78–2.10 *m**
2b	2.24 *dq*-like (9.0, 12.0)	1.78–2.10 *m**	2.00–2.40 *m**	1.78–2.10 *m**
3a	3.34 *m**	2.81–2.96 *m**	2.60–2.92 *m**	2.81–2.96 *m**
3b	3.58 *dt* (7.0, 12.0)	3.18 *ddd* (3.0, 8.5, 11.0)	3.03 *dt* (2.0, 8.5)	3.18 *ddd* (3.0, 8.5, 11.0)
5a	3.34 *m**	2.81–2.96 *m**	2.60–2.92 *m**	2.81–2.96 *m**
5b	3.86 *ddd* (2.0, 8.0, 12.0)	3.35 *br t* (7.0)	3.16 *m*	3.35 *br t* (7.0)
6	2.10 *m*	1.78–2.10 *m**	2.00–2.40 *m**	1.78–2.10 *m**
7	4.55 *br s*	4.36 *br s*	5.60 *br s*	4.33 *br s*
8	4.01 *m**	3.46 *dd* (3.5, 8.0)	3.91 *dd* (3.0, 7.5)	3.49 *dd* (3.5, 8.0)
9a	3.92 *dd* (6.0, 11.0)	4.37 *dd* (8.0, 11.0)	4.36 *dd* (7.0, 11.0)	4.71 *dd* (8.0, 11.0)
9b	4.01 *m**	4.60 *dd* (8.0, 11.0)	4.39 *dd* (7.0, 11.0)	4.86 *dd* (8.0, 11.0)
3′	—	5.16 *q* (6.5)	7.05 *dd* (1.5, 7.5)	7.00 *dd* (2.0, 8.0)
4′	—	1.32 *d* (6.5)	7.53 *dt* (1.5, 7.5)	7.45 *dt* (2.0, 8.0)
5′	—	1.40 *s*	6.90 *dt* (1.5, 7.5)	6.89 *dt* (2.0, 8.0)
6′	—	—	7.68 *dd* (1.5, 7.5)	7.83 *dd* (2.0, 8.0)
2″	—	2.30 *m*	—	—
3″	—	1.65 *m*	5.09 *q* (6.5)	—
4″	—	0.90 *t* (7.0)	1.21 *d* (6.5)	—
5″	—	1.12 *d* (7.0)	1.42 *s*	—
2‴	—	—	2.09 *s*	—

* Overlapping signals.

acids, which in most cases was not possible by GC-mass spectrometry of the ester alkaloids, the methyl esters of the necic acids were prepared from pre-purified total PA extracts of *I. hederifolia*. This was achieved by transesterification with trimethylsulfoniumhydroxide (TMSH) [15, 16] and direct analysis by GC-mass spectrometry (Table 3). The most characteristic necic acids, i.e. 2-methylbutyric acid, *erythro*-2,3-dihydroxy-2-methylbutyric acid (*e*-HMBA) and its *threo*-epimer (*t*-HMBA), as well as phenylacetic acid and salicylic acid could be identified unequivocally as methyl esters and, subsequently, were localised as ester components of the PAs (Table 2). The structures of some minor platynecine esters (ipangulines X_{1-5}) remain obscure, since their necic acids could not be identified by the methods applied. *Erythro*- and *threo*-2,3-dihydroxy-2-methylbutyric acid were found in ca equal amounts in the methyl ester fraction. As platynecine esters, the two epimers represent the respective ipangulines (*erythro*-HMBA) and isoipangulines (*threo*-HMBA) [8]. The ipanguline/isoipanguline pairs are separated by GC. The *RI* values are very close in case of the unsubstituted diesters (e.g., **16**/**17**, **25**/**26** and **27**/**28**) but the separation is much better if the HMBA moiety is further acylated (e.g., pairs **9**/**10**, **20**/**21**, **29**/**30**, and **31**/**32**). Separation of the isoipanguline/ipanguline pairs **25**/**26**, **27**/**28**, and **31**/**32**, which in the case of **26**, **27**/**28** and **32** were available as reference compounds, revealed, in all cases, a lower *RI* for the isoipangulines. From this, we deduce that necine esters with the *threo*-epimer of HMBA leave the column before the respective *erythro*-epimer. Where only one HMBA-ester was detected, differentiation between the HMBA-epimers was not possible (Table 2).

The major PAs found in *I. hederifolia* are the typical ipangulines A (i.e., **25**/**26**, **29**, **33**/**34**, and **36**) and B (i.e., **27**/**28** and **35**) (Table 4). Only a few ipangulines C (i.e., **16**/**17**, **20**, and **22**) and D (i.e., **14**) are present in reasonable amounts, the great majority of structures are detectable in trace amounts (< 1% of total PAs) only. Differences in the PA patterns exist between stems and leaves; whereas **27**/**28** are the dominating alkaloids in stems, **29** and **33**/**34** are major PAs in leaves and shoot tips. The highest total alkaloid concentrations are found in young leaves and shoot tips, being ca two-fold higher than those in mature leaves and more than seven-fold those in stems (Table 4). With PA levels up to 0.45% of dry weight the alkaloid concentrations are within the range of typical PA plants, such as *Senecio* species [17, 18]. Most interestingly, the ipangulines are largely, if not exclusively, present as free bases and not in the form of their *N*-oxides, as in species of the Asteraceae, Boraginaceae and Fabaceae [10]. This may be related to the fact that the tertiary platynecine derivatives do not necessarily need to be stored as nontoxic *N*-oxides, since they are not potentially mutagenic and cytotoxic, like the 1,2-unsaturated necine derivatives (e.g., retronecine derivatives) [19–21].

Analysis of alkaloids in seeds and of related species

Ipomoea hederifolia seeds of different provenance were compared in their alkaloid patterns (Table 5).

Table 2. GC-MS analysis of the pyrrolizidine alkaloids from shoots of *Ipomoea hederifolia* (**1**–**38**) shoots of *I. neei* (**2**, **3**, **7**, **10**, **14**–**17**, **21**, **40**–**43**) and roots of *Ipomoea* × *perigrenium* (**16**, **17**, **44**–**47**)

	Alkaloid	R_1 (at O^7)	R_2 (at O^9)	*RI*	$[M]^+$, characteristic ions (rel. int.)	CI-MS[a]
1	Ipanguline D_1	H	COC_3H_7	1703	227 (1), 209 (10), 183 (4), 140 (7), 95 (96), 82 (100), 71 (5), 55 (8), 43 (15)	
2	7-(2-Methylbutyryl)-platynecine	MeBu	H	1778	241 (0.2), 210 (0.4), 156 (34), 139 (63), 122 (5), 113 (17), 82 (100), 57 (12)	
3	9-(2-Methylbutyryl)-platynecine	H	MeBu	1803	241 (0.6), 223 (9), 197 (2), 140 (9), 124 (6), 95 (100), 82 (89), 55 (8)	N, I
4	Ipanguline D_2[b]	H	COC_4H_9O?	1886	257 (1), 239 (3), 213 (3), 185 (4), 140 (21), 95 (55), 82 (100), 73 (15), 55 (14)	I
5	9-Tigloylplatynecine	H	Tigloyl	1898	239 (0.2) 221 (15), 195 (3), 140 (5), 122 (6), 95 (100), 82 (74), 55 (20)	N
6	Isoipanguline D_3	H	*t*-HMBA	2022	273 (–), 255 (3), 212 (13), 158 (26), 140 (24), 114 (14), 95 (46), 82 (100)	
7	Ipanguline D_3	H	*e*-HMBA	2037	273 (–), 255 (7), 212 (14), 158 (29), 140 (28), 114 (12), 95 (51), 82 (100)	
8	Ipanguline D_4[b]	H	$COC_6H_{11}O_3$?	2085	299? (0.3), 281 (3), 255 (3), 212 (3), 140 (13), 95 (100), 82 (79), 43 (13)	
9	Isoipanguline D_5	H	Ac-*t*-HMBA	2108	315 (1.4), 297 (2), 271 (5), 254 (2), 228 (10), 200 (7), 158 (9), 140 (29), 95 (72), 82 (100), 43 (23)	N, I
10	Ipanguline D_5	H	Ac-*e*-HMBA	2157	315 (2), 297 (2), 271 (9), 254 (2), 228 (13), 200 (6), 158 (10), 140 (30), 95 (68), 82 (100), 43 (41)	N, I
11	Ipanguline D_6	PAA	H	2168	275 (–), 156 (19), 139 (65), 124 (3), 113 (16), 91 (20), 82 (100), 55 (7)	N, I
12	Ipanguline D_7	H	PAA	2212	275 (1), 257 (5), 231 (8), 140 (5), 95 (64), 91 (18), 82 (100), 55 (18)	N, I
13	Ipanguline D_8[b]	H	$COC_8H_{15}O_3$?	2226	343? (2), 299 (2), 228 (25), 158 (8), 140 (40), 122 (11), 96 (89), 95 (62), 82 (100), 71 (40), 43 (33)	
14	Ipanguline D_9[c]	H	DiAc-HMBA	2246	357 (1), 339 (1), 313 (8), 228 (6), 140 (17), 138 (8), 122 (7), 95 (100), 82 (91), 43 (34)	N, I
15	Ipanguline X_1[b]	?	?	2302	385 (–), 297 (17), 254 (27), 228 (20), 210 (34), 139 (58), 138 (61), 122 (53), 95 (56), 82 (100), 71 (19), 43 (48)	N, I
16	Isoipanguline C_1	MeBu	*t*-HMBA	2308	357 (0.1), 313 (1), 255 (8), 242 (21), 238 (16), 224 (18), 140 (100), 138 (59), 122 (57), 96 (22), 95 (33), 82 (67), 57 (29)	N, I
17	Ipanguline C_1	MeBu	*e*-HMBA	2311	357 (–), 312 (0.5), 255 (7), 242 (19), 238 (13), 224 (19), 140 (100), 138 (64), 122 (63), 96 (24), 95 (37), 82 (79), 57 (30)	
18	Ipanguline D_{10}	H	MeBu-*e*-HMBA	2327	357 (3), 313 (3), 242 (14), 228 (16), 158 (12), 140 (44), 122 (13), 96 (100), 95 (61), 85 (30), 82 (93), 57 (41)	N, I
19	Ipanguline X_2[b]	Tigloyl?	HMBA?	2376	355? (0.2), 340 (0.2), 311 (2), 255 (12), 240 (25), 238 (23), 222 (19), 140 (100), 138 (91), 122 (65), 96 (31), 95 (36), 83 (44), 82 (77), 55 (28)	
20	Isoipanguline C_2	MeBu	Ac-*t*-HMBA	2391	399 (0.1), 313 (8), 297 (22), 254 (37), 242 (14), 224 (32), 140 (32), 138 (90), 122 (77), 95 (65), 82 (100), 57 (39), 43 (38)	N, I
21	Ipanguline C_2	MeBu	Ac-*e*-HMBA	2426	399 (0.1), 313 (6), 297 (18), 254 (46), 242 (13), 224 (32), 140 (32), 138 (88), 122 (76), 95 (65), 82 (100), 57 (41), 43 (39)	N, I
22	Ipanguline C_3[c]	MeBu	DiAc-HMBA	2506	441 (0.1), 339 (20), 280 (22), 224 (27), 210 (11), 138 (100), 122 (79), 95 (90), 82 (82), 57 (27), 43 (34)	
23	Ipanguline C_4[c]	MeBu	MeBu-HMBA	2606	441 (0.1), 339 (10), 254 (29), 242 (12), 224 (25), 138 (66), 122 (100), 96 (40), 95 (30), 85 (44), 82 (53), 57 (63)	N, I
24	Ipanguline X_3[b]	?	?	2667	439 (–), 339 (16), 280 (8), 254 (22), 240 (9), 222 (14), 210 (7), 138 (99), 122 (100), 96 (36), 95 (45), 85 (19), 82 (65), 57 (27), 55 (17)	I
25	Isoipanguline A_1	PAA	*t*-HMBA	2702	391 (0.1), 276 (14), 258 (16), 255 (12), 238 (25), 140 (100), 138 (60), 122 (57), 96 (28), 95 (41), 91 (38), 82 (95)	N, I
26	Ipanguline A_1	PAA	*e*-HMBA	2707	391 (0.1), 276 (12), 258 (14), 255 (8), 238 (16), 140 (78), 138 (57), 122 (52), 96 (29), 95 (45), 91 (54), 82 (100)	N, I

27	Isoipanguline B_1	Sal	*t*-HMBA	2770	393 (0.1), 278 (16), 260 (6), 255 (18), 238 (25), 140 (100), 138 (70), 122 (47), 121 (29), 96 (20), 95 (26), 82 (62)	
28	Ipanguline B_1	Sal	*e*-HMBA	2772	393 (0.1), 278 (13), 260 (5), 255 (15), 238 (21), 140 (100), 138 (67), 122 (46), 121 (31), 96 (21), 95 (28), 82 (64)	
29	Isoipanguline A_2	PAA	Ac-*t*-HMBA	2782	433 (0.2), 297 (31), 276 (8), 258 (20), 254 (29), 140 (24), 138 (68), 122 (54), 96 (26), 95 (59), 91 (36), 82 (100), 43 (35)	N, I
30	Ipanguline A_2	PAA	Ac-*e*-HMBA	2810	433 (0.1), 297 (25), 276 (7), 258 (20), 254 (34), 140 (24), 138 (70), 122 (58), 96 (25), 95 (62), 91 (35), 82 (100), 43 (33)	N, I
31	Isoipanguline B_2	Sal	Ac-*t*-HMBA	2837	435 (–), 297 (42), 260 (6), 254 (36), 210 (8), 140 (75), 138 (100), 122 (64), 121 (51), 96 (27), 95 (57), 82 (98), 43 (30)	N, I
32	Ipanguline B_2	Sal	Ac-*e*-HMBA	2873	435 ( ), 297 (34), 260 (7), 254 (42), 210 (8), 140 (74), 138 (100), 122 (67), 121 (49), 96 (26), 95 (57), 82 (99), 43 (32)	I
33	Isoipanguline A_3	PAA	DiAc-*t*-HMBA	2886	475 (0.1), 389 (1), 339 (28), 280 (21), 258 (21), 210 (8), 138 (94), 122 (65), 95 (99), 91 (28), 82 (100), 43 (35)	N, I
34	Ipanguline A_3	PAA	DiAc-*e*-HMBA	2891	475 ( ), 339 (18), 280 (5), 258 (19), 210 (7), 138 (87), 122 (67), 95 (67), 91 (52), 82 (100), 43 (72)	
35	Ipanguline B_3[c]	Sal	DiAc-HMBA	2927	477 (–), 339 (24), 280 (17), 260 (5), 210 (7), 140 (32), 138 (100), 122 (60), 121 (40), 95 (69), 82 (70), 43 (21)	N
36	Ipanguline A_4[c]	PAA	MeBu-HMBA	2985	475 (0.5), 339 (14), 276 (7), 258 (19), 254 (28), 140 (21), 138 (69), 122 (100), 96 (55), 95 (36), 91 (35), 85 (40), 82 (80), 57 (55)	N, I
37	Ipanguline X_4[b]	?	?	3055	497 (0.1), 339 (12), 298 (5), 280 (13), 254 (27), 141 (12), 140 (17), 138 (43), 122 (100), 96 (35), 95 (22), 85 (21), 82 (42), 71 (9), 57 (28)	N
38	Ipanguline X_5[b]	?	?	3083	499 (0.1), 456 (4), 384 (5), 339 (11), 300 (2), 282 (10), 254 (25), 140 (30), 138 (39), 122 (100), 96 (33), 95 (22), 85 (23), 82 (45), 57 (31)	
40	9-Acetylplatynecine	H	Ac	1557	199 (1), 181 (5), 155 (5), 140 (3), 95 (56), 82 (100), 55 (10), 43 (19)	
41	Ipanguline X_6[b]	?	?	2100	315? ( ), 300 (0.1), 200 (21), 182 (22), 140 (50), 138 (41), 122 (45), 95 (42), 82 (100), 55 (16), 43 (47)	
42	Ipanguline D_{12}[b,c]	Ac-HMBA?	H	2125	315 (–), 228 (4), 200 (4), 156 (34), 139 (87), 122 (12), 113 (25), 82 (100), 43 (24)	I
43	Ipanguline X_7[b]	?	?	2217	315? ( ), 297 (6), 254 (11), 182 (26), 138 (46), 122 (42), 95 (53), 82 (100), 43 (78)	
44	Ipanguline D_{13}[b,c]	Tig-HMBA?	H	2358	355 (4), 312 (1), 296 (2), 196 (15), 156 (29), 139 (100), 122 (9), 113 (18), 96 (11), 83 (46), 82 (79), 55 (27)	
45	Ipanguline D_{14}[b,c]	H	Tig-HMBA?	2375	355 (11), 337 (2), 240 (10), 228 (12), 196 (56), 140 (30), 122 (10), 96 (52), 95 (47), 83 (72), 82 (100), 55 (43)	
46	Ipanguline D_{15}[b,c]	H	Tig-HMBA?	2387	355 (12), 337 (1), 240 (10), 228 (12), 196 (29), 140 (32), 122 (10), 96 (60), 95 (50), 83 (74), 82 (100), 55 (43)	
47	Ipanguline D_{16}[b,c]	Tig-HMBA?	H	2395	355 (3), 240 (4), 228 (5), 196 (5), 156 (29), 139 (100), 122 (9), 113 (19), 96 (13), 83 (49), 82 (82), 55 (25)	

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; Tig = tigloyl.

[a] Analysed by CI-MS, N = NH_3, I = isobutane.

[b] According to GC-MS, a platynecine ester but identity of necic acids uncertain or tentative.

[c] Orientation of hydroxyl groups of HMBA moiety unclear.

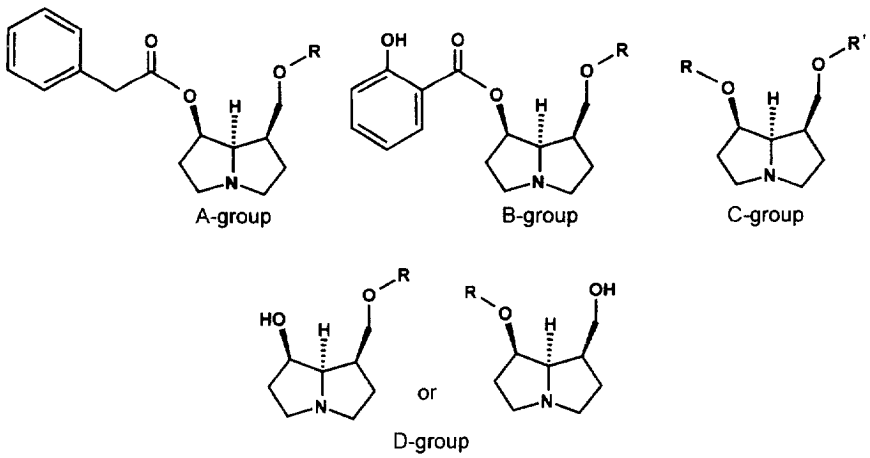

Fig. 2. The four structural types of the ipangulines. R = aliphatic necic acid.

Table 3. GC-MS analysis of necic acid methyl esters prepared from a purified crude extract of *I. hederifolia* by transesterification with TMSH

Necic acid methyl ester	*RI*[a]	*m/z* $[M]^+$	Relative intensity
2-Methylbutyric acid methyl ester	755	116	26
Tiglic acid methyl ester	855	114	2
Hexanoic acid methyl ester	915	130	1
threo-2,3-Dihydroxy-2-methylbutyric acid methyl ester	968	148	7
erythro-2,3-Dihydroxy-2-methylbutyric acid methyl ester	973	148	8
Benzoic acid methyl ester	1063	136	< 1
Octanoic acid methyl ester	1112	158	< 1
Phenylacetic acid methyl ester	1142	150	51
Salicylic acid methyl ester	1163	152	1
O-Methylsalicylic acid methyl ester[b]	1298	166	3

[a] DB-1 column.
[b] *O*-methylated by TMSH.

Although there are some differences, the A_1 ipangulines (**25**/**26**) are always the dominating PAs. In contrast to the vegetative plant parts (see Table 4), which accumulate preferentially **25**/**26**, further esterified with acetic acid (**29**, **33**/**34**) or methylbutyric acid (**36**), seeds are devoid of such esters. Furthermore, the B ipangulines are absent from the seed pattern.

Within the Convolvulaceae, *I. hederifolia* belongs taxonomically to the sectio Quamoclit [22]. Thus, two additional species, i.e. *I. neei* and *I.* × *perigrenium*, an assumed hybrid of *I. hederifolia* (D. F. Austin, personal communication) and presumably *I. quamoclit*, were included in a preliminary screening for PAs. No PAs were detected in seeds of *I. quamoclit* and *I. neei* but vegetative plant material available from *I. neei* and *I.* × *perigrenium* were found to contain PAs (Table 6). Like *I. hederifolia*, the two species contain only platynecine esters and, among the various necic acids, HMBA is most prominent. In contrast to *I. hederifolia*, aromatic necic acids (i.e., phenylacetic acid and salicylic acid) are absent from the PAs of these two species which, in turn, produce some PAs not found in *I. hederifolia*, i.e. **40**–**47** (Tables 2 and 6). More detailed studies are needed to evaluate whether the presence of ipanguline-type PAs is a general feature and, thus, a chemosystematic marker of the sectio Quamoclit within the Convolvulaceae. However, to date, ipangulines have not been detected in numerous species of the genus *Ipomoea* outside this sectio or of other convolvulaceous genera.

EXPERIMENTAL

General

^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) were recorded on a Bruker AC 300 or AC 400. EIMS: direct inlet system, 70 eV. HRMS: 80 eV. CC: silica gel 60 (70–230 mesh). TLC and prep. TLC: precoated silica gel plates 60 F_{254} (0.25 mm).

Plant material

Seeds of *I. hederifolia* L. (syn.: *I. angulata* Lamk.) were collected near Ella, Sri Lanka; Chiang Mai,

Table 4. Relative abundance and total concentrations of pyrrolizidine alkaloids in stems, shoot tips and leaves of greenhouse-grown *Ipomoea hederifolia*

	Alkaloid	*RI*	PAs, relative abundance (%)		
			Stems	Shoot tips	Mature leaves
1	Ipanguline D_1	1703	tr	< 1	< 1
2	7-(2-Methylbutyryl)-platynecine	1778	tr	< 1	< 1
3	9-(2-Methylbutyryl)-platynecine	1803	< 1	< 1	< 1
4	Ipanguline D_2	1886		tr	
5	9-Tigloylplatynecine	1898		tr	
6	Isoipanguline D_3	2022	< 1	< 1	< 1
7	Ipanguline D_3	2037	< 1	< 1	
8	Ipanguline D_4	2085		tr	tr
9	Isoipanguline D_5	2108	tr	< 1	< 1
10	Ipanguline D_5	2157	< 1	< 1	< 1
11	Ipanguline D_6	2168			< 1
12	Ipanguline D_7	2212	< 1	< 1	< 1
13	Ipanguline D_8	2226	tr	tr	
14	Ipanguline D_9	2246	4	1	7
15	Ipanguline X_1	2302	tr	tr	tr
16/17	Isoipanguline/Ipanguline C_1	2308–11	8	3	6
18	Ipanguline D_{10}	2327	< 1	< 1	< 1
19	Ipanguline X_2	2376	tr	< 1	
20	Isoipanguline C_2	2391	2	4	7
21	Ipanguline C_2	2426	tr	< 1	< 1
22	Ipanguline C_3	2506	5	3	5
23	Ipanguline C_4	2606	tr	< 1	< 1
24	Ipanguline X_3	2667	> 1	tr	tr
25/26	Isoipanguline/Ipanguline A_1	2702–7	14	13	12
27/28	Isoipanguline/Ipanguline B_1	2770–2	21	2	1
29	Isoipanguline A_2	2782	8	25	20
30	Ipanguline A_2	2810	< 1	< 1	< 1
31	Isoipanguline B_2	2837	< 1	< 1	< 1
32	Ipanguline B_2	2873	tr	< 1	< 1
33/34	Isoipanguline/Ipanguline A_3	2886–91	15	26	23
35	Ipanguline B_3	2927	4	tr	2
36	Ipanguline A_4	2985	5	9	9
37	Ipanguline X_4	3055	> 1	tr	tr
38	Ipanguline X_5	3083	> 1	> 1	> 1
Total PAs, mg g^{-1} (dry wt)					
Tertiary bases + *N*-oxides:			0.595	4.458	1.841
Tertiary bases:			0.525	3.618	1.956

Thailand; Guayaquil, Ecuador; Santa Ana, Panama. Seeds of *I. quamoclit* L. were collected near San Lorenzo, Panama, and those of *I. neii* (Spreng.) O'Donell near Boquete, Panama. *Ipomoea* × *perigrenium cv.* Cardinal was purchased from Gartencenter Pluta, Berlin-Dahlem. Plants were grown from seed in the greenhouse and provided roots and shoots for alkaloid extraction. Voucher specimens are deposited at the Institut für Pharmazie II, Freie Universität Berlin.

For detailed GC-MS analysis sterile cultures of differentiated plantlets of *I. hederifolia* (origin: Sri Lanka) were established on MS medium (phytohormones omitted) [23]. Plantlets are kept as stock-cultures and used during the growing season to initiate greenhouse plants. For PA analysis, the long shoots (1 m) were divided into (a) shoot tips (10 cm) with the youngest leaves; (b) mature leaves; (c) stems without leaves. Harvested material was immediately frozen, lyophilised and kept cold until used.

Semisynthesis of (−)-platynecine

Monocrotaline (300 mg) was refluxed with 800 mg of BaOH in H_2O for 1 h. The mixt. was acidified and extracted with $CHCl_3$. After evapn of the aq. layer, retronecine was dissolved in EtOH (60°) and hydrogenated for 2 h (Pd/C-catalyst). Evapn gave 20 mg (−)-platynecine. Colourless gum. $[\alpha]_D^{20}$ −51° (EtOH, *c* 0.9). EIMS *m/z* (rel. int.): 157 $[M]^+$ (13), 113 (24),

Table 5. GC-MS analysis of PA extracts from seeds of *Ipomoea hederifolia* of different provenances

	Alkaloid	*RI*	$[M]^+$ (*m/z*)	Relative abundance (%)			
				Sri Lanka	Panama	Ecuador	Thailand
	Anhydroplatynecine	1130	139	2	3	4	5
5	9-Tigloylplatynecine	1898	239		2	< 1	
11	Ipanguline D_6	2168	275	< 1	2	2	1
12	Ipanguline D_7	2212	275	< 1	6	6	6
14	Ipanguline D_9	2246	357	< 1	< 1		
17	Ipanguline C_1	2308	357	< 1	2	9	4
19	Ipanguline X_2	2376	355?	< 1	31	6	< 1
25/26	Isoipanguline/Ipanguline A_1[a]	2702–7	391	97	54	71	80
Total PAs, mg g^{-1} (dry wt)					0.15	0.16	0.28

[a] Two epimers poorly resolved.

Table 6. Pyrrolizidine alkaloids in two additional species of the sectio Quamoclit (Convolvulaceae) compared with *Ipomoea hederifolia*

	Alkaloid	*RI*	$[M]^+$ (*m/z*)	*Ipomoea neei* (shoots)	*Ipomoea* × *perigrenium* (roots)	*Ipomoea hederifolia* (whole plant)
40	9-Acetylplatynecine	1557	199	+		
2	7-(2-Methylbutyryl)-platynecine	1778	241	+		+
3	9-(2-Methylbutyryl)-platynecine	1803	241	+		+
7	Ipanguline D_3	2037	273	+		+
41	Ipanguline X_6	2100	315?	+		
42	Ipanguline D_{12}	2125	315	+		
10	Ipanguline D_5	2157	315?	+		+
43	Ipanguline X_7	2217	315	+		
14	Ipanguline D_9	2246	357	+		+
16	Isoipanguline C_1	2308	357	+	+	+
17	Ipanguline C_1	2311	357	+	+	+
44	Ipanguline D_{13}	2358	355		+	
45	Isoipanguline D_{14}	2375	355		+	
46	Ipanguline X_8	2387	355		+	
47	Ipanguline D_{15}	2395	355		+	
21	Ipanguline C_2	2426	399	+		+

82 (100). ^{1}H NMR (Table 1). ^{13}C NMR (MeOH): δ 28.9 (*t*, C-2), 37.1 (*t*, C-6), 44.4 (*d*, C-1), 55.3 (*t*, C-5), 56.3 (*t*, C-3), 60.7 (*t*, C-9), 72.0 (*d*, C-7), 74.4 (*d*, C-8), identical with data in Ref. [24].

Isolation of alkaloids

Aerial parts of *I. hederifolia* (2 kg) were extracted with 20 l of MeOH (70%). After evapn, the extract was acidified and partitioned between H_2O and organic solvents. The aq. layer was then made alkaline and extracted with $CHCl_3$ to yield a crude alkaloid mixt., which was fractionated on silica gel [MeOH–$CHCl_3$–NH_3 (33%)-mixts]. Frs 18–29 were further purified by prep. TLC ($CHCl_3$–MeOH, 4 : 1) to give ipanguline B_2 (**32**). Ipanguline D_{10} (**18**) and ipanguline D_{11} (**39**) were isolated from frs 68–80, again using prep. TLC in $CHCl_3$–MeOH–NH_3 (33%) (75 : 23 : 2).

Ipanguline B_2 (**32**)

EIMS *m/z* (rel. int.): 435 $[M]^+$ (0.1), 391 (4), 320 (8), 297 (60), 254 (34), 210 (7), 140 (47), 138 (70), 122 (47), 95 (48), 82 (100); HRMS, *m/z*: 435.1891 ($C_{22}H_{29}NO_8$, calc. 435.1893), 391.1634 ($C_{20}H_{25}NO_7$, calc. 391.1631), 297.1575 ($C_{15}H_{23}NO_5$, calc. 297.1576). ^{1}H NMR (Table 1).

Ipanguline D_{10} (**18**)

EIMS *m/z* (rel. int.): 357 $[M]^+$ (3), 313 (6), 242 (4), 228 (6), 158 (3), 140 (23), 139 (30)*, 96 (34), 95 (86),

* Due to an impurity with a 7-monoester.

82 (100); HRMS, *m/z*: 357.2153 ($C_{18}H_{31}NO_6$, calc. 357.2151). ^{1}H NMR (Table 1).

Ipanguline D_{11} (**39**)

EIMS *m/z* (rel. int.): 277 $[M]^+$ (13), 259 (7), 233 (8), 140 (23), 139 (30)*, 95 (86), 82 (100); HRMS, *m/z*: 277.1313 ($C_{15}H_{19}NO_4$, calc. 277.1314). ^{1}H NMR (Table 1).

Alkaloid extraction for GC and GC-MS [14]

Ground lyophilised plant material (i.e., shoot tips, mature leaves and stems), 0.25 g each, was extracted twice in a mortar with 2 ml 1 M HCl. After centrifugation, a prepurified total alkaloid fr. was obtained from the supernatant by liquid–liquid extraction using silica earth columns (Extrelut, Merck) [14]. Samples were stored in MeOH prior to analysis. In expts where PA *N*-oxides were expected, the acidic aq. extracts were reduced by adding Zn dust in excess and left to stand for 5 h, prior to liquid–liquid extraction.

Preparation of necic acid methyl esters by transesterification [15, 16]

A 0.2 M soln of trimethylsulfoniumhydroxide (TMSH, Macherey & Nagel, Düren) (20 μl) was added to 10 μl MeOH containing 10 μg alkaloids. The mixt. was directly injected into the GC-MS-instrument.

GC-MS analysis [14]

A GC equipped with a 30 m × 0.32 mm fused-silica capillary column (DB-1, J & W Scientific) was used. Conditions: injector 250°; split ratio 1:20; temp. programme 70–300°, 6° min^{-1}; carrier gas He 0.5 bar. The capillary column was directly coupled to the quadrupole mass spectrometer. EI-mass spectra were recorded at 40 eV. CI mass spectra were recorded with the same GC-MS system using NH_3 or *iso*butane as reagent gas, at 100 eV. Conditions for GC-MS analysis of the necic acids were the same as those given for the alkaloids but with a temp. programme 30–200°, 6° min^{-1}. Retention indices (*RI*): Kovats indices [10, 25] were calculated with reference to a set of co-injected hydrocarbons.

GC analysis [14]

PAs were separated on a 15 m × 0.25 mm fused-silica capillary column (DB-1, J & W Scientific). Conditions: injector 250°, split ratio 1:20, temp. programme 150–300°, 6° min^{-1}, carrier gas He 0.75 bar, detector: dual FID, PND.

* Due to an impurity with a 7-monoester.

Acknowledgements—The authors are indebted to Mrs E. Bäumel-Eich (Berlin) for essential support in exploring and collecting the plant material and to Mrs C. Theuring (Braunschweig) for skilful technical assistance. The work was supported by grants from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie to T.H.

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