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Research papers

Tropane and pyrrolizidine alkaloids in the ithomiines *Placidula euryanassa* and *Miraleria cymothoe* (Lepidoptera: Nymphalidae)

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Summary. Larvae of the ithomiine butterfly *Placidula euryanassa* sequester tropane alkaloids (TAs) from the host plant *Brugmansia suaveolens* and pass them through the pupae to freshly emerged adults. Wild caught adults also show in their tissues, variable amounts of pyrrolidizine alkaloids (PAs), probably sequestered from variable plant sources and subject to dynamics of incorporation, accumulation and utilization of PAs by ithomiine butterflies. The ratio TAs/PAs is also variable between different populations. *Miraleria cymothoe*, another ithomiine that feeds on *B. suaveolens* as larvae, does not sequester TAs from the host-plant, but sequesters PAs from plant sources visited by the adult butterflies. The main alkaloid found in both butterflies is lycopsamine, which also is the principal PA found in all genera of Ithomiinae.

Key words. chemical defense – tropane alkaloids – pyrrolizidine alkaloids – lycopsamine – Ithomiinae – *Placidula euryanassa* – *Miraleria cymothoe* – Solanaceae – *Brugmansia suaveolens*

Introduction

The butterfly subfamily Ithomiinae is well known for being aposematic and unpalatable. Most ithomiinae larvae feed on Solanaceae; only some primitive groups are restricted to Apocynaceae as larval food plants (Brown 1984, 1985, 1987; Drummond & Brown 1987). The protection of adults against predation by the giant orb weaving spider *Nephila clavipes* is due to pyrrolizidine alkaloids (PAs) sequestered by adults from nectar and other sources (*e.g. Eupatorium, Heliotropium*) (Brown 1984, 1985). Many Solanaceae toxins are inactive against this predator (Brown 1985, 1987), and the role of solanaceous compounds in the chemical ecology of Ithomiinae is poorly understood. In this work, we investigated two different species of ithomiine butterflies (*Placidula euryanassa* and *Miraleria cymothoe*) that feed as larvae on a solanaceous plant (*Brugmansia suaveolens*) containing tropane alkaloids (TAs) and show that these species of Ithomiinae use the same chemical source in different ways.

Material and methods

Collection of organisms

Larvae of *Placidula euryanassa* (Felder & Felder) were collected from leaves of *Brugmansia suaveolens* (Wild.) Sweet (Solanaceae) in the region of São Bernardo do Campo, São Paulo State, Brazil, and reared in the laboratory on leaves of the host-plant. Fifth instar larvae, near the pre-pupal stage (without feces remaining in the gut), pupae and freshly-emerged adults were placed individually in vials with 2 ml MeOH and kept at −15°C until alkaloid analysis. Wild-caught adults were collected in other places (Ilha do Cardoso, São Paulo; Serra do Japi, Jundiai, São Paulo; Morro do Japuí, São Vicente, São Paulo; Mongaguá, São Paulo; Boca do Mato, Rio de Janeiro; Represa Suiça, Santa Leopoldina, Espirito Santo; Morema, Três Coroas, Rio Grande do Sul) and preserved as above. Eggs and larvae of *Miraleria cymothoe* (Hewitson) were collected in Mérida, Venezuela and wild adults were collected in the regions of Barinitas, La Parada (San Cristóbal) and Rio Frio, SW Venezuela, and San Bernardo (Norte de Santander) and Saladito (Valle), Colombia. Larvae were reared in the laboratory on leaves of *B. suaveolens*, and all stages of the life cycle were preserved in MeOH.

Extraction and purification of alkaloids

Leaves of *B. suaveolens*, pharate larvae (with empty gut), pupae, freshly emerged and wild caught adults, preserved in MeOH, were ground with Seasand Extra Pure (Merck) and centrifuged at 4,000 rpm for 10 min. This procedure was repeated 3 times and the methanolic supernatants were united and the MeOH was evaporated in an air stream. If colorimetric assay was done (see below) the dried methanolic extract was taken up in 2 ml MeOH and two aliquots of 100 ml each were removed for analysis of N-oxides and total alkaloid; the remaining methanolic extract (1.8 ml) was dried again. The residue was taken up in 2 ml 1 N H_2SO_4, extracted three times with 2 ml CH_2Cl_2 and the organic phase was set aside. Slightly modified according to the method given by Witte *et al.* (1993), the acid aqueous solution was reduced with Zn dust for 3 h, alcalinized with NH_4OH, applied on an Extrelut (Merck) column (1.4 g/ml) and eluted 10 times with 5 ml CH_2Cl_2. The organic extract was dried over anhydrous Na_2SO_4 and the solvent evaporated. The residue represented the total alkaloids as free bases.

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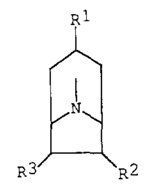


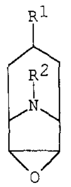
scopoline (1)
$M^{+}155\text{-}R^1 = R^2 = \text{-O-}; R^3 = OH$

3,6-dihydroxy-tropane (3)
$M^{+}157\text{-}R^1 = R^2 = OH; R^3 = H$

3-hydroxy-6-propionyl-oxytropane (4)
$M^{+}213\text{-}R^1 = OH; R^2 = O_2CC_2H_5; R^3 = H$

3-hydroxy-6-methyl-butenoyl-oxytropane (5)
$M^{+}239\text{-}R^1 = OH; R^2 = O_2CC_4H_7; R^3 = H$

3-hydroxy-6-methyl-butanoyl-oxytropane (6)
$M^{+}241\text{-}R^1 = OH; R^2 = O_2CC_4H_9; R^3 = H$



scopine (2)
$M^{+}155\text{-}R^1 = OH; R^2 = CH_3$

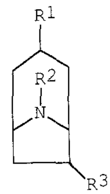
aposcopolamine (12)
$M^{+}285\text{-}R^1 = O_2CC = CH_2Ph; R^2 = CH_3$

dihydroaposcopolamine (13)
$M^{+}287\text{-}R^1 = O_2CCHCH_3Ph; R^2 = CH_3$

norscopolamine (16)
$M^{+}289\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = H$

scopolamine (18)
$M^{+}303\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = CH_3$

O-methyl-scopolamine (20)
$M^{+}317\text{-}R^1 = O_2CCHCH_2OCH_3Ph; R^2 = CH_3$



apoatropine (7)
$M^{+}271\text{-}R^1 = O_2CC = CH_2Ph; R^2 = CH_3; R^3 = H$

dihydroapoatropine (9)
$M^{+}273\text{-}R^1 = O_2CCHCH_2Ph; R^2 = CH_3; R^3 = H$

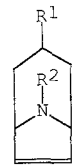
norhyoscyamine (11)
$M^{+}275\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = H; R^3 = H$

6-hydroxyapoatropine (14)
$M^{+}287\text{-}R^1 = O_2CCHCH_3Ph; R^2 = CH_3; R^3 = OH$

hyoscyamine (15)
$M^{+}289\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = CH_3; R^3 = H$

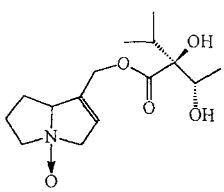
6-(*S*/*R*)-hydroxynorhyoscyamine (17)
$M^{+}291\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = H; R^3 = OH$

6-(*S*/*R*)-hydroxyhyoscyamine (19)
$M^{+}305\text{-}R^1 = O_2CCHCH_2OHPh; R^2 = CH_3; R^3 = OH$

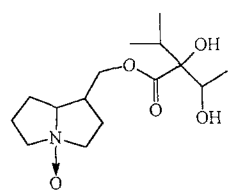


unknown (8)
$M^{+}271\text{-}R^1 = O_2CC_2H_4Ph; R^2 = CH_3$

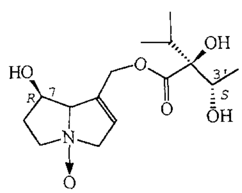
6,7-dehydronoratropine (10)
$M^{+}273\text{-}R^1 = O_2CC_2H_3OHPh; R^2 = H$



Amabiline N-oxide (21)
$M^{+}283$



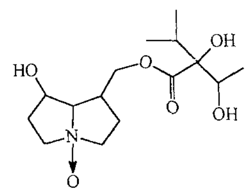
Viridiflorine N-oxide or isomers (22)
$M^{+}285$



Intermedine N-oxide (23)
$M^{+}299$. 7*R*,3′*R*

Lycopsamine N-oxide (24)
$M^{+}299$. 7*R*,3′*S*

Rinderine N-oxide (25)
$M^{+}299$. 7*S*,3′*S*



1,2-Dihydrointermedine N-oxide or isomers (26)
$M^{+}301$

Fig. 1 Tropane alkaloids (TAs) and pyrrolizidine alkaloids (PAs) found in *Brugmansia suaveolens*, *Placidula euryanassa* and *Miraleria cymothoe*

Colorimetric assay for pyrrolizidine alkaloids

Wild caught adults of *Placidula euryanassa* and *Miraleria cymothoe* were analysed quantitatively for N-oxides and total PAs (free bases + N-oxides) by a colorimetric assay modified from Mattocks (1967, 1968), Bingley (1968), Brown (1985) and Trigo *et al.* (1993). Butterflies were placed individually in vials with 2 ml of MeOH and two aliquots of one twentieth of the total liquid volume were taken up for assay.

GC-MS analysis

In the analyses done in Germany (Witte *et al.* 1993) the alkaloidal extracts were redissolved in MeOH and directly used for GC-MS analysis. A Carlo Erba Mega 5160 gas chromatograph equipped with a fused silica column (WCOT, 30 m × 0.32 mm, DB-1, J&W Scientific) was directly coupled to a quadrupole mass spectrometer Finnigan MAT 4515. Conditions: injector 250°C; temperature program 150–300°C (PAs) or 70–300°C (TAs), 6°C/min; split ratio 1:20, carrier gas He 0.5 bar. In the analyses done in Brazil the samples were analysed on a Hewlett Packard-5988 serie II gas-chromatograph with a capillary column (30 m × 0.25 mm Rtx-1, Crossbond 100% polysiloxane, Restek Co.) directly coupled to a selective mass detector Hewlett Packard 5970. Conditions: injection temperature 250°C; temperature program 150–300°C (PAs) or 70–300°C (TAs), 4°C/min; split ratio 1:100 carrier gas He 0.7 bar, 1 ml/min. RIs were calculated according to van den Dool & Kratz (1963) in both columns and the Rtx-1 RIs were corrected to the DB-1 RIs (DB-1 RIs = Rtx-1 RIs = 15). The fragmentation features of tropane and pyrrolizidine alkaloids (Table 5) were compared with those reported by Ethier & Neville (1986) and Witte *et al.* (1987, 1993).

Results

Tropane alkaloids

The tropane alkaloids were separated and identified by GC-MS from extracts of *P. euryanassa* and its larval food plant *B. suaveolens* from São Bernardo do Campo

Table 1 TAs[a] in *Brugmansia suaveolens* leaves and in the life cycle of *Placidula euryanassa*

Alkaloids	RI	M+	Relative abundance (%)				
			Larval food plant	*Placidula euryanassa*[b]			
				Larvae	Pupae	Freshly-emerged adults	
						males	females
scopoline (1)	1260	155	nd	3	2	1	nd
scopine (2)	1290	155	nd	1	1	nd	4
3,6-*dihydroxytropane* (3)	1365	157	nd	nd	1	nd	nd
3-*hydroxy*-6-*propionyl-oxytropane* (4)	1602	213	tr	nd	nd	nd	nd
3-*hydroxy*-6-*methyl-butenoyl-oxytropane* (5)	1785	239	5	nd	nd	nd	nd
3-*hydroxy*-6-*methyl-butenoyl-oxytropane* (5)	1825	239	6	nd	nd	nd	nd
3-*hydroxy*-6-*methyl-butenoyl-oxytropane* (5)	1836	239	6	nd	nd	nd	nd
3-*hydroxy*-6-*methyl-butanoyl-oxytropane* (6)	1730	241	1	nd	nd	nd	nd
apoatropine (7)	2032	271	tr	nd	tr	1	4
dihydroapoatropine (9)[c]	1956	273	nd	nd	nd	8	6
6,7-*dehydronoratopine* (10)[c]	2073	273	nd	nd	nd	1	nd
norhyoscyamine (1)	2165	275	nd	9	nd	nd	nd
aposcopolamine (12)	2131	285	nd	tr	4	9	4
dihydroaposcopolamine (13)[c]	2072	287	nd	3	3	35	39
6-*hydroxyapoatropine* (14)	2205	287	nd	nd	tr	5	8
hyoscyamine (15)	2170	289	51	7	9	23	23
norscopolamine (16)	2277	289	21	18	8	nd	nd
6*S*-*hydroxynorhyoscyamine* (17)	2318	291	nd	1	nd	nd	nd
6*R*-*hydroxynorhyoscyamine* (17)	2340	291	nd	6	nd	nd	nd
scopolamine (18)	2288	303	8	35	42	19	12
6*S*-*hydroxyhyoscyamine* (19)	2334	305	nd	3	2	nd	nd
6*R*-*hydroxyhyoscyamine* (19)	2355	305	nd	12	30	nd	nd

[a] see the alkaloid structures in Figure 1; **nd** not detected;
[b] from São Bernardo do Campo, São Paulo;
[c] tentatively identified (see also Table 5)

Table 2 Amounts of TAs in the life stages of *Placidula euryanassa* and in leaves of its larval food plant *Brugmansia* suaveolens from São Bernardo do Campo, São Paulo. Quantification by GC analysis

Stages	N	Dry weight/individual (mg)	Tropane[a]	
			μg/individuum	μg/mg dr.wt
larvae	311	20	63.00	3.150
pupae	51	43	31.00	0.720
freshly emerged adults	40	30	0.41	0.014

[a] pooled individuals

(Table 1, Fig. 1). They are present mostly as free bases. Leaves of *B. suaveolens* contain hyoscyamine (15), norscopolamine (16) and scopolamine (18) as major TAs. These TAs are also present in larvae and pupae of *P. euryanassa*, although the relative proportions are different (Table 1). Some TAs present in the host plant as minor components, such as the isomeric tropine 6-hydroxy esters (5), are not sequestered by insects. On the other hand, larvae and pupae (in part) contain a number of minor TAs which are not found in the food plant. These compounds, such as scopoline (1), scopine (2), dihydroaposcopolamine (13) and the isomeric 6-hydroxyhyoscyamines (19), might be degradation or transformation products from plant derived precursors.

In the population of São Bernardo do Campo, about half of the total TAs sequestered by larvae are transferred into the pupal stage (Table 2). But more than 98% of total TAs found in the pupae are lost with the emergence of adults. Nevertheless, TAs are always detectable in different concentrations in wild caught adults of *P. euryanassa* from different localities (Table 3).

All stages of the life cycle of *Miraleria cymothoe*, also feeding on *B. suaveolens*, were found to lack even trace amounts of TAs; the larval feces contained a wide variety of TAs.

Pyrrolizidine alkaloids

The larval host plant, as well as early stages and freshly-emerged adults of *P. euryanassa* and *M. cymothoe* were devoid of even traces of PAs. Wild-caught adults of both species, however, were found to contain lycopsamine (24) in most populations, with single occurrences of amabiline (21) and a 1,2-saturated PA (26) (Table 3, Fig. 1). PAs are predominantly present as N-oxides.

Different populations of *P. euryanassa* have a non-normal distribution of PAs in their tissues and show significant differences in amount of PAs (Kruskall-Wal-

Table 3 TAs and PAs[a] in populations of wild-caught *Placidula euryanassa* and *Miraleria cymothoe* adults

Alkaloids	RI	M^+	Relative abundance (%)							
			Placidula euryanassa							*Miraleria cymothoe*
			Ilha do Cardoso		Serra do Japi		Japuí		Morema[b]	La Parada
			M	F	M	F	M	F	F	M
TROPANE ALKALOIDS										
apoatropine (7)	2032	271	nd	nd	nd	nd	nd	1	nd	nd
unknown (8)[c]	2046	271	nd	nd	nd	nd	1	nd	nd	nd
dihydroapoatropine (9)[c]	1956	273	nd	nd	nd	nd	2	1	nd	nd
6,7-*dehydronoratopine* (10)[c]	2073	273	nd	nd	nd	nd	2	2	nd	nd
aposcopolamine (12)	2131	285	nd	14	nd	nd	nd	nd	nd	nd
dihydroaposcopolamine (13)[c]	2072	287	5	nd	16	2	6	3	nd	nd
6-*hydroxyapoatropine* (14)	2205	287	nd	nd	nd	nd	2	nd	nd	nd
hyoscyamine (15)	2170	289	8	23	12	20	16	13	nd	nd
norscopolamine (16)	2277	289	nd	nd	nd	nd	6	3	nd	nd
scopolamine (18)	2288	303	nd	nd	nd	nd	3	1	nd	nd
6*S*-*hydroxyhyoscyamine* (19)	2334	305	nd	nd	nd	nd	3	1	nd	nd
6*R*-*hydroxyhyoscyamine* (19)	2355	305	nd	nd	nd	nd	3	5	nd	nd
O-*methyl-scopolamine* (20)[c]	2251	317	4	nd	18	17	nd	nd	17	nd
Others (*unknown*)	–	–	nd	nd	1	1	1	nd	1	nd
PYRROLIZIDINE ALKALOIDS										
amabiline (21)	1984	283	nd	tr	nd	nd	29	11	nd	nd
viridiflorine or isomer (22)	1987	285	nd	nd	nd	nd	3	nd	nd	nd
intermedine (23)	2130	299	nd	nd	nd	nd	nd	4	nd	nd
lycopsamine (24)	2145	299	83	56	23	12	12	46	tr	100
rinderine (25)	2157	299	nd	nd	nd	nd	nd	1	nd	nd
1,2-*dihydrointermedine or isomer* (26)[c]	2152	301	nd	nd	nd	nd	nd	nd	36	nd

[a] see alkaloid structures in Figure 1;
[b] males were not analysed;
[c] tentatively identified (see also Table 5)

Table 4 Amount of PAs in wild-caught adults of *Placidula euryanassa* and *Miraleria cymothoe* from different localities. Quantification of PAs by colorimetric analysis, and TAs by GC

Species/Locality	PAs (μg/individual)			Tropane/PA ratio[a]
	N	$X \pm S$	Min-Max	
Placidula euryanassa				
Males – Boca do Mato, RJ	02	–	2, 16	nc
Females – Boca do Mato, RJ	01	–	5	nc
Both sexes – Ilha do Cardoso, SP	15	76 ± 65	17–266	nc
Males	10	91 ± 72	18–226	0.20
Females	05	47 ± 39	17–101	0.78
Both sexes – Serra do Japi, SP	36	12 ± 18	0–84	nc
Males	20	13 ± 16	0–54	3.39
Females	16	11 ± 20	1–84	7.51
Mongaguá, SP – Males	01	–	148	nc
Mongaguá, SP – Females	02	–	1, 26	nc
Both sexes – Morema, Três Coroas, RS	40	7 ± 5	1–21	nc
Males	23	7 ± 6	2–21	nc
Females	17	6 ± 4	1–15	1.80
Males and females – Represa Suiça, Santa Leopoldina, ES	02	–	106, 242	nc
Miraleria cymothoe				
Males – La Parada, Venezuela	12	24 ± 20	4–73	0
Males – Barinitas, Venezuela	02	–	4, 53	nc
Females – Rio Frio, Venezuela	02	–	2, 5	nc
Male – San Bernardo, Colombia	01	–	5	nc
Male – Saladito, Colombia	01	–	840	nc
Females – San Bernardo, Colombia	03	–	83, 230, 305	nc

[a] nc: not calculated

Table 5 MS-EI fragmentation of TAs and PAs found in plants and butterflies[a]

Tropane alkaloids	m/z (relative abundance, %)
scopoline (1)	$[M]^+$ **155** (29), 138 (3), 126 (14), 110 (9), 98 (10), 97 (8), 96 (60), 94 (34), 81 (34), 70 (20), 57 (43), 42 (100)
scopine (2)	$[M]^+$ **155** (24), 138 (4), 126 (5), 110 (23), 98 (3), 97 (2) 96 (4), 94 (17), 86 (7), 84 (8), 82 (7), 70 (10), 68 (12) 57 (41), 42 (100)
3,6-*dihydroxytropane* (3)	$[M]^+$ **157** (21), 140 (6), 113 (100), 98 (10), 96 (71), 94 (19), 84 (13), 82 (17), 70 (14), 57 (46), 44 (24), 42 (74)
3-*hydroxy*-6-*propanoyl-oxytropane* (4)	$[M]^+$ **213** (12), 140 (10), 122 (11), 113 (100), 98 (6), 96 (39), 94 (29), 84 (5), 82 (12), 70 (6), 57 (19), 44 (13) 42 (33)
3-*hydroxy*-6-*methyl-butenoyl-oxytropane* (5)	$[M]^+$ **239** (13), 156 (7), 140 (6), 122 (10), 113 (100), 96 (31), 94 (29), 83 (19), 70 (6), 57 (10), 55 (12), 42 (26)
3-*hydroxy*-6-*methyl-butanoyl-oxytropane* (6)	$[M]^+$ **241** (7), 156 (10), 140 (12), 122 (10), 113 (100), 96 (30), 94 (21), 82 (9), 70 (5), 57 (17), 42 (23),
apoatropine (7)	$[M]^+$ **271** (10), 140 (7), 124 (100), 103 (13), 96 (39), 94 (23), 83 (29), 82 (37), 77 (6), 67 (14), 42 (22),
unknown (8)[a]	$[M]^+$ **271** (2), 247 (27), 122 (100), 121 (58), 120 (56)
dihydroapoatropine (9)[a]	$[M]^+$ **273** (9), 140 (10), 124 (100), 105 (13), 96 (10), 94 (14), 83 (26), 82 (26), 67 (10), 42 (7)
6,7-*dehydronoratropine* (10)	$[M]^+$ **273** (3), 140 (9), 122 (100), 105 (46), 94 (28), 80 (55), 67 (9), 55 (8), 42 (9)
norhyoscyamine (11)	$[M]^+$ **275** (3), 136 (3), 110 (100), 103 (7), 91 (6), 80 (17), 68 (16), 55 (3), 42 (7)
aposcopolamine (12)	$[M]^+$ **285** (40), 154 (54), 138 (71), 136 (46), 120 (14), 108 (49), 103 (56), 94 (100), 81 (17), 77 (21), 68 (10), 57 (15), 42 (71)
dihydroaposcopolamine (13)[a]	$[M]^+$ **287** (23), 154 (48), 138 (74), 136 (41), 120 (13), 108 (50), 105 (48), 138 (74), 136 (41), 120 (13), 108 (50), 105 (50), 94 (100), 42 (40)
6-*hydroxyapoatropine* (14)	$[M]^+$ **287** (12), 243 (10), 140 (24), 110 (5), 103 (16), 93 (12), 94 (100), 77 (8), 42 (25)
hyoscyamine (15)	$[M]^+$ **289** (20), 140 (12), 124 (100), 103 (6), 96 (11), 94 (19), 83 (24), 82 (24), 67 (12), 42 (20)
norscopolamine (16)	$[M]^+$ **289** (3), 140 (5), 124 (31), 123 (42), 122 (100), 105 (16), 103 (22), 94 (45), 80 (57), 67 (13), 55 (10), 42 (20)
6-*hydroxynorhyoscyamine* (17)	$[M]^+$ **291** (1), 247 (6), 126 (18), 103 (10), 81 (96), 80 (100)
scopolamine (18)	$[M]^+$ **303** (23), 154 (50), 138 (90), 136 (45), 120 (26), 108 (51), 103 (21), 96 (24), 94 (100), 81 (20), 57 (24),) 42 (77)
6-*hydroxyhyoscyamine* (19)	$[M]^+$ **305** (8), 261 (12), 140 (37), 103 (7), 95 (92), 94 (100)
O-methyl-scopolamine (20)[a]	$[M]^+$ **317** (76), 286 (14), 261 (8), 154 (59), 138 (65).
amabiline (21)	$[M]^+$ **283** (<1), 268 (<1), 239 (<1), 140 (7), 123 (26), 122 (100), 121 (36), 120(45), 108 (14), 93 (20), 80 (11), 70 (10), 53 (9), 43 (21).
viridiflorine or isomers (22)	$[M]^+$ **285** (<1), 267 (3), 252 (2), 241 (1), 240 (3), 226 (1), 142 (51), 124 (100), 83 (26), 70 (9), 55 (23), 43 (16)
intermedine and isomers (23, 24, 25)	$[M]^+$ **299** (<1), 255 (<1), 254 (<1), 156 (7), 139 (35), 138 (97), 120 (9), 108 (3), 94 (63), 93 (100), 80 (18), 67 (13), 53 (8), 43 (30).
1,2-*dihydrointermedine or isomers* (26)[a]	$[M]^+$ **301** (not observed), $[M]^+$-H_2O 283 (3), 268 (3), 258 (1), 257 (2), 256 (3), 240 (6), 158 (75), 140 (35), 120 (12), 122 (14), 114 (20), 97 (21), 96 (34), 95 (51), 82 (100), 70 (6), 69 (6), 55 (13).

[a] compounds 8, 9, 10, 13, 17, 20 and 26 are tentatively identified

lis test, $P < 0.01$, Table 4). In several populations with samples of $n \geq 8$, comparison between sexes showed no significant differences in the amount and concentration of PAs (Table 4). The ratio of tropanes/PAs was also variable among *Placidula* populations (Table 4).

Discussion

Analysis by GC-MS of alkaloidal fractions of tissues of *P. euryanassa* free from intestinal contents reveals a great variety of TAs between stages of the life cycle and populations of wild-caught adults; these are almost surely derived from the tropane-rich larval host plant *B. suaveolens* (Tables 1–3). The qualitative variability among stages could result from metabolic transformation of host-plant alkaloids, and the qualitative variability among populations could be a result of alkaloidal variability in host-plants. Experiments with labelled TAs are being undertaken to clarify the qualitative and quantitative uptake of these alkaloids.

On the other hand, larvae, pupae and adults of *Miraleria cymothoe* do not show any traces of stored TAs, but large amounts appear in the frass.

These results show that *Placidula* has its own specific alkaloid acquisition syndrome, which is different from that of other previously studied Ithomiinae (see Brown *et al.* 1991). The larvae of a number of primitive ithomiines feed on plants containing TAs and are aposematic, while adults store variable amounts of PAs from plant sources. *Placidula* is one case in this group: the gregarious larvae are moderately aposematic in color and behavior (Freitas 1993; Brown & Freitas 1994) and the adults store variable, usually small amounts of PAs (see Table 4). The storage of TAs by *Placidula* larvae leads to their unpalatability to vertebrate predators: preliminary trials showed a strong rejection of these by chickens and monkeys (J. R. Trigo & A. V. L. Freitas unpubl.). In contrast, the orbweaving spider *Nephila clavipes*, which rejects PA-insects, accepted and fed on tropane-containing freshly emerged adults and also palatable prey painted with 200 μg of atropine (J. R. Trigo & A. V. L. Freitas unpubl.)

The capacity of storing tropane alkaloids from the host-plant is probably a primitive trait in Ithomiinae, since primitive genera like *Tellervo* and *Tithorea* store PAs from their host-plants and even advanced Ithomiinae can store PAs if they are fed to the larvae (Trigo & Motta 1990; Trigo *et al.* 1996a; Orr *et al.* 1996). In these primitive taxa, larvae bear an aposematic pattern (chemical protection probably begins after feeding), while other ithomiine larvae, probably not thus protected, show a cryptic pattern. The latter case includes *Miraleria* (Brown & Freitas 1994); although this species feeds on *Brugsmansia suaveolens* as larval host-plant, no sequestration or storage of these alkaloids was observed, and the larvae are cryptic. The variable concentration of PAs and the variable ratio TAs/PAs in adults of *Placidula* (Table 4) could be related to the availability of PA sources for different populations and the dynamics of incorporation, accumulation and utilization of PAs by adults of ithomiines (Trigo & Brown 1990); the concentration of TAs in larval host-plant also could have a role in the variable ratios of TAs/PAs. However, the variable concentration of PAs could be also related to the observed low attraction of adults of *Placidula* to PA sources (although in Morro de Japuí a reasonable attraction was observed, with a male bias; A. V. L. Freitas unpubl.), since TAs would provide some chemical protection for freshly emerged adults against predators (but not *Nephila*). This low attraction is also observed in *Tithorea harmonia*, that sequesters PAs from the larval host plant, and likewise is not always protected against *Nephila* as freshly emerged adult (Pliske 1975; Trigo & Brown 1990; Trigo *et al.* 1996a).

Lycopsamine is the main PA found in both *Placidula* and *Miraleria*, as in all other genera of Ithomiinae analysed (Trigo *et al.* 1994, 1996b). This PA can be sequestered without modification from plant sources or be produced by stereochemical inversion of its isomers also present in plant sources (Trigo *et al.* 1994).

Other intermediate syndromes are to be expected in the primitive Ithomiinae genera *Methona*, *Athesis*, *Melinaea* and *Olyras*, all Solanaceae-feeders with aposematic larvae. These taxa need detailed studies on the immautre biology, adult behavior and chemistry in order to understand better the evolutionary history of chemical defense in the Ithomiinae.

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