Pyrrolizidine Alkaloids from *Heliotropium megalanthum*

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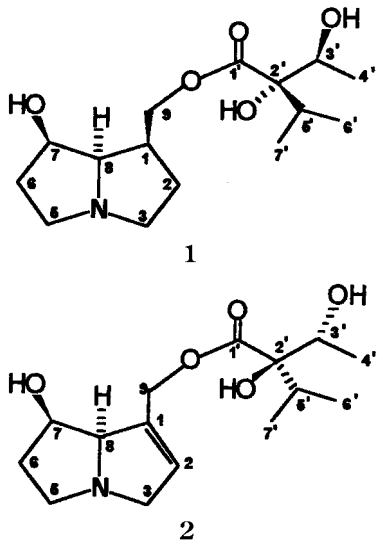
Two pyrrolizidine alkaloids, megalanthonine (**1**) and lycopsamine (**2**), have been isolated from *Heliotropium megalanthum*. The structure of the novel compound **1** was determined by spectroscopic methods. The insecticidal, antifeedant, and antifungal effects of compounds **1** and **2** have been evaluated.

The genus *Heliotropium* (Boraginaceae) is known to be a source of pyrrolizidine alkaloids which exhibit a number of biological activities.[1−3] This has promtped us to carry out phytochemical research on *Heliotropium megalanthum*, a shrub which grows in the northern part of Chile.[4] Here we describe the separation and structural elucidation of megalanthonine (**1**), a new saturated pyrrolizidine monoester alkaloid, along with the already known compound lycopsamine (**2**) (Chart 1). We have also studied the antifeedant, insecticidal, and fungicidal effects of **2** and the *H. megalanthum* alkaloidal fraction against the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), the lepidopteran *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae), and the plant pathogen *Fusarium moniliforme* (Sheld).

Column chromatography and preparative TLC on silica gel of the alkaloidal extract of *H. megalanthum* provided two alkaloids. Megalanthonine (**1**) was isolated as an oil, and its IR spectrum showed bands at 3418 cm^{-1} (OH) and 1734 cm^{-1} (ester). The HREIMS gave the molecular ion peak at *m*/*z* 301.1864 (2.2%) corresponding to the molecular ion $C_{15}H_{27}NO_5$ (calcd 301.1889), along with major fragmentation ions at *m*/*z* 158.1185, $C_8H_{16}NO_2$ (calcd 158.1181), *m*/*z* 140.1027 $C_8H_{14}NO$ (calcd 140.1075), *m*/*z* 95.0701, C_6H_9N (calcd 95.0734), and the base peak at *m*/*z* 82.0618, C_5H_8N (calcd 82.0656), which are characteristic of saturated pyrrolizidine monoester alkaloids with a C_7 acid esterified at position C-9-O and a hydroxyl group on C-7.[5,6]

The ^{1}H NMR spectrum of **1** showed signals at δ_H 0.83 (d, J = 6.8 Hz, H-7′), 0.93 (d, J = 6.8 Hz, H-6′), 2.08 (m, H-5′), 3.98 (q, J = 6.8 Hz, H-3′), and 1.33 (d, J = 6.7 Hz, H-4′), attributed to two isopropyl methyl groups, two methine signals, and one methyl signal, respectively, corresponding to the necic acid part of the alkaloid. These protons were correlated with the carbon signals at δ_C 17.8 (q, C-7′), 15.6 (q, C-6′), 32.0 (d, C-5′), 72.1 (d, C-3′), and 17.0 (q, C-4′) in the HMQC experiment (Table 1), and are in agreement with **1** having a (2′*R*, 3′*R*)-(−)-viridiflorate ester unit ($\Delta\delta H_2$-9 = 0.37 and $\Delta\delta$C-6′/C-7′ = 2.2).[7−9] Additionally, three methine protons were observed at δ_H 4.29 (br t, J = 3.6 Hz), 3.48 (dd, J = 7.8, 4.0), and 2.78 (m). The downfield signal could be attributed to a proton geminal to the hydroxyl group. The double doublet was assigned to H-8 and the multiplet at δ_H 2.78 was characteristic of H-1 in saturated pyrrolizidine alkaloids with esterified platynecine type bases.[10] These signals were

Chart 1



shown to be coupled to each other by a homonuclear ^{1}H−^{1}H COSY experiment, and were correlated with the carbon signals at δ_C 69.5 (d), 72.8 (d), and 36.0 (d) by a HMQC experiment. Therefore, we could assign the ^{13}C NMR signals to C-7, C-8, and C-1, respectively. The HMBC experiment confirmed those assignments. The remaining methylene protons were assigned by HMQC, HMBC, and ^{1}H−^{1}H COSY experiments (Table 1).

The necine base was identified as platynecine by spectroscopic methods, and by comparing the chemical shifts, the coupling constants of the protons of the asymmetric centers and the values of $\sum J_7$ of **1**, (δ_H 3.48 dd, J = 7.8, 4.0 Hz, H-8, δJ_7 = 6.3 Hz), with those of the saturated pyrrolizidine alkaloids, sarracine, and 9-angelylplatynecine (δ_H 3.69 dd, J = 8.0, 3.7 Hz, H-8, $\sum J_7$ = 6.5 Hz) and (δ_H 3.52 dd, J = 8.0, 2.7 Hz, H-8, $\sum J_7$ = 6.4 Hz), respectively.[10] Conclusive evidence of the stereochemistry of alkaloid **1** was provided by an alternative of the 1D version of the NOESY experiment, the so-called GOESY 1D experiment, with pulsed-field gradients (PFG).[11] A selective excitation at δ_H 3.48 (dd, H-8) gave a quick and clean 1D spectrum, with only the protons having positive NOE [δ_H 2.78 (m, H-1α), 4.29 (brt, H-7α), and 4.09 (dd, H-9u)] being observed. The point of attachment of the ester at C-9 was determined by an HMBC experiment with pulsed-field gradients (PFG).[12,13]

Alkaloid **2** was isolated as oil. Its mass spectrum showed a molecular ion at *m*/*z* 299 with a base peak at 138 and the typical fragmentation of an 1,2-unsaturated necine esterified at C-9 such as retronecine and heliotridine.[14] The

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Table 1. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR Data of Megalanthonine (**1**)

proton	δ (J_{H-H} in Hz)	COSY	correlated carbon HMQC	correlated carbon HMBC
1α	2.78 m	H-8,H-9u,H-9d,H-2α,H-2β	36.0 d	
2α	1.89 m	H-2β,H-3a,H-3β,H-1α	31.2 t	
2β	2.15 m	H-2α,H-3β	31.2 t	
3a	3.26 m	H-2α,H-3β	51.1 t	C-1,C-8
3β	2.61 ddd (10.8,8.6,6.0)	H-2α,H-2β,H-3a	51.1 t	C-1,C-5
5α	3.21 m	H-5β,H-6α,H-6β	54.6 t	C-8
5β	2.73 m	H-5α,H-6α,H-6β	54.6 t	C-3
6α	2.03 m	H-5α,H-5β,H-6β,H-7a	36.8 t	
6β	2.14 m	H-5α,H-5β,H-6β,H-7a	36.8 t	
7a	4.29 br t (3.6)	H-6α,H-6β,H-8	69.5 d	C-1,C-8
8	3.48 dd (7.8, 4.0)	H-1α,H-7a	72.8 d	C-5,C-7
9u	4.09 dd (10.8, 7.8)	H-1α,H-9d	66.2 t	C-1,C-2,C-8,C-1′
9d	4.46 dd (10.8, 4.5)	H-1α,H-9u	66.2 t	C-1,C-2,C-8,C-1′
			174.5 s (C-1′)	
			84.1 s (C-2′)	
3′	3.98 q (6.8)	H-4′	72.0 d	C-1′,C-2′,C-4′
4′	1.33 d (6.7)	H-3′	17.0 q	C-2′,C-3′
5′	2.08 m	H-6′,H-7′	32.0 d	
6′	0.93 d (6.8)	H-5′	15.6 q	C-2′,C-5′,C-7′
7′	0.83 d (6.8)	H-5′	17.8 q	C-2′,C-5′,C-6′

Table 2. Antifeedant Effects of the Alkaloidal Fraction of *H. megalanthum* and Compound **2** on *S. littoralis* L6 Larvae and *L. Decemlineata* Adults

treatment	dose (μg/cm^2)	%FR[a] (average ± SE) S. littoralis choice	*L. decemlineata* choice	*L. decemlineata* no-choice
alkaloid Fraction	100.0	44.32 ± 27.50	21.00 ± 19.75	69.00 ± 17.98
2	100.0	61.08 ± 13.06	27.53 ± 10.30	68.40 ± 14.31
EC_{50} (95% C.I.)[b]		31.62 (27.69, 35.95)	−	25.97 (24.62, 27.32)
Eu[c]	5.0	67.00 ± 4.7	−	−
At[d] EC_{50} (95% C.I.)[b]		−	1.79 (0.93, 3.42)	2.92 (0.57, 17.78)

[a] Percent feeding reduction: %FR = 1 − (T/C) × 100, where T = consumption of treated leaf disks and C = consumption of control leaf disks. [b] Concentration required to give a feeding reduction of 50% (95% C.I., confidence intervals). [c] Eu, europine.[17] [d] At, 3′-acetyltrachelanthamine.[9]

^{1}H and ^{13}C NMR spectra confirmed the pyrrolizidine base as (+)-retronecine esterified with (−)-viridifloric acid, and compound **2** was therefore identified as lycopsamine.[15]

The antifeedant assays showed that the alkaloidal fraction of *H. megalanthum* had a moderately significant effect on *L. decemlineata* in no-choice tests (% FR > 60) and a lesser effect on *S. littoralis* (Table 2). The major alkaloid present in this fraction, compound **2**, showed a moderately significant antifeedant effect on both insect species in choice and no-choice assays, respectively (Table 2). Compound **1** could not be tested due to the limited amount available. The pyrrolizidine alkaloid europine and 3′-acetyltrachelanthamine have been included as reference compounds.

Table 3 shows the results of the oral and abdominal injection of the alkaloid fraction of *H. megalanthum* **1** and **2** on the target insects. *S. littoralis* consumption and growth rates (RCR and RGR) were not affected by any of the test substances. However, *L. decemlineata* mortality significantly increased when injected with the alkaloid fraction of *H. megalanthum*.

An antifungal test performed showed that the alkaloidal fraction of *H. megalantum* did not have any significant effect on *F. moniliforme* (19% mycelial growth inhibition, data not shown).

Antifeedants are gaining importance as potential components of Integrated Pest Management strategies for insect control. Unsaturated pyrrolizidine alkaloids have been reported as having antifeedant effects on polyphagous and oligophagous Lepidopterans.[16] Furthermore, europine, structurally analogous to lycopsamine, showed antifeedant effects on *S. littoralis*.[17] The saturated pyrrolizidine alkaloid 3′-acetyltrachelanthamine, however, has been reported as a strong Colorado potato beetle antifeedant without affecting the feeding behavior of *S. littoralis*.[9] Here we have shown that the unsaturated pyrrolizidine alkaloid **2** has similar although moderate antifeedant effects on both insect species tested, suggesting a broader and less specific action for this compound. Furthermore, **2** was more effective on Colorado potato beetle in no-choice than in choice tests, indicating a deterrent effect without associated behavioral avoidance for this insect.

This is the first report on the insect antifeedant effects of lycopsamine (**2**). Other biological effects mediating insect−plant interactions have also been attributed to this compound. It plays a role in the sexual communication of

Table 3. Oral and Haemolymph Injection Effects of the Alkaloidal Fraction of *H. megalanthum* and Compounds **1** and **2** on *S. littoralis* L6 Larvae (72 h, RCR and RGR) and *L. decemlineata* Adults (2 days % mortality)

treatment	*S. littoralis* N[c]	RCR[a]	RGR[b]	*L. decemlineata* N[c]	% mortality[d]
control	25	19.44 ± 1.10	3.30 ± 0.27	25	0
alkaloid fraction	18	18.04 ± 1.55	2.66 ± 0.41	19	29[e]
1	n.a.[f]	−	−	19	10
2	18	21.19 ± 0.97	3.32 ± 0.23	22	12
At[g]	−	−	−	20	5

[a] RCR = I/(BI) × T, I = mg food consumed, T = feeding period (days), BI = initial insect weight (mg). [b] RGR = DB/(BI) × T, DB = change in insect body weight (mg). [c] Number of insects. [d] Corrected according to Abbott (1925). [e] $P < 0.05$, Fisher's exact test for 2 × 2 tables. [f] Insufficient compound available for this test. [g] From ref 9.

adult itomiidae, while a defensive role has been proposed for its presence in adults of the grasshopper *Zonocercus variegatus* and the arctiid moth *Hyalurga syma*.[18−20]

It has been suggested that toxic pyrrolizidine alkaloids are bioactivated via similar pathways in mammalian liver and insect cells.[21] However, we did not observe any toxic effects of the antifeedant **2** on *S. littoralis* or Colorado potato beetle at the doses tested. This lack of a relationship between deterrency and toxicity has been noted for a broad selection plant allelochemicals.[22] The lack of toxicity of **1** on *L. decemlineata* corresponds to the low toxicity attributed to saturated pyrrolizidine alkaloids,[23] while the toxicity of the alkaloidal fraction on this insect may be attributable to the presence of minor toxic components.

In conclusion, pyrrolizidine alkaloids **1** and **2** have been isolated from *H. megalantum*. Alkaloid **1** is a new saturated pyrrolizidne alkaloid monoester based on the necine base platynecine, with a hydroxyl group at C-7β and a (2′*R*,3′*R*)-(−)-viridifloric acid at C-9, while **2** is the known alkaloid lycopsamine. Antifeedant assays showed that **2** has a similar moderate effect on two insect species, *S. littoralis* and *L. decemlineata*, without associated toxicity.

Experimental Section

General Experimental Procedures. Optical rotations were measured in EtOH, on a Perkin-Elmer 137 polarimeter. IR spectra were obtained on KBr disks on a Bruker IFS66V spectrometer. NMR spectra were obtained in $CDCl_3$ on a Bruker AMX2 500 MHz spectrometer with pulsed field gradient. The programs used for DEPT, ^{1}H COSY, HMQC, HMBC (J = 7 Hz), and GOESY (mixing time, τ_M = 600 ms) experiments were those furnished in the Bruker software. MS were recorded on an Autospec instrument at 70 eV. Silica gels Merck Art. 9385 and 5717 were used for column chromatography and preparative TLC, respectively. Visualization was effected with Dragendorff's reagent.

Plant Material. *H. megalanthum*[24] was collected in October, 1991, from the north of Chile (Atacama, III Region) and identified by Dr. Sebastian Teillier from Museo de Historia Natural de Santiago. A voucher specimen is deposited in the herbarium of this museum, with the number ST 2569.

Extraction and Isolation. A portion (700 g) of dried and pulverized aerial parts of *H. megalanthum* were exhaustively extracted as described in previous work.[9] The crude alkaloid fraction (2.71 g, 0.38%) was chromatographed on a silica gel column. Elution was carried out with $CHCl_3$ and MeOH mixtures of increasing polarity. The eluate obtained with $CHCl_3$−MeOH (7:3) gave lycopsamine (**2**) and that with $CHCl_3$−MeOH (65:35) a mixture of lycopsamine (**2**) and megalanthonine (**1**). Further purification using preparative TLC over silica gel (20 cm × 20 cm, 2.0 mm) eluted with $CHCl_3$−MeOH−NH_3 (8:2:0.5) afforded lycopsamine (**2**) (48.0 mg) and megalanthonine (**1**) (22.2 mg).

Megalanthonine (1). This compound was obtained as oil; $[\alpha]_D$ −5° (*c* 0.04, EtOH); IR (KBr) ν_{max} 3418, 2960, 2928, 2874, 2857, 1734, 1579, 1464, 1384, 1233, 1158, 1073, 1010, 900, 833 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 301 (2), 283 (9), 256 (11), 240 (8), 212 (19), 158 (86), 141 (15), 140 (43), 138 (14), 124 (10), 122 (18), 114 (13), 97 (18), 96 (44), 95 (62), 83 (15), 82 (100) and 55 (32); HREIMS *m*/*z* $[M]^+$ 301.1864, calcd for $C_{15}H_{27}NO_5$ 301.1889.

Lycopsamine (2). This alkaloid was isolated as oil; $[\alpha]_D$ +2.5 (EtOH) [lit. $[\alpha]_D$ +5.7 (EtOH)],[25] and exhibited comparable spectral data (IR, ^{1}H NMR, ^{13}C NMR, EIMS) to published values.[15]

Insect Bioassays. *L. decemlineata* and *S. littoralis* colonies were reared on potato foliage and artificial diet,[26] respectively, and maintained at 24 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 h (L:D) in a growth chamber.

Choice Feeding Assays (≤6 h): These experiments were conducted with adult *L. decemlineata* and newly emerged sixth-instar *S. littoralis* larvae as described by González-Coloma et al.[27,28] The relative potencies of the test compounds (EC_{50} values, the effective dose for 50% feeding reduction) were determined by standard regression analysis (%FR on log dose).

Oral Cannulation: This experiment was performed with preweighed newly emerged *S. littoralis* L6-larvae (average wt 400 mg). The relative consumption rate (RCR) and the relative growth rate (RGR) were calculated on a dry weight basis according to Farrar et al.[29] All dry larval weight measures were log-transformed prior to an ANOVA analysis to test for treatment effects.

Haemolymph Injection: Adult *L. decemlineata* beetles (average weight 130 mg) were injected with the test compounds as described in previous work.[30] Toxicity symptoms and mortality were recorded up to 2 days. Percent mortality was analyzed with contingency table analysis and corrected according to Abbott.[31]

Antifungal Activity Assays. The antifungal activity of the alkaloids was tested at a single dose (0.5 mg/mL) against the plant pathogen *F. moniliforme*, and estimated as mycelial growth inhibition.[9,28] This pathogen was selected because we have shown that other *Heliotropium* alkaloids significantly inhibited its mycelial growth.[9]

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