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ISO-LYCOPSAMINE, A PYRROLIZIDINE ALKALOID FROM *HELIOTROPIUM KERALENSE*

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Key Word Index—*Heliotropıum keralense*, Boragınaceae, pyrrolızıdıne alkaloıds; *ıso*-lycopsamıne, 1,2-dehydro-1-hydroxymethyl-7β-(+)-vırıdıfloroxy-8α-pyrrolızıdine; ıntermedine, retronecıne

Abstract—The pyrrolızıdıne alkaloıds of *Heliotropium keralense* have been ısolated and characterized. They are *ıso*-lycopsamine, a new pyrrolızıdıne ester alkaloid, ıntermedıne and retronecine. *iso*-Lycopsamine was formulated as 1,2-dehydro-1-hydroxymethyl-7β-(+)-vırıdıfloroxy-8α-pyrrolızıdıne on the basıs of spectroscopic measurements and hydrolysis.

INTRODUCTION

The toxic properties of many pyrrolizıdıne alkaloids are well-known [1–7]. Although they have been isolated from representatives of about 13 plant famılies they occur most abundantly in the large genera *Senecıo* (Compositae) and *Crotalaria* (Fabae) and ın many members of Boragınaceae including *Heliotropium* More recently they have been detected in Lepıdoptera [3–7].

Heliotropium keralensıs ıs a new species found ın southern Kerala State, India [8]. Morphologically it is similar to *H. indıcum* but dıffers from ıt in its prominently white smaller flowers, shorter and differently shaped corolla tube and profuse long setose bulbous-based hairs on the calyx and corolla tube [8, 9]. *Heliotropium indicum* ıs toxıc to animals due to the presence of two unsaturated pyrrolizıdıne ester alkaloıds, indıcıne (**1**) and acetylindıcine (**2**) [10], whıle *H. keralense* has been used as a medicinal herb and as a substitute for *H. indıcum* ın South Indıa. Hence a systematıc ınvestıgation of *H. keralense* was undertaken to evaluate ıts toxicity and to see whether it was chemically dıstınguishable from *H ındıcum* Thıs has led to the isolatıon of *ıso*-lycopsamıne (**3**), a new pyrrolizidıne ester alkaloid, as well as intermedıne (**4**) and retronecine (**5**).

RESULTS AND DISCUSSION

Trıal experiments showed that *H. keralense* collected ın the flowering season (July) gave optımal yields of alkaloids (0.05% from aerial parts, 0 04% from roots). The yield was less than from *H ındıcum* [7]. Extraction of the whole plant, fractıonatıon and purificatıon afforded three homogeneous basıc substances. The first base, *ıso*-lycopsamıne, a previously unreported ısomer of lycopsamıne (**6**), was obtaıned as a pale yellow gum (0.066% of dry wt); $[\alpha]_D^{20°}$ + 19.5° (EtOH). High resolution mass spectrometry revealed the molecular formula, $C_{15}H_{25}O_5N$, the IR spectrum ($CHCl_3$) exhıbited intense bands at 3400 cm^{-1} (OH), 1660 cm^{-1} (C=C), and a carbonyl band at 1720 cm^{-1} reminıscent of an unsaturated pyrrolızıdıne ester alkaloid.

Consistent wıth the IR spectrum the 270 MHz ^{1}H NMR spectrum of *ıso*-lycopsamıne measured ın $CDCl_3$ (Table 1) exhıbıted signals characterıstic of a 1,2-dehydropyrrolizidınedıol esterıfıed wıth vırıdıfloric acid. Doublets at δ0.89, 0 96 (each 3H, J = 7 Hz, C-5′ methyls) and δ1 21 (3H, J = 6.5 Hz, C-3′-methyl), a one proton septet at δ1 87 (J = 7 Hz, H-5′) and a one proton quartet at δ4.00 (J = 6.5 Hz, H-3′), were characterıstic of an ester of vırıdıfloric acid [11]. All assignments were confirmed

Table 1 ^{1}H NMR spectrum of compound **3** (270 MHz)

H	$CDCl_3$	$CDCl_3 + D_2O$
2	5 69 *br s*	5 70 *br s*
3a	~4 00	3 96 *br d* (15 5)
3b	~3 45 *m*	~3 40 *m*
5a	~3 45 *m*	~3 40 *m*
5b	2 72 *ddd* (11 5, 10 7)	2 68 *ddd*
6a	2 18 *m* (14, 10, 3 5)	2 15 *m*
6b	2 11 *br dd* (14, 6 5)	2 07 *br dd*
7	5 56 *br t* (3)	5 53 *br dd* (3, 2 5)
8	4 38 *m*	4 26 (obsc)
9a	4 29 *br d* (15)	4 29 *br d*
9b	4 03 *br d* (15)	4 03 *br d*
3′	4 03 *q* (6 5)	4 04 *q*
4′*	1 21 *d* (6 5)	1 31 *d*
5′	1.87 *sept* (7)	1 87 *sept*
6′*	0 96 *d* (7)	0 96 *d*
7′*	0 89 *d* (7)	0 89 *d*
-OH	3 39 *br*	
	3 30 *br*	

*Intensity three protons

Table 2 ^{13}C NMR spectrum of compound **3** (67 89 MHz, $CDCl_3$)*

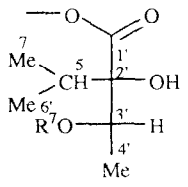
C		C	
1	139 13 *s*	1	174 10 *s*
2	123 25 *d*†	2′	82 83 *s*
3	63 01 *t*†	3′	69 40 *s*†
5	53 44 *t*†	4	16 55 *q*†
6	34 90 *t*†	5	33 38 *d*†
7	76 36 *d*	6	17 21 *q*†
8	76 50 *d*	7′	17 27 *q*†
9	59 50 *t*†		

*Multiplicities by DEPT pulse sequence

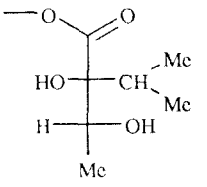
†Assignment by heteronuclear decoupling

by spin decoupling. The acid moiety esterified a secondary hydroxyl on C-7 of the necine base as the spectrum exhibited a somewhat broadened doublet of doublets at δ5 53 (J = 3, 2 5 Hz, H-7) characteristic of a proton under an acyloxy residue Signals of two mutually coupled protons of a methoxyl group (H-9a,b), broadened by allylic coupling to vinylic H-2 of the unsaturated necine base at δ5 70 (*quintet*, J = 1 Hz), were observed near δ4 38 and 3 95, these were partially superposed on the broadened singlet of H-8 at δ4.26, and the broadened doublet of H-3a at δ4 0 The latter was in turn coupled to H-3b at δ3.41 which was superposed on the H-5a resonance of δ3 38 The signal of H-5b, a *ddd* (J = 11, 9 5, 7 Hz) appeared at δ2 68 while the strongly coupled system of H-6a,b (J's = 14, 10, 3 5, 3 5 and 14,7 Hz, respectively) was centred at δ2.12 Chemical shifts and coupling constants of the necine moiety were consonant with those of a 7-ester of retronecine [1] Hence, *iso*-lycopsamine was one of the two possible stereoisomers of 7-*O*-viridiflorylretronecine

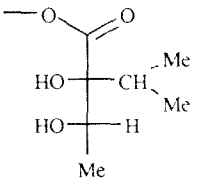
Corroborative evidence for structure **3** was obtained from the ^{13}C NMR spectrum (Table 2) which exhibited signals indicating the presence of a C-7 ester of retronecine and viridiflonc acid The mass spectrum displayed, in addition to ions corresponding to $[M]^+$ (m/z 299), $[M-H_2O]^+$ (m/z 281) and $[M-C_2H_5O_2]^+$ (m/z 236), significant peaks at m/z 154, 137, 124, 111, 106, 94 and 80 which parallel fragment ions reported for 7-angelylretronecine [12]



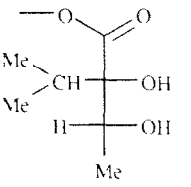
R^3



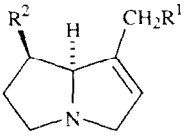
R^4



R^5



R^6



1 R^1=R^3, R^2=OH, R^7=H
2 R^1=R^3, R^2=OH, R^7=Ac
3 R^1=OH, R^2=R^6
4 R^1=R^4, R^2=OH
5 R^1, R^2=OH
6 R^1=R^5, R^2=OH

Alkaline hydrolysis of *iso*-lycopsamine afforded a crystalline necine base, mp 116–117°, $[\alpha]_D^{20°}+40°$ (EtOH), which was identified as retronecine by direct comparison with an authentic sample. The esterifying acid, also a crystalline solid, mp 123–124°, $[\alpha]_D^{20°}+2.3°$ (EtOH), was characterised as (+) viridifloric acid. Thus, *iso*-lycopsamine can be formulated as 1,2-dehydro-1-hydroxymethyl-7β-(+)-viridifloroxy-8α-pyrrolizidine, a new pyrrolizidine alkaloid.

The second base (0.15% dry wt) was also a gum, $[\alpha]_D^{20°}+6.6°$ (EtOH). Its IR spectrum ($CHCl_3$), 3400 cm^{-1} (OH), 1600 cm^{-1} (C=C), 1720 cm^{-1} (C=O), was suggestive of the presence of an unsaturated pyrrolizidine ester alkaloid. The molecular formula $C_{15}H_{25}O_5N$ from the HR mass spectrum and the ^1H NMR spectrum characterized it as intermedine. The signals of the necic acid moiety at δ0.91, 0.95 (ea. 3H, *d*, $J=7.4$ Hz, C-5′ methyls), δ1.17 (3H, *d*, $J=6.3$ Hz (C-3′ methyl), δ2.15 (1H, *q*, $J=7.4$ Hz, H-5′) and δ4.0 (1H, *q*, $J=6.3$ Hz, H-3′) characterized it as trachelanthic acid [11]. The two unequal doublets observed at δ5.1 and 4.6 could be attributed to the C-9 hydrogens. Hence, the alkaloid was 9-*O*-trachelanthylretronecine or intermedine. The structure was confirmed by hydrolysis, which afforded retronecine and (+) trachelanthic acid, mp 91–92°, $[\alpha]_D^{20°}+3.2°$ (EtOH). This is the second report of the isolation of intermedine from Boraginaceae. Earlier it was isolated from *Amsinckia hispida* and *A. intermedia* [1].

The third base (0.009% dry wt) was a crystalline compound, mp 116–117°, $[\alpha]^{20°}+40°$ (EtOH), which was identical in all respects with retronecine. This appears to be the second report of its occurrence in a *Heliotropium* species. Previously it has been reported from *H. ovalifolium* [13].

iso-Lycopsamine and intermedine have the structural features considered necessary for hepatotoxicity [3], hence *H. keralense* can be considered as a toxic species. The structure of *iso*-lycopsamine is unusual in that C_7-necic acids normally esterify the primary, not the secondary, hydroxyl group of pyrrolizidine 7,9-diols. The chemistry of *H. keralense* appears to differentiate it from the closely related *H. indicum* although there are conflicting reports on the chemistry of the latter. Collections of *H. indicum* from Ghana and Australia gave indicine, acetylindicine and a third ester of retronecine with an unidentified acid apparently different from viridifloric or trachelanthic acid [14] while isolation of heliotrine, lasiocarpine and echinatine has been claimed by workers in Bangla Desh [15].

EXPERIMENTAL

Herbarium species of *H. keralense* Sivaraj *et* Manilal collected in 1986, 1987 and 1988 are preserved in our department. Rotations were measured in EtOH. NMR spectra were recorded at 270 MHz. MS were run at 70 eV. TLC was performed on silica gel G (ACME, Bombay). S_1 refers to MeOH as developing solvent, S_2 to $CHCl_3$–MeOH–25% NH_4OH (32:8:1). Spots were detected with I_2. Basic Al_2O_3 was prepared by slurrying 100 g neutral Al_2O_3 with 250 ml of 0.1 N NaOH soln, pouring into an evaporating basin, allowing to stand for 2 hr and activating at 110° for 16 hr. Silica gel for CC was prepd by slurrying 150 g of TLC grade absorbent with 300 ml 0.1 M NaOH and/or 0.5 M NaOH soln, pouring into an evaporating basin, allowed to stand for 4 hr, activating at 110° for 16 hr, powdering and storing at least 2 days before use.

Extraction and fractionation of alkaloids. Dried aerial parts of *H. keralense* (3 kg) collected during August 1986 in Calicut were defatted with hexane and then extracted ×3 with EtOH by percolation at room temp. The combined extracts were evapd *in vacuo*. The syrupy residue was agitated with 50 ml 1 M H_2SO_4 for 1 hr, allowed to stand for 24 hr at 0° and filtered. The clear filtrate was washed with Et_2O (4 × 100 ml). The Et_2O washings after washings, drying and evapn yielded a brown gummy non-alkaloidal residue which was not investigated.

The aq. phase was adjusted to pH 10.5 with NH_4OH and extracted with $CHCl_3$ (6 × 100 ml). Evapn of the washed and dried solvents yielded fraction A (200 mg) as a gum. The aq. layer was acidified with dil. H_2SO_4 to pH 2. Zn dust (4 g) was added and the mixt. stirred for 48 hr at room temp. The soln was filtered, made alkaline with NH_4OH and extracted with $CHCl_3$ (5 × 100 ml). Evapn of the washed and dried solvent gave a brown gum (550 mg, fraction B). The aq. basic fr. was then extracted continuously with $CHCl_3$ in a liquid–liquid extractor for 4 days. Evapn of the washed and dried extract afforded a dark brown gum (900 mg, fraction C). TLC of frs A–C revealed the presence of two major components with R_f 0.6 (base X), R_f 0.45 (base Y) and one minor component, R_f 0.2 (base Z). The total yield of crude alkaloids was 0.51%. Extraction and fractionation of the dried root of the plant (500 g) gave 200 mg (fr. D) of crude alkaloid. TLC revealed the presence of three bases X–Z. The yield of alkaloids was 0.04%.

Isolation of base X (iso-*lycopsamine*). Frs A–D (1.8 g), dissolved in a min. vol. of $CHCl_3$, were applied to a column of basic Al_2O_3 (50 g) prepd in $CHCl_3$ and eluted with $CHCl_3$ followed by mixts. of $CHCl_3$–MeOH (10 ml frs, each monitored by TLC in S_2). Frs 1–6 ($CHCl_3$) gave non-basic impurities. Frs 7–12 ($CHCl_3$), 12–15 ($CHCl_3$–MeOH, 99:1) and 16–20 ($CHCl_3$–MeOH, 49:1) gave some basic material which showed three spots on TLC. The combined frs (7–20) were therefore dissolved in a min. vol. of $CHCl_3$, absorbed on a column of alkaline (N/10) silica gel (150 g) prepd in $CHCl_3$ and eluted with the same solvent. Elution with $CHCl_3$–MeOH–NH_4OH (32:8:1) in 5 ml frs monitored by TLC in S_2 produced in frs 17–25 a brown gum (180 mg, base X) which showed one spot (R_f 0.6) but could not be induced to crystallize. $[\alpha]_D^{20°}+19.5°$ (EtOH); IR bands ($CHCl_3$) at 3400 (OH), 1660 (C=C) and 1720 cm^{-1} (C=O), ^1H NMR spectrum in Table 1, HRMS[M]$^+$ at *m/z* 298.87 (Calc. for $C_{15}H_{25}O_5N$ 299.01). Other significant peaks in the MS were at *m/z* (rel. int.) 281 [M − H_2O]$^+$ 12.3), 236 ([M − H_2O–C_2H_5O]$^+$ 10.3), 138 (67.1), 137 (41.9), 136 (33.7), 124 (21.3), 121 (14.7), 120 (69.5), 119 (15.3), 118 (15.4), 117 (13.6), 111 (68.0), 110 (21.2), 109 (11.0), 108 (49.2), 106 (80.5), 95 (17.1), 94 (42.8), 93 (27.7), 80 (100).

Isolation of base Y (*intermedine*). Frs 40–70 yielded a yellow gum (200 mg base Y) which showed one spot, R_f 0.4, but could not be induced to crystallize. $[\alpha]_D^{20°}+6.6°$ (EtOH). IR bands ($CHCl_3$) at 3400 cm^{-1} (OH), 1600 cm^{-1} (C=C), 1720 cm^{-1} (C=O), ^1H NMR spectrum in Table 1, MS *m/z* (rel. int.) 299 [M]$^+$ 281 (2), 143 (2.2), 120 (5), 118 (6), 87 (23.5), 85 (99), 83 (100).

Isolation of base Z (*retronecine*). Frs 95–114 yielded 90 mg of a crystalline base. Recrystallization from Me_2CO gave colourless cubes of retronecine, mp 116–117°. $[\alpha]_D^{20°}+40°$ (EtOH); IR ($CHCl_3$) 3310 cm^{-1} (OH), 1640 cm^{-1} (C=C), ^1H NMR spectrum identical with that of authentic material.

Hydrolysis of base X (iso-*lycopsamine*). Base X (40 mg) dissolved in 1 ml EtOH was heated with 15% NaOH (2 ml) at 100° for 2 hr. The soln was allowed to cool and extracted with $CHCl_3$. Evapn of the washed and dried solvent afforded a brown gum (12 mg). Recrystallization from Me_2CO gave colourless cubes of retronecine, mp 116–117°; $[\alpha]_D^{20°}+40°$ (EtOH). The remaining aq. layer was neutralized with 2 M HCl and extracted with Et_2O. Evapn of the dried solvent gave 11 mg of (+)-viridifloric acid,

mp. 123–124°, $[\alpha]_D^{20°}$ + 2.3 (EtOH), after recrystallization from EtOH–C_6H_6

Hydrolysis of base Y (intermedine) Intermedine (70 mg) was dissolved in 1 ml EtOH and heated with 15% NaOH (2 ml) at 100° for 2 hr The soln was allowed to cool and extracted with $CHCl_3$. Evapn. of the solvent yield a brown gum. (30 mg). Recrystallization from Me_2CO gave colourless cubes of retronecine, mp. 116–117°; $[\alpha]_D^{20°}$ + 40° (EtOH). The remaining aq layer was neutralised with 2 M HCl and extracted with Et_2O Evapn of the solvent gave (20 mg) of (+) trachelanthic acid, mp 91–92°, $[\alpha]_D^{20}$ + 3.2° (EtOH) after recrystallization from EtOH–C_6H_6

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