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HELIFOLINE, A PYRROLIZIDINE ALKALOID FROM *HELIOTROPIUM OVALIFOLIUM**

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Key Word Index—*Heliotropium ovalifolium*; Boraginaceae; pyrrolizidine alkaloids; helifoline; 1α-angelyloxymethyl-8α-pyrrolizidine-2β,7β-diol; retronecine; structural determination.

Abstract—The alkaloidal fraction of *Heliotropium ovalifolium* yielded retronecine and the new pyrrolizidine alkaloid helifoline. Helifoline was formulated as 1α-angelyloxymethyl-8α-pyrrolizidine-2β,7β-diol on the basis of spectroscopic measurements and hydrolysis to the necine base which appears to be identical with croalbinecine.

INTRODUCTION

Hepatotoxic pyrrolizidine alkaloids of some *Heliotropium* species have been shown to be responsible for outbreaks of liver disease and for some serious livestock losses in certain areas [1, 2]. As part of a systematic search for alkaloids in *Heliotropium* species occurring in the vicinity of Madras [3], we have investigated *H. ovalifolium* Forsk which is said to be poisonous, causing diarrhea and vomiting [4]. This has led to the isolation of a new pyrrolizidine alkaloid, helifoline, for which we propose structure **1a**, as well as retronecine (**3**). An earlier study of the same plant [5] gave β-sitosterol and β-amyrin.

RESULTS AND DISCUSSION

H. ovalifolium gave a positive test for alkaloids; trial experiments showed that collections made in August gave optimal yields of alkaloids but the alkaloid fraction was much less than previously obtained from *H. curassavicum* [3]. Fractionation and purification by the method described in the Experimental gave two homogeneous basic substances. The first of these (base X) was 30 mg (0.003% of dry wt) of a new crystalline alkaloid, $C_{13}H_{21}NO_4$ (high resolution mass spectrum), mp 131–132°, $[\alpha]_D^{25}$ + 25.4°, which we have named helifoline. The IR spectrum (KBr) exhibited an intense band at 3380 cm^{-1} (OH) and a carbonyl band at 1725 cm^{-1} reminiscent of a pyrrolizidine ester alkaloid.

Consistent with the mass spectrum the ^1H NMR spectrum of helifoline measured in $CDCl_3$ (Table 1) integrated for a total of 21 protons. Addition of D_2O reduced the integrated intensity by two protons, thus permitting the inference that two OH groups were present. The nature of the ester moiety was also clear from the ^1H NMR spectrum which showed signals characteristic of an angelate. This was attached to C-9 of the necine residue as the spectrum exhibited no other Me signals and as at 270 MHz the characteristic two-proton doublet of H-9 (J = 7 Hz) was sharply resolved from a two-proton multiplet arising from the protons under two secondary OH groups.

The disposition of the two OH groups on the necine moiety was established by double resonance experiments at 270 MHz. Identification of the H-1 signal was achieved first by irradiation at the frequency of H-9. This resulted in collapse of a one-proton multiplet at δ 2.65 (H-1) to a *dd*. Irradiation at the frequency of H-1 collapsed not only the H-9 doublet at δ 4.16 to a singlet, but reduced a one-proton triplet at 3.37 (J = 4.3 Hz) to a doublet and effected simplification of the two-proton multiplet centered at 4.24. The signal at δ 3.37 was therefore that of H-8; this frequency was in agreement with that found in other pyrrolizidine alkaloids. Irradiation at the frequency of H-8 simplified not only the H-1 multiplet at δ 2.65, but again also the two-proton multiplet at 4.24. These results are compatible only with attachment of the two OH groups at C-2 and C-7.

Helifoline could thus be formulated as **1a** exclusive of stereochemistry, a structure consistent with the chemical shifts of the remaining signals. The *dd*'s at δ 3.29 and 2.76 were appropriate for H-3α and H-3β, respectively. The multiplet at 3.2 could be assigned to H-5α and the distorted quartet at 2.9 to H-5β, while the H-6 signals were superimposed on the vinyl Me at 1.99. These assignments were confirmed by double resonance experiments; coupling constants determined in this manner are given in Table 1.

Corroboration for structure **1a** was obtained from the mass spectrum which in addition to the M^+ (m/z 255)

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Table 1. ^{1}H NMR spectral data of helifoline, helifolinecine and croalbinecine*

	Helifoline†		Helifolinecine‡		Croalbinecine‡§	
H-1	2.65 *m*	$J_{1,8} = 4.3$	2.33 *dist. quint.*	$J_{1,8} = 7$	2.35	$J_{1,8} = 8$
H-2	4.24 *m*	$J_{1,2} = 4.2$	4.18 *dist. quint.*	$J_{1,2} = 7.5$	4.18	$J_{1,2} = 8$
H-3α	3.29 *dd*	$J_{2,3\alpha} = 4.8$	3.26 *dd*	$J_{2,3\alpha} = 4$	3.29	$J_{2,3\alpha} = 5.8$
		$J_{3\alpha,3\beta} = 11.2$		$J_{3\alpha,3\beta} = 10$		$J_{3\alpha,3\beta} = 10$
H-3β	2.76 *dd*	$J_{2,3\beta} = 3.9$	2.54 *dd*	$J_{2,3\beta} = 8.5$	2.55	$J_{2,3\beta} = 8$
H-5α	3.20 *m*	$J_{5\alpha,5\beta} = 9.5$	3.10 *m*	$J_{5\alpha,5\beta} = 11$	3.14	$J_{5\alpha,5\beta} = 10$
		$J_{5\alpha,6} = 4.4$		$J_{5\alpha,6} = 4, 7.5$		$J_{5\alpha,6} = 4, 5.5$
H-5β	2.90 *dist. quart*	$J_{5\beta,6} = 8.5$	2.73 *m*	$J_{5\beta,6} = 6, 10$	2.76	$J_{5\beta,6} = 7, 10$
H-6‖	~1.99		2.02¶		~2.03	
H-7	4.24 *m*	$J_{7,8} = 4.3$	4.28 *m*	$J_{6,7} = 3$	4.29	$J_{6,7} \sim 4$
				$J_{7,8} = 6$		$J_{7,8} \sim 4$
H-8	3.37 *t*		3.23 *dd*		3.28	
H-9u	4.16 *d*	$J_{1,9u} = 7$	3.63 *dd*	$J_{1,9u} = 7.5$	3.63	$J_{1,9u} = 6.4$
H-9d		$J_{1,9d} = 7$	3.75 *dd*	$J_{1,9d} = 5.5$	3.73	$J_{1,9d} = 5.8$
H-3′	6.10 *qbr*	$J_{3',4'} = 7$				
H-4′**	1.99 *d*					
H-5′**	1.89 *br*					

* Run at 270 MHz with TMS as internal standard, unless indicated otherwise. Chemical shifts in ppm. Coupling constants, all established by double irradiation, in Hz.

† In $CDCl_3$ solution.

‡ In D_2O solution, internal standard DDS.

§ From ref. [9], in D_2O solution at 100 MHz.

‖ Two-proton intensity.

¶ AB part of ABCDX system.

** Three-proton intensity.

displayed significant peaks at *m/z* 156, 111, 99, 98, 82 and 80. Scheme 1 depicts the fragmentation processes leading to these ions; they are in agreement with the observations of Aasen *et al.* [6] which showed that whilst the initial fissions in saturated pyrrolizidines with several OH groups are those of bonds β to a nitrogen atom (fragment *m/z* 156), there is a strong tendency for two such bonds in one ring to break together. The ^{13}C NMR spectrum of helifoline corroborated formula **1a**; the assignments shown in Table 2 are in accordance with those of Mody *et al.* [7].

Previously encountered ester alkaloids derived from 1-hydroxymethyl-2,7-dihydroxypyrrolizidines are rosmarinine [8] and croalbidine [9]. Helifoline could be a 9-angelate of rosmarinecine (**2**), croalbinecine (**1b**) or one of their, as yet, unknown diastereomers. To narrow the choice among these possibilities, helifoline was subjected to acid hydrolysis. This afforded a necine hydrochloride of mp 164–165° which corresponds to the mp (165–166°) reported [10] for croalbinecine hydrochloride, thus suggesting the possible identity of helifolinecine with croalbinecine.* Free helifolinecine was obtained as a gum, $C_8H_{15}NO_3$ (high resolution mass spectrum), $[\alpha]_D^{25} + 45.7°$, whose fragmentation pattern was in agreement with that reported [9] for croalbinecine (Table 3). Unfortunately, the rotation of croalbinecine is not recorded nor were we able to secure authentic samples of croalbinecine or its hydrochloride for a direct comparison with helifolinecine in order to establish the suspected identity.

Double resonance experiments on helifolinecine were therefore undertaken to define the stereochemistry at the various centers and to provide a comparison with croalbinecine which had been subjected to similar studies earlier [9]. Chemical shifts and coupling constants

Table 2. ^{13}C NMR spectral data of helifoline*

Carbon	
1	44.93 *d*
2	71.66 *d*
3	62.50 *t*
5	53.42 *t*
6	37.27 *t*
7	75.71 *d*
8	71.61 *d*
9	64.78 *t*
1′	169.53
2′	128.96
3′	139.06 *d*
4′	15.98 *q*
5′	20.65 *q*

* Run in $CDCl_3$ at 67.9 MHz with TMS as internal standard. Assignments made by using noise-decoupled spectra, off-resonance spectra and single frequency proton off-resonance decoupling to identify multiplets.

* The mp of rosmarinecine hydrochloride has not been recorded, nor is the NMR spectrum of rosmarinecine in the literature.

Table 3. Mass spectral fragmentation of helifolinecine and croalbinecine

m/z	% Abundance Helifolinecine*	Croalbinecine†
173	21.0	72‡
155	9.5§	—
129	67.1	78
112	8.1‖	—
99	51.5	65
98	100¶	100
82	58.7	60

*Run on MS-902 instrument (high resolution) at 70 eV. Relative intensities were recorded by computer.

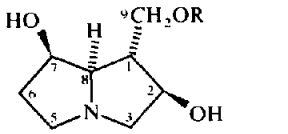
†Data from ref. [10] using low-resolution instrument.

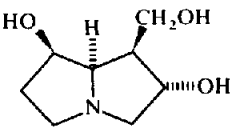
‡Possible misprint in ref. [10].

§Metastable ion at *m/z* 138.9 for $M^+ - H_2O$.

‖Metastable ion at *m/z* 97.2 for 129 − OH.

¶Metastable ion at *m/z* 74.5 for 129 − CH_2OH.





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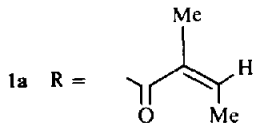
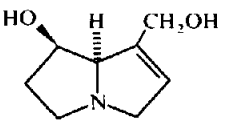


1a R =

1b R = H



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derived from double resonance studies on helifolinecine at 270 MHz were in excellent agreement with those reported for croalbinecine at 100 MHz (Table 1) except for variations in $J_{7,8}$ and $J_{2,3a}$. At first glance the observed difference in $J_{7,8}$ suggests the possibility that helifolinecine could be the C-7 epimer of croalbinecine; however, the coincidence of chemical shift values renders this possibility rather unlikely. Hence, we conclude that helifolinecine and croalbinecine are identical and attribute the literature report for $J_{7,8}$ and $J_{2,3\alpha}$ to operation at lower field strengths where signal separation is a problem. Hence helifoline should be formulated as 1α-angelyloxymethyl-8α-pyrrolizidine-2β,7β-diol (**1a**).†

Helifoline is the first example of a pyrrolizidine alkaloid derived from a triol necine to be encountered in the Boraginaceae. The previously known alkaloids derived from triol necines, viz. rosmarinine [8], croalbidine [9], angularine [11], madurensine [12–14], 7-acetylmadurensine and 7-acetyl-*cis*-madurensine [14], anacrotine [12–15], 6-acetylanacrotine, 6-acetyl-*trans*-anacrotine, 6-angelyl-*trans*-anacrotine and crotaflorine [14] are all macrocyclic diesters, whereas helifoline is the first monoester alkaloid derived from such a triol.

The minor alkaloid of *H. ovalifolium* (base Y) was obtained in 20 mg yield. The molecular formula $C_8H_{13}NO_2$ (mass spectrum), the mp (116–117°), the rotation, $[\alpha]_D^{25} + 42°$, an IR band (KBr) at 3320 cm^{-1} (OH) and significant ^1H NMR signals at δ 5.68 (*br*, vinyl H) and 4.68 (*br*, 2 H, $-CH_2OH$) suggested that it was retronecine. Direct comparison with an authentic sample of retronecine (**3**) showed that the two samples were identical.

Although *ca* 200 pyrrolizidine alkaloids have been isolated and characterized to-date, the occurrence of free necines in nature is rare. Retronecine seems to have been isolated only once previously, from *Crotolaria retusa* L. (Leguminosae) [16]. Thus the discovery of retronecine in *H. ovalifolium* represents only its second isolation and the first isolation from the Boraginaceae.

Although *H. ovalifolium* is reputed to be poisonous [4] the present work which has resulted in the isolation of helifoline and retronecine as the major alkaloids of this species suggests that the report is in error, unless some other toxic alkaloid is present in small amounts. Helifoline is the ester of a saturated necine lacking the C-1, C-2 double bond and as such lacks the structural requirement for hepatotoxicity. This inference is reinforced by earlier observations that croalbidine [10] and rosmarinine [1] are not hepatotoxic. Retronecine although possessing the required C-1, C-2 double bond is a free necine; such amino alcohols have also been found to be non-toxic.

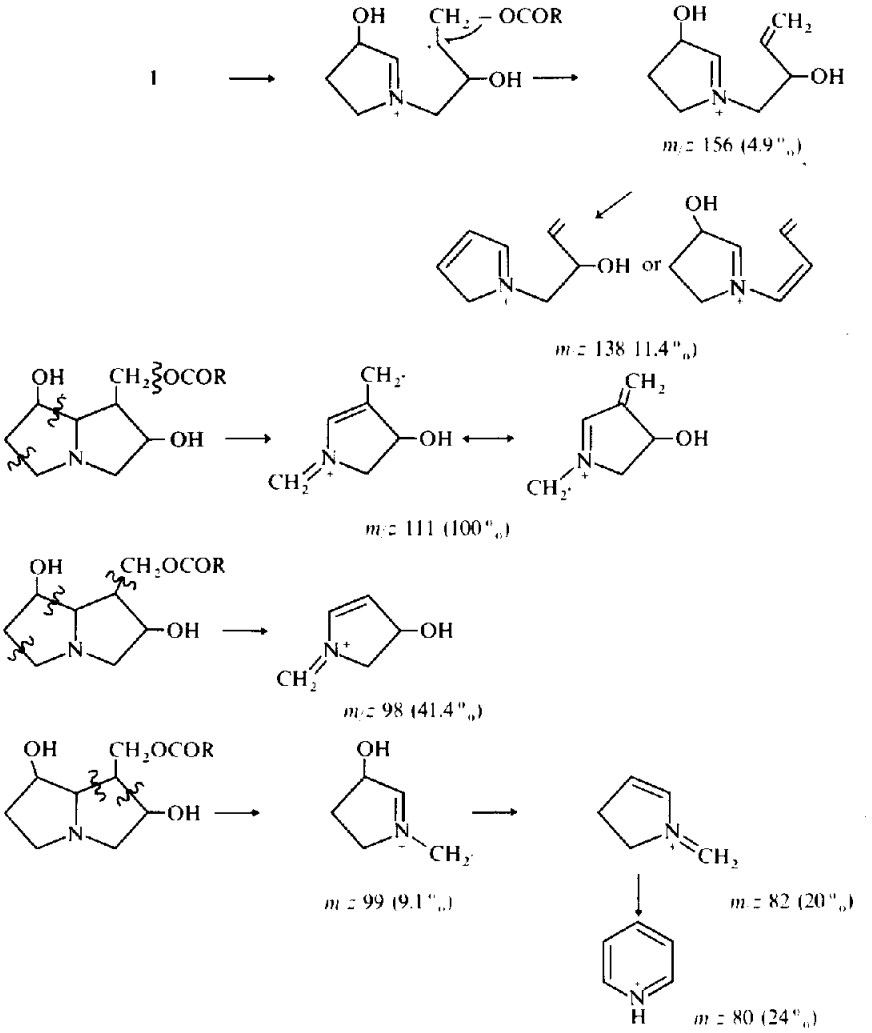
EXPERIMENTAL

Rotations were measured in EtOH. NMR spectra were recorded at 90 and at 270 MHz (or 67.9 for ^{13}C). High resolution MS were run at 70 eV. TLC was performed on Si gel C(Acme, Bombay). S_1 refers to MeOH as developing solvent, S_2 to $CHCl_3$–MeOH–25% NH_4OH (17:3.8:0.25). Spots were detected with I_2 and/or spraying with Dragendorff reagent. Neutral Al_2O_3 was prepared by washing Activity I, II Centron adsorbent with 50% HNO_3 followed by distilled H_2O until neutral and then MeOH (twice), followed by activation at 110° for 16 hr. Si gel for column chromatography was prepared by slurrying 30 g of TLC grade adsorbent with 60 ml 0.1 N NaOH soln, pouring into an evaporating basin, allowing to stand for 4 hr, activating at 110° for 12 hr, powdering and storing at least 2 days before use.

Extraction and fractionation of alkaloids. Dried whole plant (1 kg) collected during August 1978 in the environs of Madras was defatted with hexane and then extracted × 3 with EtOH by percolation at room temp. The combined extracts were evaporated *in vacuo*. The sirupy residue was agitated with 100 ml 0.5 N H_2SO_4 for 1 hr, allowed to stand for 48 hr at 0° and filtered. The clear filtrate was washed with Et_2O (4 × 200 ml). The Et_2O washings after washing, drying and evaporation 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 successively with Et_2O (5 × 200 ml) and $CHCl_3$ (5 × 200 ml). Evapn of the washed and dried solvents yielded fractions A (50 mg) and B (70 mg) as gums. TLC of fractions A

†Significant differences in the coupling constants of helifoline and helifolinecine indicate that the conformations of the alkaloid and the necine base are somewhat different. Part of this could be due to the required change of solvent ($CDCl_3$ for helifoline, D_2O for helifolinecine).



Scheme 1. MS fragmentation of helifoline.

and B revealed the presence of one major component with R_f 0.50 (base X) and 3 minor components, R_f 0.65, 0.35 and 0.26, respectively. The aq. basic fraction was then extracted continuously with $CHCl_3$ in a liquid–liquid extractor for 7 days. Evapn of the washed and dried solvent afforded a dark brown gum (165 mg, fraction C). It contained very little basic material and was rejected. The remaining aq. layer was acidified with dil. HCl to pH 2. Zn dust (5 g) was added and the mixture stirred for 24 hr at room temp. The soln was filtered, made alkaline with NH_4OH and extracted with $CHCl_3$ (5 × 200 ml). Evapn of the washed and dried solvent gave a brown gum (12 mg, fraction D). Finally, the aq. soln was extracted continuously with $CHCl_3$ for 5 days. Evapn of the washed and dried extract gave a brown gum (140 mg, fraction E). Fractions D and E exhibited essentially identical TLC profiles with two main components of R_f 0.50 (minor, base X) and 0.24 (major, base Y). The total yield of crude alkaloids was 0.045%.

Isolation of helifoline. Fractions A and B (120 mg), dissolved in the minimum vol. of $CHCl_3$, were applied to a column of neutral Al_2O_3 (50 g) prepared in $CHCl_3$ and eluted with $CHCl_3$ followed by mixtures of $CHCl_3$–MeOH (10 ml fractions, each monitored by TLC in S_1). Fractions 1–3 ($CHCl_3$) and 4–6 ($CHCl_3$–MeOH, 99:1) gave non-basic impurities. Fractions 7 and 8 ($CHCl_3$–MeOH, 49:1) gave a few mg of basic material which showed several spots on TLC. Fractions 9–15 ($CHCl_3$–MeOH, 19:1) yielded a brown gum (55 mg, base X) which showed mainly one spot, R_f 0.05, but it could not be induced to crystallize. The material was therefore dissolved in a little $CHCl_3$, adsorbed on a column of alkaline (N/10) Si gel prepared in $CHCl_3$ and eluted with the same solvent. Elution with $CHCl_3$–MeOH–NH_4OH (17:3.8:0.25) in 10 ml fractions monitored by TLC (S_1 and S_2) produced in fractions 2–7 40 mg of pure base X which solidified on trituration with ice cold Me_2CO. Recrystallization from the same solvent afforded 30 mg of helifoline, colorless cubes, mp

131–132°, $[\alpha]_D^{25}$ + 25.4° (*c* 0.0006, EtOH), IR bands (KBr) at 3380 (OH), 1725 cm^{-1} (ester); ^1H and ^{13}C NMR spectra Tables 1 and 2. (Calc. for $C_{13}H_{21}NO_4$: MW, 255.1490. Found: MS, 255.1465). Other significant peaks in the high resolution MS were at *m/z* (composition, rel. int.): 237 ($C_{13}H_{19}O_3$, 3.2), 156 ($C_8H_{14}NO_2$, 4.9), 138 ($C_8H_{21}NO$, 11.4), 137 ($C_8H_{11}NO$, 3.4), 112 ($C_6H_{10}NO$, 27.1), 111 (C_6H_9NO, 100), 99 (C_5H_9NO, 9.1), 98 (C_5H_8NO, 41.5), 94 (C_6H_8N, 4.1), 83 (C_5H_9N, 8.9), 82 (C_5H_8N, 20.2), 80 (C_5H_6N, 24.0).

Hydrolysis of helifoline. Helifoline (40 mg) was heated with 10% HCl (10 ml) at 80° for 24 hr. The soln was allowed to cool, washed with $CHCl_3$ and the aq. layer taken to dryness *in vacuo*. The residue was taken up in EtOH. Recrystallization from EtOH–Me_2CO afforded feathery needles of the necine HCl (30 mg), mp 165–166°, lit. mp 166–167° [10]. The hydrochloride (20 mg) was dissolved in H_2O and passed through a column of Amberlite IRA-400 resin (OH^- form). Elution with H_2O yielded 12 mg of the necine base as a gum, homogeneous on TLC (S_1 and S_2), which could not be induced to crystallize, $[\alpha]_D^{25}$ + 45.7° (*c* 0.0024, EtOH). For ^1H NMR spectrum see Table 1. (Calc. for $C_8H_{15}NO_3$: MW, 173.1051. Found: 173.1058). Other significant peaks in the high resolution MS were at *m/z* (composition, rel. int.), 155 ($C_8H_{13}NO_2$, 9.5), 129 ($C_6H_{11}NO_2$, 67.1), 124 ($C_7H_{10}NO$, 2.8), 116 ($C_5H_{10}NO_2$, 6.1), 112 ($C_6H_{10}NO$, 8.2), 98 (C_5H_8NO, 100), 82 (C_5H_8N, 58.7), 80 (C_5H_6N, 10.1).

Isolation of retronecine. Fractions D and E (152 mg), dissolved in the minimum vol. of $CHCl_3$, and two drops of MeOH, were adsorbed on a column of neutral Al_2O_3. The column was eluted with 10 ml portions of $CHCl_3$ and $CHCl_3$–MeOH of increasing MeOH content, all fractions being monitored by TLC (S_1). Fractions 11–20 ($CHCl_3$–MeOH, 4:1) yielded 15 mg of solid material homogeneous by TLC (S_1 and S_2). Recrystallization from Me_2CO gave colorless cubes of retronecine, mp 117–118°, $[\alpha]_D^{25}$ + 42.0° (*c* 5, EtOH); IR (KBr) 3320 cm^{-1} (OH); ^1H NMR signals ($CDCl_3$) at 1.96 *m* (2 H), 2.75 *m* (1 H), 3.24 *m* (1 H), 3.4 *m* (1 H), 3.88 *m* (1 H), 4.24 *m* (4 H), 4.68 *br* (2 H) and 5.68 *br* (1 H); MS *m/z* (rel. int.): 155 (M^+, 32), 112 (7), 111 (78), 110 (7), 94 (18), 93 (9), 82 (9), 81 (16), 80 (100), 68 (20), 67 (7), 53 (12). The substance was identical with authentic retronecine (mmp, IR).

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