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# HELISCABINE, A PYRROLIZIDINE ESTER ALKALOID FROM *HELIOTROPIUM SCABRUM*

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**Key Word Index**—*Heliotropium scabrum*; Boraginaceae; pyrrolizidine alkaloids; heliscabine; 1α-angelyloxymethyl-8α-pyrrolizidine-1β,7β-diol.

**Abstract**—Two pyrrolizidine alkaloids, heliscabine and retronecine, have been isolated from *Heliotropium scabrum* and their structures elucidated by physicochemical methods. Heliscabine, a new ester alkaloid is 1α-angelyloxymethyl-8α-pyrrolizidine-1β,7β-diol (9-angelyl helibractinecine).

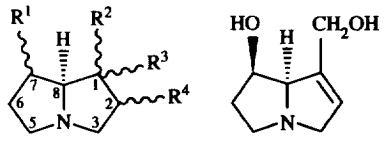
## INTRODUCTION

Unsaturated pyrrolizidine ester alkaloids are potentially hepatotoxic [1, 2]. The plant species which contain such toxic alkaloids are responsible for outbreaks of liver disease among livestock in certain areas [3]. Many *Heliotropium* species are toxic due to the presence of unsaturated ester alkaloids [4, 5]. In our systematic work on alkaloids of Indian *Heliotropium* species [6], we have investigated *H. scabrum*. It is a spreading or procumbent herb, distributed in the foothills of peninsular India and also in Sri Lanka up to 1500 m on the exposed slopes, especially on the thin layer of soil by rocks [7].

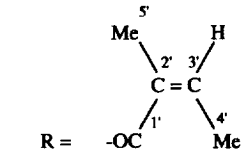
## RESULTS AND DISCUSSION

Trial experiments showed the presence of alkaloids in the title species and the alkaloid content was found to be optimal (0.025%) during the flowering season (June/July). Extraction and fractionation of the alkaloids of *H. scabrum* were done by the method described earlier [8]. This has resulted in the isolation of a new ester alkaloid, heliscabine (**1**), and a known necine, retronecine (**2**).

Heliscabine was an optically active gum $[\alpha]_D^{25}$ − 19.5° (EtOH; *c* 0.1). Its molecular formula was deduced as $C_{13}H_{21}NO_4$ [calc. $M_r$, 255.1490, found: 255.1435]. Its IR spectrum was similar to a saturated pyrrolizidine ester alkaloid containing one or more hydroxyl groups. $^1$H NMR of this compound integrated for a total of 21 protons. The reduction in the intensity by two protons upon the addition of $D_2O$ indicated the presence of two hydroxyl groups. The nature of the acid part was established as angelic from its $^1$H NMR signals (Table 1).




**2**




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| **1** | -βOH | -βOH | -α$CH_2$OR | |
| **3** | -βOH | H | -α$CH_2$OR | -βOH |
| **4** | -βOH | -βOH | -α$CH_2$OH | |

Esterification at the C-9 hydroxyl group was revealed by the downfield resonance position of the H-9 protons, which resonated at δ4.35 and δ4.25 as doublets (*J* = 12 Hz). The downfield one proton quartet signal at δ4.66 was diagnostic of a H-7 proton under a secondary hydroxyl group. The one proton doublet at δ3.64 (*J* = 5 Hz) was characteristic of the H-8 proton. The assignment of other proton signals was established by double resonance studies. The fact that the H-8 signal was coupled only to the H-7 proton established the absence of a proton at C-1 [8–10]. Based on this evidence, heliscabine could be tentatively formulated as a 1-angelyloxymethyl-pyrrolizidine-1,7-diol. The mass spectrum of heliscabine was reminiscent of helifoline (**3**), a monoester alkaloid of a saturated triol necine. The mass spectrum displayed significant fragment ions at, in addition to the $[M]^+$, *m/z* 156, 138, 111, 99, 98, 82 and 80 which parallel the fragment ions reported for helifoline [11].

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Table 1. $^1$H NMR spectral data of heliscabine (1)

| Proton | $CDCl_3 + D_2O$* | |
|---|---|---|
| H-2 | 1.86 *m* (2H) | |
| H-3$\beta$ and H-5$\beta$ | 3.05 *m* (2H) | |
| H-3$\alpha$ | 3.42 *dt* (1H) | $J_{3\alpha,3\beta} = 9$ |
| | | $J_{2,3\alpha} = 7.5$ |
| H-5$\alpha$ | 3.30 *dt* (1H) | $J_{5\alpha,5\beta} = 10$ |
| H-6 | | $J_{5\alpha,6} = 7$ |
| | 2.16 *m* (2H) | |
| H-7 | | |
| | 4.66 *dist q* (1H) | $J_{6,7} = 5.3$ |
| | | $J_{7,8} = 5$ |
| | 3.64 *d* (1H) | $J_{7,8} = 5$ |
| H-9$_u$ | | |
| | 4.25 *d* (1H) | $J_{9u,9d} = 12$ |
| H-9$_d$ | | |
| | 4.35 *d* (1H) | $J_{9u,9d} = 12$ |
| H-5′ | | |
| | 1.89 *s* (3H) | |
| H-4′ | | |
| | 1.99 *d* (3H) | $J_{3',4'} = 7$ |
| H-3′ | | |
| | 6.10 *q* (1H) | $J_{3',4'} = 7$ |

*Measured at 300 MHz with TMS as internal standard. Chemical shifts in ppm. Coupling constants in Hz all established by double irradiation.

Corroborative evidence for the structure of heliscabine was obtained from its $^{13}$C NMR spectrum (*vide* Experimental). The PND spectrum exhibited signals for 13 carbon atoms. The SFORD spectrum exhibited three singlets, three doublets, five triplets, and two quartets. Both the $^1$H and $^{13}$C NMR signals for its pyrrolizidine nucleus closely resembled helibractinecine (**4**), a free necine isolated from *H. bracteatum* [8]. Indeed, acid hydrolysis of heliscabine afforded a gummy free necine which was identical in all respects with helibractinecine. Thus, heliscabine was formulated as 1$\alpha$-angelyloxymethyl-8$\alpha$-pyrrolizidine-1$\beta$,7$\beta$-diol (**1**).

The second alkaloid (*ca* 7.2% of total alkaloids) was identified as retronecine on the basis of IR, $^1$H NMR data and physical constants.

Heliscabine is a new ester alkaloid of a triol necine, helibratinecine. *Heliotropium scabrum* contains ester alkaloid (**1**) while *H. bracteatum* contain the corresponding free necine. The latter also contains ester alkaloids which are diastereoisomers of heliscabine [12]. This finding suggests that the two species behave like chemotypes. Besides this chemotoxonomic relationship between them, they are non-toxic as they do not contain unsaturated ester alkaloids.

## EXPERIMENTAL

Herbarium specimens of *H. scabrum* Retz. collected around Madras during 1982 are preserved in our department. Air-dried aerial parts of *H. scabrum* (2 kg) collected during the flowering season in and around Madras were processed for the isolation of alkaloids as reported in ref. [11]. Other experimental details were given in our earlier report [8]. Fr. A (60 mg) and Fr. B (100 mg) exhibited identical TLC ($S_1$, N/10 alkalized silica gel). Fr. C (70 mg) was essentially devoid of basic compounds. Frs D (120 mg) and E (150 mg) contained free necines.

*Isolation and heliscabine.* Combined Frs A and B (160 mg) dissolved in a min vol. of $CHCl_3$ were adsorbed on a very short column of neutral $Al_2O_3$ (15 g); $CHCl_3$ (50 ml) eluted most of the non-basic materials. $CHCl_3$–MeOH (99:1) eluted the bases (90 mg). This was rechromatographed over a column of alkalized (N/10 NaOH) silica gel in $CHCl_3$. Elution with $S_2$ afforded a fr. rich in heliscabine (30 mg). Further purification was done by rechromatography over N/10 alkalized silica gel. This gave 10 mg of heliscabine $R_f$ 0.6 ($S_1$, N/10 alkalized silica gel) as a gum. $[\alpha]_D^{25} - 19.5°$ (EtOH; *c* 0.1). IR $\nu_{max}$ ($CHCl_3$) cm$^{-1}$ 3390 (–OH), 1710 (ester carbonyl), 1150 (C–O). $^1$H NMR spectrum (Table 1). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$81.1 *s* (C-1), 38.4 *t* (C-2), 52.8 *t* (C-3), 52.0 *t* (C-5), 36.0 *t* (C-6), 72.8 *d* (C-7), 69.8 *d* (C-8), 70.0 *t* (C-9), 168.1 *s* (C-1′), 127.2 *s* (C-2′), 139.7 *d* (C-3′), 16.0 *q* (C-4′), 20.6 *q* (C-5′). HRMS *m/z* rel. int. $[M]^+$ at *m/z* 255.1435 (4) (calcd for $C_{13}H_{21}O_4N$: MW, 255. 1490). Other peaks at 156 (42), 138 (12), 111 (100), 99 (33), 98 (92), 82 (77) and 80 (10).

*Hydrolysis of heliscabine.* Heliscabine (50 mg) was heated with 10% aq. HCl (10 ml) at 80° for 12 hr. The reaction mixt. was then cooled and washed with $CHCl_3$. The aq. phase was evapd *in vacuo* to give a gummy necine HCl (40 mg). This was dissolved in $H_2O$ and passed through a bed of Amberlite resin IRA-400 (–OH) which gave 18 mg of the necine base as a gum, homogeneous on TLC ($S_1$, N/10 & N alkalized silica gel). $[\alpha]_D^{25} - 63°$ (EtOH; *c* 0.1). IR: $\nu_{max}$ (neat) cm$^{-1}$ 3350 (–OH), 1050 (C–O). $^1$H and $^{13}$C NMR identical with that of authentic helibractinecine.

*Isolation of retronecine.* Frs C and D (270 mg) dissolved in a min. vol. of $CHCl_3$–MeOH (99:1) were chromatographed on a short column of basic $Al_2O_3$ (40 g) prepd in $CHCl_3$. Frs 1–6 ($CHCl_3$, 10 ml each, monitored by TLC in $S_1$) gave non-basic impurities. Frs 7–12 [$CHCl_3$–MeOH (99:1), 10 ml each] on TLC showed a single spot ($R_f$ 0.22, N/10 alkalized silica gel). Evapn of these frs yielded a gummy residue (40 mg). This was rechromatographed over a column of alkalized (N/2 NaOH) silica gel (10 g) in $CHCl_3$. The column was eluted with $S_2$. Frs 5–11 (2 ml, each) yielded 18 mg of a solid material homogeneous by TLC ($S_1$ and $S_2$). Recrystallization from $Me_2CO$ gave pure retronecine, mp 116–117°. $[\alpha]_D^{20} + 50°$ (EtOH; *c* 0.1). IR: $\nu_{max}$ (KBr) cm$^{-1}$ 3340 (OH), 1640 (C=C). $^1$H NMR identical with that of authentic material.

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