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ESTER ALKALOIDS OF *HELIOTROPIUM BRACTEATUM*

AKONI J. LAKSHMANAN* and SINGARAM SHANMUGASUNDARAM

Department of Chemistry, Presidency College, Madras 600005, India

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Key Word Index—*Heliotropium bracteatum*; Boraginaceae; pyrrolizidine ester alkaloids; helibracteatinine; 1β-hydroxymethyl-7α-angelyloxy-8α-pyrrolizidine-1α-ol; helibracteatine; 1β-angelyloxymethyl-8α-pyrrolizidine-1α,7α-diol.

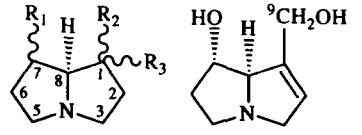
Abstract—Isolation and structure determination of the two new ester alkaloids of *Heliotropium bracteatum*, viz. helibracteatinine and helibracteatine, are described. Helibracteatinine was formulated as 1β-hydroxymethyl-7α-angelyloxy-8α-pyrrolizidine-1α-ol and helibracteatine as 1β-angelyloxy-8α-pyrrolizidine-1α,7α-diol. Structures were established by spectrometry of the alkaloids and by hydrolysis to their necine bases.

INTRODUCTION

In a continuation of our studies [1–4] on the chemical constituents of of *Heliotropium* species, we have reported the isolation and characterisation of a novel triol necine, helibractinecine [2] from *Heliotropium bracteatum* R.Br. (syn. *H. laxiflorum* Roth). Further examination of the ester alkaloid fraction led to the identification of helibracteatinine and helibracteatine.

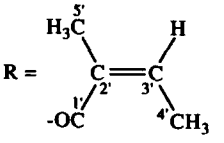
RESULTS AND DISCUSSION

Fractionation of the alkaloids of *H. bracteatum* gave four fractions [2]. Fraction A was mainly composed of ester alkaloids. Chromatographic separation of fraction A over a column of neutral alumina led to the isolation of two minor gummy bases. Rechromatography over alkalized silica gel columns afforded two gummy isomeric ester alkaloids, helibracteatinine (**1**) and helibracteatine (**2**). The mass spectra of **1** and **2** revealed their molecular formulae as $C_{13}H_{21}NO_4$ and the fragmentation patterns of both the alkaloids were identical to that of a monoester alkaloid derived from saturated triol necine [5]. Their IR spectra mainly consisted of hydroxyl and ester carbonyl groups. The mass spectral fragmentations showed that alkaloids **1** and **2** are esterified at different positions with a C-5-linked acid. Consistent with the molecular formulae deduced from their mass spectra the ^{1}H NMR spectra of **1** and **2** integrated for a total 21 protons each and their integral values were reduced by two upon the addition of D_2O indicating the presence of two free



(8)

	R_1	R_2	R_3
1a	OR	OH	CH_2OH
1b	OH	OH	CH_2OR
1	-αOR	-αOH	-βCH_2OH
2	-αOH	-αOH	-βCH_2OR
3	-αOH	-αOH	-βCH_2OH
4	-βOH	-βOH	-αCH_2OH
5	-βOH	-αOH	-βCH_2OH
6	H	-βOH	-αCH_2OH
7	H	-αOH	-βCH_2OH



hydroxyl groups. The nature of the esterifying acid was established as angelic acid by the diagnostic signals and thus the two alkaloids are angelyl esters of a triol necine.

In ^{1}H NMR spectrum of helibracteatinine (**1**), the H-7 signal resonated as a distinct quartet at δ5.09. The absence of a proton at C-1 was established by the resonance signal of the H-8 proton which resonated as a doublet at δ3.30. This information served to characterise helibracteatinine as 1-hydroxymethyl-7-angelyloxy-pyrrolizidine-1-ol (**1a**) but excluded the stereochemistry. This assignment was consistent with the remaining signals of its ^{1}H NMR spectrum (Table 1). Acetylation of helibracteatinine afforded a monoacetate. The ^{1}H NMR spec-

* Present address: Department of Chemistry, Government Arts College, Udhagamandalam, The Nilgiris, Tamil Nadu 643002, India.

Table 1. ^{1}H NMR spectral data of compound, **1–3** (in $CDCl_3 + D_2O$, 270 MHz)

H	**1**	**2**	**3** (in $CDCl_3$)
2u	1.80 *m*	1.85 *m*	1.63 dist. *q* (5.0, 5.8)
2d	2.10 *m* (8.6, 14)		
3α	3.35 *m*	3.22 *m*	3.26 *m*
3β	2.78 *m* (8.6, 11)	2.54 *m*	2.55 *dt* (5.8, 10.8)
5α	3.20 *m* (7.6, 11)	3.22 *m*	3.26 *m*
5β	2.60 *m* (7.6, 11)	2.54 *m*	2.52 *dt* (4.1, 9.6, 9.6)
6u	1.80 *m*	1.85 *m*	1.84 *m* (12)
6d		2.05 *m*	2.11 *m*
7	5.09 dist. *q* (5.5, 6)	4.08 dist. *q* (5.5, 6.5)	3.94 *q* (6.9, 6.9, 6.9)
8	3.30 *d* (6)	3.30 *d* (6.5)	3.12 *d* (6.9)
9u	3.64 *d* (12)	4.35 *d* (12)	3.79 *d* (10.6)
9d	3.84 *d* (12)	4.46 *d* (12)	3.84 *d* (10.6)
3′	6.12 *q* (7)	6.10 *q* (7)	
4′†	1.98 *d* (7)	1.99 *d* (7)	
5′†	1.88 *s*	1.91 *s*	
-OH			3.81 *br*

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

† Three proton intensity.

trum of this monoacetate clearly showed that it was a C-9 acetate as the two H-9 doublets (J = 12 Hz) at δ3.64 and 3.84 in helibracteatinine were shifted downfield and resonated at δ4.62. In the case of helibracteatine (**2**) the H-9 protons resonated at δ4.35 and 4.46 as doublets (J = 12 Hz) indicating the esterification at C-9. The resonance positions of other signals (Table 1) and mass spectral fragmentations also reinforced helibracteatinine as 9-angelyloxymethyl-pyrrolizidine-1,7-diol exclusive of stereochemistry (**1b**). To unravel the nature of the necine moiety, both the ester alkaloids were subjected to alkaline hydrolysis. These afforded the identical free necine, helibracteatinecine (**3**). On comparison of helibracteatinecine (**3**) with helibractinecine (**4**), a free necine isolated from *H. bracteatum* [2] revealed that this necine (**3**) is a diastereoisomer of helibractinecine (**4**) and its structure was elucidated.

Helibracteatinecine (**3**) was obtained as a semi-solid, $[\alpha]_{Hg}^{25}$ + 12.1° (EtOH; *c* 0.60). The molecular formula of this compound was deduced as $C_8H_{15}NO_3$ (HR-MS) and its IR spectrum was devoid of C=O and C=C groups, indicating it was a saturated necine. The mass spectral fragmentations of helibracteatinecine were similar to that of helibractinecine and differed only in the relative intensity, suggested a diastereomeric relationship between them. This was borne out by the 300 MHz ^{1}H NMR spectrum of helibracteatinecine (in $CDCl_3 + CD_3OD$, Table 1). The diagnostic H-7 signal which resonated at δ3.94 as a quartet, the C-9 methylene was at δ3.79 and 3.84 as the characteristic doublet and the H-8 signal which resonated at δ3.12 as a doublet (J = 6.9 Hz) were in favour of the structure **3**. The assignments for the remaining protons were confirmed by double resonance studies (Table 2). The structure **3** assigned to helibracteatinecine was established by the ^{13}C NMR spectral data. The assignments shown in Table 3 were made in comparison with ^{13}C NMR values of reference compounds helibractinecine (**4**), hadienecine (**5**), curassanecine (**6**) and its diastereoisomer (**7**) which possess a C-1 hydroxyl group [2, 7–9]. Corroborative evidence for structure **3** was obtained from the mass spectrum. The mass spectral fragmentation process was reminscent of a saturated pyrrolizidine alkaloid with several hydroxyl groups reported by Aasen *et al.* [6]. The stereochemistry of **3** at C-7 and C-8 was deduced from the chemical reactions of helibracteatinine (**1**). This on dehydration gave a product which was identical in all respects with 7-angelyl heliotridine (**8**) [10]. This confirmed the stereochemistry of the hydrogens at C-7 and C-8 are α in helibracteatinine and hence in **3**. Establishment of the stereochemistry at C-1 was made with the help of ^{13}C NMR spectral data. The ^{13}C NMR values of **6** and **7** (Table 3) which present a structural analogy with **3** and **4** have been furnished by Gramain *et al.* [7]. The stereochemistry at C-1 is characterized by the + 5.63 and + 2.14 ppm shifts at C-2 and C-9 in the pyrrolizidine nucleus with the α-CH_2OH when compared with β-CH_2OH configuration at C-1 which is due to the close proximity of the C-1/C-9 bond to C-2 and thus causing a deshielding effect. Shifts of − 4.15 and − 1.44 ppm for C-2 and C-9 in helibracteatinecine as opposed to helibractinecine is an indication that C-1 has a β-CH_2OH and thus establishing the structure of helibracteatinecine (**3**) as 1β-hydroxymethyl-8α-pyrrolizidine-1α,7α-diol. Therefore, the structure of helibracteatinine and helibracteatine are **1** and **2**, respectively.

Table 2. NMR irradiation data of compound 3

Sl. no.	Signal irradiated	Signal affected	Signal observed
1	δ1.63 (H-2, dist. *q*)	δ2.55 (H-3β, *dt*)	*d*
		δ3.26 (H-3α, *m*)	simplified
2	δ2.55 (H-3β, *dt*)	δ3.26 (H-3α, *m*)	simplified
		δ1.63 (H-2, dist. *q*)	*d*
3	δ2.52 (H-5β, *dt*)	δ3.26 (H-5α, *m*)	simplified
		δ1.84 (H-6u, *m*)	simplified
		δ2.11 (H-6d, *m*)	*dd*
4	δ3.26 (H-3α & H-5α, *m*)	δ2.52 (H-5β, *dt*)	simplified
		δ2.55 (H-3β, *dt*)	simplified
		δ1.84 (H-6u, *m*)	simplified
		δ2.11 (H-6d, *m*)	simplified
		δ1.63 (H-2, dist. *q*)	*d*
5	δ1.84 (H-6u, *m*)	δ2.11 (H-6d, *m*)	simplified
		δ3.94 (H-7, *q*)	*t*
		δ3.26 (H-5α, *m*)	simplified
		δ2.52 (H-5β, *dt*)	*dd*
6	δ2.11 (H-6d, *m*)	δ1.84 (H-6u, *m*)	simplified
		δ3.94 (H-7, *q*)	*t*
		δ2.52 (H-5β, *dt*)	*t*
		δ3.26 (H-5α, *m*)	simplified
7	δ3.94 (H-7, *q*)	δ3.12 (H-8, *d*)	*s*
		δ1.84 (H-6u, *m*)	simplified
		δ2.11 (H-6d, *m*)	*dd*
8	δ3.12 (H-8, *d*)	δ3.94 (H-7, *q*)	*t*
9	δ3.82 (near the centres of H-9u, *d* & H-9*d*,*d*)		

Table 3. ^{13}C NMR spectral data for compound, 3 and its related alkaloids

C	3*	4 [2]*	5 [8]	6 [7]	7 [7]
1	82.6 *s*	82.8	81.9	80.2	82.5
2	34.1 *t*	38.3	36.9	38.9	33.3
3	53.0 *t*	53.4	54.3	55.7	64.1
5	52.9 *t*	54.5	54.3	53.8	64.0
6	33.3 *t*	36.2	35.7	25.1	25.1
7	79.7 *d*	73.7	69.7	27.7	28.5
8	73.0 *d*	71.2	80.7†	70.9	70.9
9	67.2 *t*	68.6	65.2	67.8	65.6

* Run in $CDCl_3$–CD_3OD at 75.43 MHz with TMS as internal standard. Assignments made by using proton-noise decoupled spectra and single frequency off-resonance decoupling to identify multiplets.

† High value.

Usually a plant contains the pyrrolizidine ester and its corresponding free necine. Occurrence of helibracteatinine (1) and helibracteatine (2) in *H. bracteatum* along with its diastereoisomeric free necine, helibractinecine (4) is hitherto not reported. This infers that the plant elaborates both the ester alkaloid and free necine independently and probably free necines are not esterified to produce pyrrolizidine ester alkaloids.

EXPERIMENTAL

Heliotropium bracteatum. R.Br. (2 kg dry wt) collected during its flowering season (July/August) was processed for alkaloids as described previously [2]. Other experimental details were also presented in ref. [2].

Isolation of new alkaloids. Alkaloid fr. A (220 mg) was dissolved in a min. vol of $CHCl_3$ and applied to a column of neutral Al_2O_3 (50 g) set in $CHCl_3$. The column was initially eluted with $CHCl_3$ and then with a $CHCl_3$–MeOH gradient. Fractions (10 ml) were monitored by TLC on silica gel impregnated with 0.1 M NaOH, developed with MeOH. Frs 1–3 ($CHCl_3$) yielded mainly non-basic impurities. Frs 6–9 ($CHCl_3$–MeOH, 49:1) contained mainly helibracteatininine (40 mg) and 12–17 ($CHCl_3$–MeOH, 49:1) helibracteatine (60 mg). Helibracteatinine and helibracteatine were further purified by rechromatography of their combined fractions on alkalized (0.1 M NaOH) silica gel as described in ref. [2] to yield helibracteatinine (18 mg) and helibracteatine (23 mg).

Helibracteatinine was a pale yellow gum, $[\alpha]_{Hg}^{25}$ + 10.85° (EtOH; *c* 0.64) R_f 0.54 (S_1) and 0.8 (S_2). IR ν_{max}^{Neat} cm^{-1}: 3360, 2940, 1710, 1650, 1470, 1390, 1370, 1240, 1170, 1055, 930, 860, 770. ^{13}C NMR in Tables 1 and 3. HRMS *m/z* (rel. int.): 255.1462 $[M]^+$ (2) (Calc for $C_{13}H_{21}O_4N$ 255.1490), 238 (3), 237 (3.5), 224 (9), 198 (7), 181 (60), 156 (16), 155 (93), 154 (10), 124 (12), 122 (21), 100 (23), 98 (20), 83 (23), 82 (100).

Acetylation of helibracteatinine. Helibracteatinine (20 mg) and Ac_2O (0.5 ml) were mixed and kept at room temp. for 6 hr. Then H_2O (1 ml) was added and basified to pH 10.5 with NH_4OH. Acetate was extracted with $CHCl_3$, washed with aq. $NaHCO_3$, dried and purified by prep. TLC on alkalized (0.1 M) silica gel (S_2). A pale yellow gum (14 mg) homogeneous by TLC (R_f 0.63, S_1) was obtained and could not be induced to crystallize IR: ν_{max}^{Neat} cm^{-1}: 3330, 1740, 1710, 1230. ^1H NMR ($CDCl_3$). 1.9 (4H, *m*), 2.04 (7H, *br s*), 2.25 (2H, *m*), 2.5 (1H, *m*), 2.75 (1H, *m*), 3.22 (2H, *m*), 3.81 (1H, *d*, *J* = 5 Hz), 4.62 (2H, *br s*), 5.30 (1H, *q*, *J* = 5 Hz) and 6.12 (1H, *q*, *J* = 7 Hz).

Dehydration of helibracteatinine. To a soln of **1** (30 mg) in pyridine (3 ml) was added MsCl (75 μl), and the mixture was boiled under reflux for 30 min. After removing excess reagent *in vacuo*, the residue was partitioned between aq. Na_2CO_3 (*ca* 10 ml) and $CHCl_3$ (25 × 4 ml). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evapd to yield a solid residue (12 mg). Upon purification on prep TLC on alkalized (0.1 M) silica gel with S_2, afforded **8** (4 mg) mp 115–116°, undepressed with authentic 7-angelyl heliotridine. $[\alpha]_D^{25}$ + 10.9° (*c* 0.2; EtOH); IR ν_{max}^{KBr} cm^{-1} 3380 (OH), 1700 (ester).

Helibracteatine (**2**) was a brown gum, $[\alpha]_{Hg}^{25}$ + 3.64° (EtOH; *c* 0.33) R_f 0.43 (S_1) and 0.52 (S_2); IR: ν_{max}^{Neat} cm^{-1}: 3380, 2950, 1710, 1650, 1470, 1390, 1360, 1240, 1165, 1055, 860, 770. ^1H and ^{13}C NMR spectra in Tables 1 and 3. HRMS *m/z* (rel. int.) 255.1467 $[M]^+$ (0.5), 238 (6.2), 237 (1), 156 (43.4), 155 (46.5), 138 (16.3), 122 (2.3), 111 (99.7), 99 (31), 98 (100), 82 (35).

Hydrolysis of ester alkaloids. The ester alkaloids (40 mg) dissolved in 1 ml of EtOH was refluxed with 2 ml of 15% NaOH on a steam bath for 1 hr. The reaction mixt was cooled and extracted exhaustively with $CHCl_3$. The $CHCl_3$ extract dried and evapd to afford free necine (15 mg).

Both helibracteatinine (**1**) and helibracteatine (**2**) on hydrolysis gave identical necine, helibracteatinecine (**3**), gum, $[\alpha]_{Hg}^{25}$ + 12.1° (EtOH; *c* 0.6). IR: ν_{max}^{Neat} cm^{-1} 3350, 2930, 2860, 1560, 1420, 1200, 1100, 1060 and 840. ^1H and ^{13}C NMR in Tables 1 and 3. HRMS *m/z* (rel. int.): 173.1058 $[M]^+$ (19.5) (Calc. for $C_8H_{15}O_3N$ 173.1052), 156 (4.5), 155 (14), 142 (2.7), 129 (12), 99 (84.9), 98 (100), 82 (83.5).

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