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PYRROLIZIDINE ALKALOIDS FROM *CYNOGLOSSUM CRETICUM*

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Key Word Index—*Cynoglossum creticum*; Boraginaceae; pyrrolizidine alkaloids; gas chromatography-mass spectrometry; NMR.

Abstract—3′-Acetylrinderine, 3′-acetylechinatine, 3′-acetylheliosupine and heliosupine were isolated from the aerial parts of *Cynoglossum creticum* and characterized by mass spectrometry and ^{1}H and ^{13}C NMR. A GC-mass spectrometric analysis revealed the presence of 13 alkaloids altogether, among them the previously reported alkaloids, echinatine, rinderine and 7-angeloylheliotridine, and traces of 7-senecioylheliotridine, supinine and trachelanthamine.

INTRODUCTION

The broad range of pharmacological and toxicological activities [1–6], such as carcinogenic, hepatotoxic, antitumour, antispasmodic and mydriatic properties, associated with pyrrolizidine alkaloids (PAs) has continued to generate extensive studies on these compounds. Besides their presence in some traditional herbal medicines, they may also occur as low-level contaminants in some human diets, such as honey and milk [1, 2, 5].

In previous investigations [7, 8], heliosupine and echinatine were isolated as major alkaloids and rinderine, 7-angeloylheliotridine and cynoglossamine as minor alkaloids from *Cynoglossum creticum*. The present study reports the isolation and structural elucidation of 3′-acetylrinderine as a major component (41.5%), as well as of 3′-acetylechinatine and 3′-acetylheliosupine, which have not been recorded before from this species. In addition, a GC-mass spectrometric study revealed the presence of 13 PAS altogether.

RESULTS AND DISCUSSION

Four pyrrolizidine alkaloids (**1–4**) were isolated by prep. TLC and their structures determined by mass spectrometry, ^{1}H and ^{13}C NMR (Tables 1 and 2). Alkaloid **1** was obtained as a colourless oil and its mass spectrum showed a $[M]^{+}$ at m/z 341, corresponding to the formula $C_{17}H_{27}NO_6$. The mass spectrum exhibited a base peak at m/z 138 and the typical fragmentation of an unsaturated necine with a free hydroxyl group at C-7, such as retronecine or heliotridine. In order to corroborate the structure of **1**, its ^{1}H and ^{13}C NMR spectra were recorded and compared with those of a structurally related compound, such as rinderine [9]. The ^{13}C NMR spectrum exhibited signals at δ 135.5 and δ 128.4, which indicated a double bond between C-1 and C-2. The signals at δ 80.4 (C-8), δ 75.1 (C-7) and δ 34.0 (C-6) suggested the presence of a heliotridine C-9 monoester. The ^{1}H NMR spectrum of **1** showed a one proton quartet at δ 5.28 coupled only to a three-proton doublet at δ 1.29. This indicates that the secondary alcohol at C-3′, is, in fact, the acetylated one, since the quartet for the corresponding methine occurs at δ 4.08, as in rinderine [9], the parent alkaloid. Proton-signals at δ 2.04 (3 H, *s*, 3′-H) indicate an acetyl ester moiety. Thus, alkaloid **1** was identified as 3′-acetylrinderine. Only the GC-mass spectrometry data of this compound have been reported so far [10, 11], which are consistent with those of our compound. The same fragmentation pattern as in compound **1** was observed in the mass spectrum of compound **2**, indicating that **2** is a stereoisomer of **1**. Based upon mass spectra and ^{1}H NMR, compound **2** was identified as 3′-acetylechinatine, and alkaloids **3** and **4** as heliosupine [7, 8] and 3′-acetylheliosupine [12], respectively.

GC-mass spectrometry is the method of choice for separation and convenient identification of PAs, even in minute quantities or in diastereoisomeric forms [13–18]. An alkaloid extract of aerial parts of *C. creticum* was analysed in this way. Thirteen PAs were detected and identified by direct comparison (mass spectrum and

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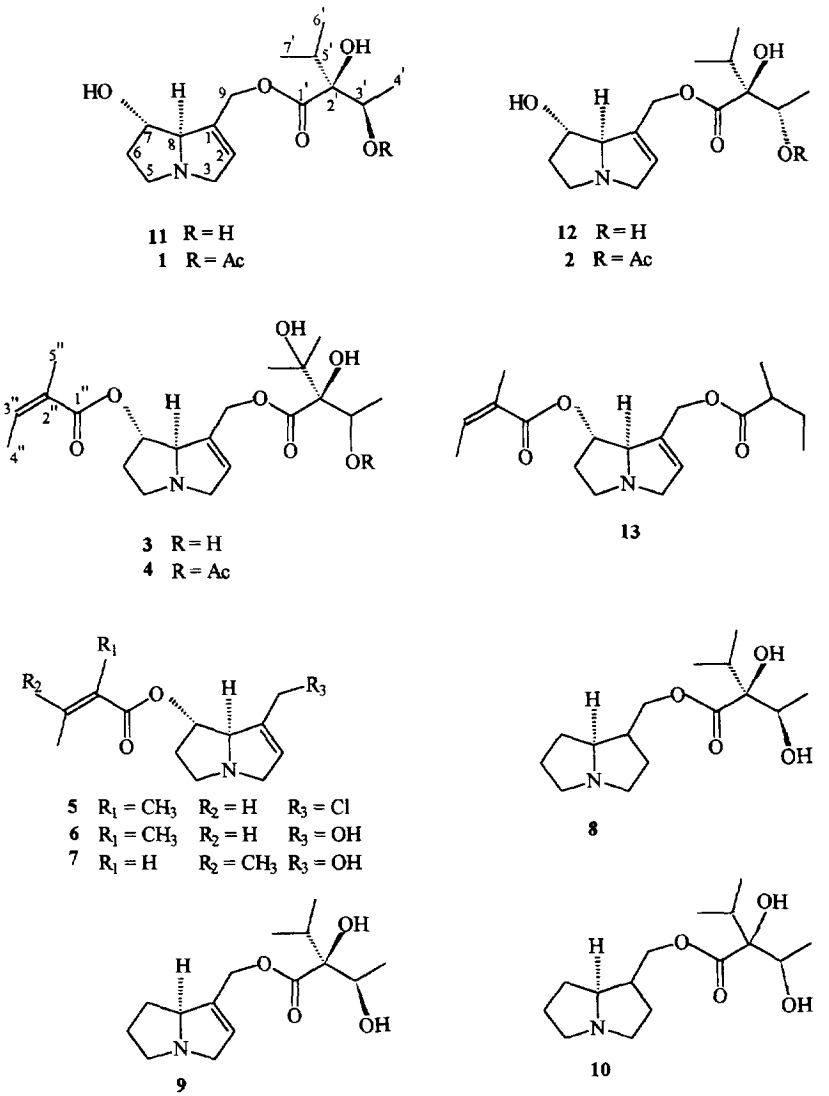

Fig. 1. Structures of pyrrolizidine alkaloids found in *Cynoglossum creticum*.

retention index) with authentic material or by comparison of their mass spectra with literature data [13, 17, 18] (Tables 3 and 4). Nine alkaloids could be unambiguously identified as 3′-acetylrinderine (**1**), 3′-acetylechinatine (**2**), heliosupine (**3**), 3′-acetylheliosupine (**4**), 7-angeloylheliotridine (**6**), 7-senecioylheliotridine (**7**), supinine (**9**), rinderine (**11**) and echinatine (**12**). The remaining four alkaloids, **5**, **8**, **10**, and **13** were only tentatively identified, since amounts were too limited for a thorough spectroscopic analysis.

Alkaloid **5** showed a $[M]^+$ at *m/z* 255, which is consistent with the molecular formula $C_{13}H_{18}NO_2Cl$. The ion series *m/z* 136 to 80 were characteristic of the 1,2-unsaturated pyrrolizidine nucleus [19]. The fragment ion at *m/z* 220 $[M - 35]^+$ is probably due to the loss of chloride at C-9 and the fragment ion at *m/z* 155 due to the loss of the acid attached to C-7 $[M - 100]^+$. These fragmentations support the tentative structure of compound **5** as 7α-angeloyl-1-chloromethyl-1,2-dehydropyrrolizidine. Whether this halogenated compound is a natural product or an artefact needs to be confirmed.

The mass spectrum of compound **10** was virtually identical to **8** indicating that it is a diastereoisomer of **8**. Both compounds were detected as trace components with a base peak at *m/z* 124, indicating the presence of a saturated necine of the pyrrolizidine-1-yl methanol type [20]. Based upon mass spectral fragmentation and GC elution sequence reported in ref. [21], compound **8** was tentatively identified as trachelanthamine and **10** as a stereoisomer of **8**.

Compound **13** showed a $[M]^+$ at *m/z* 321 corresponding to the formula $C_{18}H_{27}NO_4$. The fragment *m/z* 220 possibly refers to 7-angeloyldehydroxyheliotridine $[M - C\text{-}9 \text{ ester}]^+$ and *m/z* 221 to $[M - \text{angelic acid}]^+$ [19]. A peak ion at *m/z* 57 (C_4H_9) must be derived

Table 1. ^{1}H NMR data of compounds **1–4** (300 MHz, $CDCl_3$)

H	1	2	3	4
2	5.76, 1H, *br s*	+	5.88, 1H, *br s*	5.78, 1H, *br s*
3u	3.37, 1H, *dm*	+	3.36, 1H, *dm*	3.36, 1H, *dm*
3d	3.92, 1H, *m*	+	3.99, 1H, *br d* (15.8)	3.97, 1H, *br d* (15.8)
5u	2.68, 1H, *m*	+	2.87, 1H, *m*	2.85, 1H, *m*
5d	3.30, 1H, *m*	+	3.21, 1H, *m*	3.23, 1H, *m*
6u	1.91, 1H, *m*	+	1.94, 1H, *m*	1.94, 1H, *m*
6d	1.98, 1H, *m*	+	1.94, 1H, *m*	1.94, 1H, *m*
7	4.21, 1H, *td*	4.17, 1H, *dt*	5.19, 1H, *m*	5.05, 1H, *m*
8	3.96, 1H, *m*	+	4.15, 1H, *m*	4.09, 1H, *m*
9u	4.66, 1H, *br d* (12.1)	4.78, 1H, *br d* (12.1)	4.93, 1H, *br d* (13)	4.86, 1H, *br d* (13)
9d	4.93, 1H, *br d* (12.1)	4.96, 1H, *br d* (12.1)	4.99, 1H, *br d* (13)	4.94, 1H, *br d* (13)
3′	5.28, 1H, *q* (6.7)	5.21, 1H, *q* (6.7)	4.19, 1H, *q* (6.3)	5.43, 1H, *q* (6.3)
4′	1.25, 3H, *d* (6.7)	1.29, 3H, *d* (6.7)	1.27, 3H, *q* (6.3)	1.37, 3H, *q* (6.3)
5′	2.01, 1H, *m*	+	—	–
6′	0.92, 3H, *d* (6.7)	0.88, 3H, *d* (6.7)	1.25, 3H, *s*	1.16, 3H, *s*
7′	0.98, 3H, *d* (6.7)	0.96, 3H, *d* (6.7)	1.29, 3H, *s*	1.38, 3H, *s*
3′-OAc	2.02, 3H, *s*	2.04, 3H, *s*	—	1.96, 3H, *s*
3″	—	—	6.12, 1H, *qq* (1.4, 7.2)	6.10, 1H, *qq* (1.4, 7.2)
4″	—	—	1.97, 3H, *dq* (1.5, 7.2)	1.97, 3H, *dq* (1.5, 7.2)
5″	—	—	1.86, 3H, *quin* (1.4)	1.86, 3H, *quin* (1.4)

Figures in parentheses are coupling constants in Hz.
+ Signals identical to corresponding signals of **1**.

from a side-chain at C-9 after decarboxylation. Based upon biogenic considerations and mass fragmentation, compound **13** was tentatively identified as 7-angeloyl-9-(2-methylbutyryl)heliotridine.

The alkaloid composition of *C. creticum* (Table 4) was found to differ significantly from data published before [7, 8]. Whereas 3′-acetylrinderine, rinderine and 3′-acetylechinatine figure as major alkaloids in our study, heliosupine and echinatine (which were also found by us) had been identified as major components in a previous investigation [7, 8]. This difference could be due to the presence of ecotypes of *C. creticum*, differing environmental conditions or misidentification of species used in previous studies.

Table 2. ^{13}C NMR data of compounds **1**, **3** and **4** (75 MHz, $CDCl_3$)

C	1	3	4
1	135.5 *s*	134.3 *s*	134.5 *s*
2	128.4 *d*	129.8 *d*	128.7 *d*
3	61.9 *t*	62.2 *t*	62.1 *t*
5	54.2 *t*	54.3 *t*	54.3 *t*
6	34.0 *t*	30.2 *t*	30.3 *t*
7	75.2 *d*	76.8 *d*	76.7 *d*
8	80.4 *d*	79.2 *d*	79.8 *d*
9	62.4 *t*	62.5 *t*	62.6 *t*
1′	174.3 *s*	174.3 *s*	173.2 *s*
2′	81.8 *s*	82.7 *s*	82.8 *s*
3′	72.5 *d*	69.9 *d*	72.6 *d*
4′	21.1 *q*	18.5 *q*	20.5 *q*
5′	33.1 *d*	73.9 *s*	73.1 *s*
6′	16.8 *q*	25.9 *q*	26.6 *q*
7′	17.2 *q*	24.8 *q*	24.5 *q*
3′-OAc:C=O	170.2 *s*	—	169.5 *s*
3′-OAc:Me	13.9 *q*	—	15.2 *q*
1″	—	168.3 *s*	167.9 *s*
2″	—	127.5 *s*	127.6 *s*
3″	—	139.1 *d*	138.6 *d*
4″	—	15.9 *q*	15.8 *q*
5″	—	20.4 *q*	21.0 *q*

Multiplicities by DEPT pulse sequence.

EXPERIMENTAL

Plant material and alkaloid extraction. Aerial parts of *C. creticum* Miller, cultivated in the Botanical Garden Heidelberg, Germany, were collected in August 1995. A voucher specimen is deposited at the Institut für Pharmazeutische Biologie Heidelberg; its identity was confirmed by Prof. Dr W. Greuter (Botanical Garden, Berlin). Plant material (80 g fr. wt) was extracted twice with 0.5 N HCl through homogenization in a Ultra-turrax and left to stand for 1 hr. The extract was adjusted to 2N HCl and PA-*N*-oxides reduced with Zn dust with stirring overnight. Excess Zn was removed by filtration. Basification of the aq. acid soln with NH_4OH was followed by extraction with CH_2Cl_2, drying (Na_2SO_4) and evapn of solvent to give 290 mg of the total alkaloidal fr.; total alkaloid content = 0.36% (fr. wt). Prep. TLC [silica gel F_{254}, CH_2Cl_2–MeOH–NH_4OH (25%), 85:15:2] yielded alkaloids **1–4**.

Analysis. A fused silica capillary column (DB1) was directly coupled to a quadrupole mass spectrometer. EI-MS were recorded at 40 eV. Conditions: inj. 250°; temp. prog. 150–300°, 6° min^{-1}; split ratio 1:20; carrier gas He, 0.5 bar. Routine FID-GC measurements

Table 3. Identification of pyrrolizidine alkaloids from *Cynoglossum creticum* by GC-mass spectrometry

Alkaloid	$[M]^+$	Characteristic ions (relative abundance)	Ref.
1 3′-Acetylrinderine*	341	326 (0.1), 298 (1), 255 (1), 254 (0.5), 181 (5), 156 (5), 139 (30), 138 (100), 137 (17), 136 (17), 120 (10), 99 (10), 95 (9), 94 (35), 93 (95), 80 (13), 67 (9), 43 (36).	[10, 11]
2 3′-Acetylechinatine*	341	326 (0.1), 298 (1), 255 (2), 254 (2), 181 (2), 156 (4), 139 (21), 138 (100), 137 (10), 136 (10), 120 (6), 99 (6), 94 (20), 93 (71), 80 (8), 67 (5), 43 (22).	
3 Heliosupine	397	382 (0.1), 352 (0.1), 297 (2), 238 (4), 221 (29), 220 (100), 141 (11), 138 (10), 137 (8), 136 (50), 121 (40), 120 (95), 119 (70), 106 (10), 94 (26), 93 (52), 83 (12), 80 (10), 59 (10), 55 (15), 43 (15).	[19]
4 3′-Acetylheliosupine*	439	424 (0.1), 321 (1), 221 (28), 220 (100), 141 (10), 138 (4), 137 (5), 136 (45), 121 (14), 120 (89), 119 (82), 106 (7), 94 (21), 93 (40), 83 (11), 80 (5), 59 (8), 55 (11), 43 (22).	[12]
5 7α-Angeloyl-1-chloromethyl-1,2-dehydropyrrolizidine*†	255	220 (40), 172 (15), 155 (45), 136 (23), 130 (24), 129 (32), 128 (63), 121 (11), 120 (94), 119 (24), 106 (30), 94 (100), 93 (20), 83 (20), 80 (17), 67 (8), 55 (35).	
6 7-Angeloylheliotridine	237	219 (1), 154 (2), 137 (42), 136 (20), 124 (25), 111 (35), 106 (86), 94 (25), 83 (10), 80 (100), 68 (10), 55 (20).	[13, 23]
7 7-Senecioylheliotridine*	237	137 (35), 136 (18), 124 (20), 111 (35), 106 (90), 94 (21), 83 (15), 80 (100), 68 (8), 55, (18).	[13]
8 Trachelanthamine (or its isomer)*†	285	267 (5), 252 (4), 240 (4), 142 (50), 125 (18), 124 (100), 110 (4), 96 (6), 83 (20), 82 (11), 70 (6), 55 (14), 43 (12).	[21]
9 Supinine*	283	140 (6), 123 (25), 122 (100), 121 (40), 120 (49), 108 (11), 93 (20), 80 (8), 70 (7), 53 (5), 45 (4), 43 (12).	[13, 21]
10 Isomer of trachelanthamine*†	285	267 (4), 252 (3), 240 (4), 142 (30), 125 (12), 124 (100), 110 (5), 96 (7), 83 (22), 82 (7), 70 (5), 55 (13).	[21]
11 Rinderine	299	284 (0.1), 254 (0.6), 156 (9), 139 (35), 138 (100), 137 (10), 136 (10), 120 (5), 95 (12), 94 (23), 93 (70), 80 (12), 67 (7), 53 (5), 43 (16).	[13]
12 Echinatine	299	284 (0.1), 254 (1), 156 (10), 139 (30), 138 (100), 137 (7), 136 (4), 95 (9), 94 (25), 93 (64), 80 (7), 67 (5), 53 (3), 43 (12).	[19]
13 7-Angeloyl-9-methylbutyrylheliotridine (or its isomer)*†	321	221 (50), 220 (82), 195 (5), 141 (28), 138 (3), 137 (8), 136 (87), 121 (13), 120 (100), 119 (68), 106 (15), 94 (44), 93 (52), 83 (25), 80 (12), 67 (7), 57 (22), 55 (28).	

*New for *C. creticum*.
†Tentative identification.

were performed under the following conditions: DB1-30W fused silica capillary column 30 m × 0.317 mm inner diameter; carrier gas He; det. temp. 300°; inj. temp. 250°; oven temp. prog.; initial temp. 170°, 5 min isothermal, 170–300°, 10° min^{-1}, 300°, 15 min isothermal. Kovats retention indices [22] were calculated with respect to a set of co-injected even-numbered hydrocarbons (C_{14}–C_{28}). Each index was subjected to a library search by comparison with the reference indices stroed in a data base of the Institute of Pharmaceutical Biology. EI-MS were recorded at 70 eV by direct inlet. ^1H and ^{13}C NMR were recorded in $CDCl_3$, at 400 and 100 MHz, respectively.

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Table 4. Pyrrolizidine alkaloid profile of *Cynoglossum creticum* as determined by GC and GC-mass spectrometry

Alkaloid	Kovat's retention index	Area (%)*
1 3′-Acetylrinderine	2222	41.48
2 undetermined PA	2272	8.26
3 Heliosupine	2553	6.29
4 3′-Acetylheliosupine	2640	6.79
5 7α-Angeloyl-1-chloromethyl-1,2-dehydropyrrolizidine	1815	trace
6 7-Angeloylheliotridine	1820	5.62
7 7-Senecioylheliotridine	1870	trace
8 Trachelanthamine (or its isomer)	1970	trace
9 Supinine	1978	trace
10 Isomer of trachelanthamine	2010	trace
11 Rinderine	2155	16.21
12 Echinatine	2175	15.35†
13 7-Angeloyl-9-methylbutyrylheliotridine (or its isomer)	2180	15.35†

*Total alkaloid = 100%.

†Compound coeluted. This value refers to the sum of both compounds.

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