Pergamon

0031-9422(95)00847-0

Phytochemistry, Vol. 42, No. 1, pp. 225–230, 1996

0031-9422/96 $15.00 + 0.00

PYRROLIZIDINE AND TETRAHYDROISOQUINOLINE ALKALOIDS FROM *ECHIUM HUMILE*

A. EL-SHAZLY,*†§ T. SARG,* A. ATEYA,* E. ABDEL AZIZ,* S. EL-DAHMY,* L. WITTE‡ and M. WINK†§

*Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt; ‡Institut für Pharmazeutische Biologie der Technischen Universität, Mendelssohnstrasse 1, D-38106 Braunschweig, Germany; †Universität Heidelberg, Institut für Pharmazeutische Biologie, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

(*Received in revised form* 26 *October* 1995)

Key Word Index—*Echium humile*; Boraginaceae; pyrrolizidine alkaloids; echihumiline; pycnanthine; echihumiline *N*-oxide; 7- and 9-senecioylretronecine; lycopsamine; 7-acetyl-lycopsamine; tetrahydro-isoquinoline; carnegine.

Abstract—Three new pyrrolizidine alkaloids, echihumiline, pycnanthine and echihumiline *N*-oxide, were isolated from *Echium humile* and their structures determined by spectroscopic methods. In addition, we record the presence of the known alkaloids, 7-senecioylretronecine, 9-senecioylretronecine, lycopsamine and 7-acetyl-lycopsamine. The tetrahydro-isoquinoline alkaloid, carnegine, was recorded for the first time in the Boraginaceae.

INTRODUCTION

Echium humile is a small hispid biennial to perennial herb growing naturally in north Africa and southern Europe [1]. Members of the genus *Echium* are known to contain pyrrolizidine alkaloids, consisting of monoester and/or open-chain diesters [2–4]. These types of alkaloids are well known for their remarkable biological activities, including carcinogenic and/or hepatotoxic properties. They represent a major hazard to livestock. This study is the first phytochemical investigation of *E. humile*, and has led to the isolation of three new pyrrolizidine alkaloids, echihumiline, pycnanthine and echihumiline *N*-oxide, as major components. The minor constituents were separated and identified by capillary GC–mass spectrometry as 7-senecioylretronecine, 9-senecioylretronecine, lycopsamine and 7-acetyl-lycopsamine; we also tentatively identified 7-(2-methylbutyryl) retronecine, 7 - (2 - methylbutyryl) - 9 - (2,3 - dihydroxybutyryl)retronecine, 7-senecioylretronecine and 7-(2-methylbutyryl)-9-echimidinylretronecine (or their isomers). In addition, the tetrahydro-isoquinoline alkaloid, carnegine, was recorded for the first time in the Boraginaceae.

RESULTS AND DISCUSSION

Column chromatography and preparative TLC of the alkaloidal extract of flowering *E. humile* plants provided three pyrrolizidine and two tetrahydro-isoquinoline alkaloids. Compound **1** was an oil with a $[M]^+$ at *m/z* 397 (corresponding to $C_{20}H_{31}NO_7$), with a base peak at *m/z* 220 and the ion series, *m/z* 136, 120, 119, 93 and 80, which are chracteristic of 1,2-unsaturated diester pyrrolizidine alkaloids [5]. The base peak at *m/z* 220 is the result of cleavage of the weak allylic ester bond; the ion at *m/z* 297 is due to loss of the acid attached at C-7 [5]. The ^{1}H NMR (Table 1) showed the presence of a senecioic acid ester moiety, an olefinic proton at δ 5.59 (1H, *sept*, $J = 1.5$ Hz) coupling with H-20 at δ 1.90 (3H, *d*, $J = 1$ Hz) at δ 1.90 and H-21 at δ 2.15 (3H, *d*, $J = 1$ Hz). The chemical shifts of the second acid protons were assigned by comparison with the reported data [6, 7] for echimidinic acid. The chemical shifts of the ring protons were in close agreement with the values reported for other acyclic diester retronecine alkaloids [8, 9]. The ^{13}C NMR spectrum (Table 2) exhibited signals at δ 132.8 and δ 127.6, indicating a double bond between C-1 and C-2. Also, signals at δ 76.08 (C-8), δ 73.66 (C-7) and δ 34.23 (C-6) imply the presence of a retronecine diester [6, 10]. The senecioic acid ester moiety was also confirmed in the ^{13}C NMR spectrum by the signals at δ 115.3 for the olefinic C-18 and δ 159.0, δ 27.7 and δ 20.4 for C-19, C-20 and C-21, respectively [11, 12]. Thus, alkaloid **1** was identified as 7-senecioyl-9-echimidinylretronecine. It has not been previously described in the literature and the name, echihumiline, is proposed.

EI mass spectrometry confirmed the $[M^+]$ of com-

Table 1. ^{1}H NMR ($CDCl_3$, 400 MHz) data of alkaloids **1–3**

H	1	2	3
2	5.87, 1H, *br s*	5.83, 1H, *br s*	5.97, 1H, *br s*
3 u	3.53, 1H, *m*	3.55, 1H, *m*	4.54, 1H, *dm*
3 d	3.72, 1H, *m*	4.06, 1H, *m*	4.77, 1H, *dm*
5 u	2.83, 1H, *m*	2.80, 1H, *m*	3.73, 1H, *m*
5 d	2.83, 1H, *m*	3.50, 1H, *m*	4.13, 1H, *m*
6	2.09, 2H, *m*	2.15, 2H, *m*	2.24, 2.89, 2H, *m*
7	5.48, 1H, *m*	5.47, 1H, *m*	5.77, 1H, *m*
8	4.13, 1H, *m*	4.12, 1H, *m*	5.45, 1H, *m*
9 u	4.65, 1H, *dm* (11.8)	4.65, 1H, *m*	4.71, 1H, *dm*
9 d	4.96, 1H, *d* (3.3)	4.75, 1H, *d* (13.4)	4.94, 1H, *dm*
12	4.21, 1H, *q* (6.3)	3.99, 1H, *q* (6.6)	4.22, 1H, *q* (6.4)
13	1.26, 3H, *d* (6.4)	1.26, 3H, *d* (6.7)	1.27, 3H, *d* (6.5)
14	–	2.15, 1H, *m*	–
15	1.24, 3H, *s*	0.93, 3H, *d* (6.7)	1.25, 3H, *s*
16	1.31, 3H, *s*	0.85, 3H, *d* (6.7)	1.30, 3H, *s*
18	5.59, 1H, *s*	5.92, 1H, *s*	5.57, 1H, *s*
20	1.90, 3H, *d* (1)	3.65, 2H, *m*	1.92, 3H, *d* (1)
20-OH	–	4.68, OH, *m*	–
21	2.15, 3H, *d* (1)	2.06, 3H, *s*	2.17, 3H, *d* (1)

u = upfield, d = downfield.
Figures in parentheses are coupling constants in Hz.

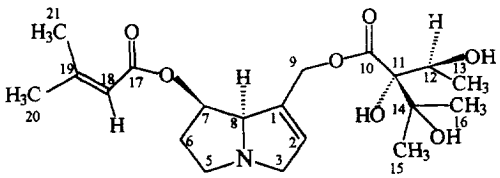
unsaturated diester pyrrolizidine alkaloids [5]. The base peak at *m/z* 236 (220 + 16) is due to allylic fission of the esterified acid at C-9 and the signal at *m/z* 281 is formed through loss of the acid attached at C-7. This acid has 16 mu more than senecioic acid (or its isomer), indicating the presence of a hydroxymethyl group. The ^{1}H NMR spectrum (Table 1) proved the presence of a hydroxysenecioic acid moiety with an olefinic proton H-18 at δ 5.92 (1H, *sept*, J = 1.5 Hz), H-21 at δ 2.06 (3H, *s*) and H-20 at δ 3.65 (2H, *m*) [12, 13]. The signals at δ 3.99 (1H, *q*, J = 6.6, Me–CH–OH) and δ 1.26 (3H, *d*, J = 6.7, Me–CH–OH) confirmed the presence of a (+)-trachelanthic acid ester. The ^{13}C NMR spectrum (Table 2) exhibited signals at δ 132.8 and δ 126.0, indicating a double bond between C-1 and C-2. The signals at δ 75.9 (C-8), δ 72.4 (C-7) and δ 34.2 (C-6) are characteristic of a retronecine diester [6, 10]. The ester moiety at C-7 was also confirmed in the ^{13}C NMR spectrum by the signal at δ 112.4, owing to the olefinic C-18, and the signal at δ 66.8 for C-20 (hydroxymethyl group) [13, 14]. Thus, alkaloid **2** was unequivocally identified as 7-(4-hydroxysenecioyl)-9-(+)-trachelanthylretronecine, a new compound; we propose the name pycnanthine, for it.

Table 2. ^{13}C NMR ($CDCl_3$, 100 MHz) data of alkaloids **1** and **2**

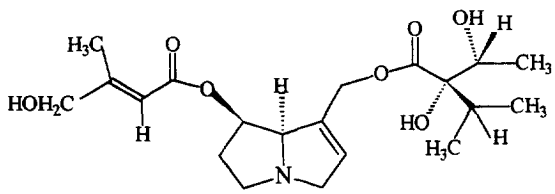
C	1	2
1	132.8 *s*	132.8 *s*
2	127.6 *d*	126.0 *d*
3	62.0 *t*	62.2 *t*
5	53.7 *t*	53.9 *t*
6	34.2 *t*	34.2 *t*
7	73.7 *d*	72.4 *d*
8	76.1 *d*	75.8 *d*
9	62.0 *t*	61.7 *t*
10	174.3 *s*	174.4 *s*
11	83.1 *s*	83.6 *s*
12	69.8 *d*	71.3 *d*
13	18.5 *q*	15.8 *q*
14	72.4 *s*	32.0 *d*
15	26.0 *q*	17.7 *q*
16	24.9 *q*	17.0 *q*
17	165.5 *s*	165.5 *s*
18	115.3 *d*	112.4 *d*

The FAB mass spectrum of compound **3** showed an $[M + H]^+$ at *m/z* 414 (for $C_{20}N_{31}NO_8$), 16 mu higher than that of compound **1**. The main fragment pattern below *m/z* 398 $[M + H - 16]^+$ was superimposable on that of alkaloid **1**. The ^{1}H NMR of compound **3** (Table 1) is almost identical to compound **1**, except for signals of the ring protons at C-3, C-5 and C-8, which are highly deshielded [15]. Thus, **3** can be described as the *N*-oxide of **1**. The existence of an *N*-oxide indicates that the reduction process was either incomplete or that echihumiline *N*-oxide (**3**) was very stable.

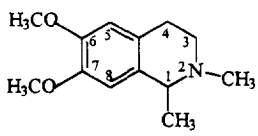
The GC–EI mass spectrum of compound **4** showed a $[M]^+$ at *m/z* 221; this corresponds to $C_{13}H_{19}NO_2$. The base peak at *m/z* 206 is formed by the loss of a methyl group $[M - 15]^+$. The most important peaks in the mass spectrum of this compound are due to $[M]^+$, $[M - 1]^+$, $[M - 15]^+$ (base peak) and $[M - 31]^+$, which are characteristic of *N*- and C-1 methylated tetrahydro-isoquinoline alkaloids [16]. The ^{1}H NMR spectrum of compound **4** showed signals for two methoxyls at δ 3.85 (6H, *s*), a doublet at δ 1.55 (J = 6.6 Hz) for a methyl attached at C-1, two aromatic *para*-oriented protons at δ 6.56 and δ 6.59, C-5 and C-8 protons and a singlet at δ 2.64 indicating a *N*-CH_3.



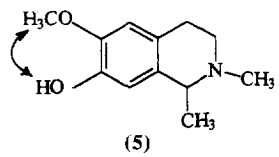
(1)*



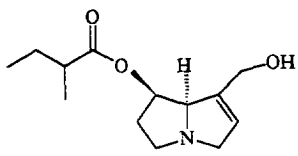
(2)



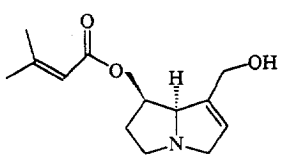
(4)



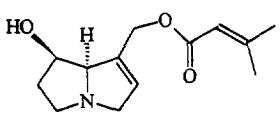
(5)



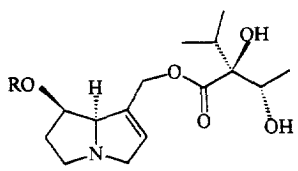
(6)



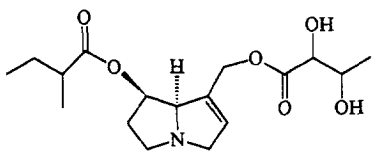
(7)



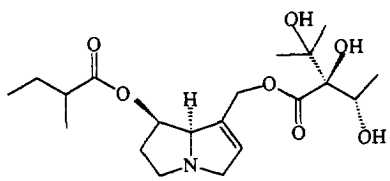
(8)



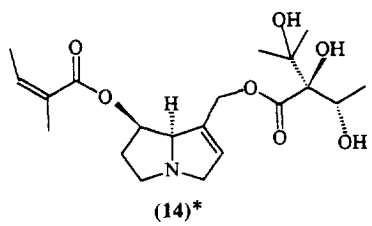
(9) R = H
(10) R = CH_3CO
(12) R = $C_5H_7O_2$



(11)



(13)*



(14)*

Fig. 1. Structures of pyrrolizidine and tetrahydro-isoquinoline alkaloids found in *Echium humile*. *Stereochemistry at position 12

methoxyl groups at C-6 and C-7. Comparison of the mass spectral and ^{1}H NMR data of compound **4** with those reported for carnegine [17], confirmed that compound **4** is carnegine (1,2-dimethyl-6,7-dimethoxyl-1,2,3,4-tetrahydro-isoquinoline).

The GC–EI mass spectrum of compound **5** showed a $[M]^+$ at *mz* 207, consistent with the formula $C_{12}H_{17}NO_2$. The base peak *m/z* 192 is formed by the loss of methyl group $[M-15]^+$; fragment ions, *m/z* 176, 164, 148, 131 and 118 are shifted by 14 mu as compared with compound **4**. The ^{1}H NMR spectrum of **5** indicated the presence of four aromatic protons at δ 6.57, δ 6.58, δ 6.59 and δ 6.66. The C-1 methyl groups showed two doublets at δ 1.54 and δ 1.70 (J = 6.6 Hz); the singlet at δ 3.85 (3H, *s*) indicates the presence of OCH_3 and the singlet at δ 2.6 (3H, *s*), *N*-CH_3. Therefore, compound **5** is a mixture of two isomers having one methoxyl group and one free hydroxyl group, instead of two methoxyl groups as in **4**. Thus, compound **5** probably consists of two isomers as shown in Fig. 1. They differ only at the asymmetric carbon C-1 and are considered to be 6- or 7-norcarnegine. Unfortunately, the quantities available were too small to carry out separation, full characterization and complete assignment of these alkaloid isomers. However, the presence of simple tetrahydro-isoquinoline alkaloids in the Boraginaceae has not previously been described.

In addition, the alkaloidal extract of *E. humile* was analysed by capillary GC–mass spectrometry, 13 alkaloids were detected and identified (Table 3). Five alkaloids could be unambiguously identified by direct comparison of their retention indices (RI) and mass spectra with authentic material or literature data, namely, 7-senecioylretronecine (**7**), 9-senecioylretronecine (**8**), lycopsamine (**9**), 7-acetyl-lycopsamine (**10**) and echimidine (**14**). Alkaloids **1–5** were isolated and further identified by ^{1}H and ^{13}C NMR spectral analysis (see above). The remaining four alkaloids were tentatively identified as 7-(2-methylbutyryl) retronecine (**6**), 7-(2-methylbutyryl)-9-(2,3-dihydroxybutyryl)retronecine (**11**), 7-senecioylretronecine (**12**) and 7-(2-methylbutyryl)-9-echimidinylretronecine (**13**).

Alkaloid **6** showed a $[M]^+$ at *m/z* 239 corresponding to the formula $C_{13}H_{21}NO_3$. The mass spectrum exhibited significant ions at *m/z* 137, 124, 111, 106 and 80 (base peak), these fragments being characteristic of 1,2-unsaturated necines with a C-7 monoester [5]. The fragment ion at *m/z* 137 is probably due to loss of the acid attached to C-7 $[M-C_5H_{10}O_2]^+$. The fragments *m/z* 85 and *m/z* 57 indicated that this acid is 2-methylbutanoic acid having 2 mu more than angelic acid, which has been previously observed in the Boraginaceae [18]. From mass fragmentation and biogenic considerations, alkaloid **6** was tentatively identified as 7-(2-methylbutyryl) retronecine.

Alkaloids **7** and **8** showed a $[M]^+$ at *m/z* 237 characteristic of 1,2-unsaturated necines with a C-7 monoester [5]. The fragment ion *m/z* 137 results from the loss of the acid moiety attached to C-7. The mass spectrum of alkaloid **8** shows significant ions at *m/z* 138, 137, 136 and 93 (base peak), characteristic of 1,2-unsaturated necines with C-9 monoester [5, 19]. The fragment ion *m/z* 138 results from the cleavage of the weak allylic ester bond. Thus, comparison of RI and mass spectra with data previously reported [14, 20], implied that alkaloid **7** is 7-senecioylretronecine and alkaloid **8** is 9-senecioylretronecine.

Alkaloid **9** showed a $[M]^+$ at *m/z* 299, corresponding to the formula $C_{15}H_{25}NO_5$. The base peak at *m/z* 138 is due to cleavage of the weak allylic ester bond and provided strong evidence for the presence of a free OH at C-7. Comparing RI and the mass spectrum of this alkaloid with those reported in the literature [20, 21], provided evidence that alkaloid **9** is lycopsamine.

Alkaloid **10** showed a $[M]^+$ at *m/z* 341 ($C_{17}H_{27}NO_6$), with a base peak at *m/z* 180 ($C_{10}H_{14}NO_2$) and an ion at *m/z* 281 $[M-60]^+$. This provided strong evidence for the presence of an acetoxyl group at C-7 [22]. Comparison of the mass spectrum of this alkaloid with that reported in the literature [18], suggested that compound **10** is 7-acetyl-lycopsamine.

Alkaloids **11–13** showed an ion series *m/z* 136, 119, 120, 94, 93 and 80, which are characteristic of 1,2-unsaturated diester pyrrolizidine alkaloids [5]. Alkaloid **12** displayed a base peak at *m/z* 220 ($C_{13}H_{18}NO_2$) due to cleavage of the allylic ester bond. Although no $[M]^+$ was detected, the ion at *m/z* 281 $[M-100]^+$ ($C_5H_8O_2$) results from the loss of the acid attached to C-7. Based upon mass spectral fragmentation and biogenic considerations, the structure of alkaloid **12** was identified as 7-senecioyl-lycopsamine (or a closely related isomer).

Alkaloids **11** and **13** show a base peak at *m/z* 222 and fragments at *m/z* 85 and 57. The presence of a saturated acid at C-7 (corresponding to $C_5H_{10}O_2$ as in alkaloid **6**) was presumed. The mass spectrum of **11** exhibited a $[M]^+$ at *m/z* 341 ($C_{17}H_{27}NO_6$). The ion at *m/z* 239 $[M-C_5H_{10}O_2]^+$ is due to the loss of the acid attached to C-7 and the base peak at *m/z* 222 $[M-C_4H_7O_2]^+$ is caused by the cleavage of the weak allylic ester bond. Based upon mass spectral fragmentation and biogenic considerations, alkaloid **11** was tentatively identified as 7-(2-methylbutyryl)-9-(2,3-dihydroxybutyryl) retronecine (or a closely related isomer).

The mass spectral fragmentation of alkaloid **13** showed a $[M]^+$ at *m/z* 399, corresponding to the formula $C_{20}H_{33}NO_7$ (2 mu more than echimidine). The fragment *m/z* 297 results from the loss of the acid attached to C-7 $[M-C_5H_{10}O_2]^+$ and the base peak at *m/z* 222 from cleavage of the weak allylic ester bond [M – echimidinic acid]$^+$. The fragment *m/z* 59 corre-

considerations alkaloid **13** was tentatively identified as 7-(2-methylbutyryl)-9-echimidinyl retronecine (or a closely related isomer).

Finally, the GC–mass spectral analysis detected alkaloid **14** in trace amounts (peak 11 in Table 3). This was identified as echimidine, previously isolated from *E. setosum* [23].

EXPERIMENTAL

Plant material. Whole flowering plants of *E. humile* Desf. (Syn. *E. pycnanthum* Pomel) were collected around Tripoli, Libya, in March 1990, and identified by Dr A. El-Gadi, Department of Botany, Faculty of Science, El-Faateh University, Libya.

Extraction and isolation. Plant material (1260 g) was shade-dried and pulverized to fine powder, then exhaustively extracted with 95% MeOH by cold maceration. The extract was concd under red. pres. and the residue extracted with 2N HCl and stirred with 20 g Zn dust overnight. The acidic soln was filtered then extracted with CH_2Cl_2 (to remove non-alkaloidal components). The acidic soln was then made alkaline with NH_4OH (pH 10) and extracted with CH_2Cl_2 to afford 325 mg of a yellowish-brown mass; alkaloidal content of plant 0.025% dry wt. Total crude alkaloids were charomatographed on a silica gel column (1.5 × 50 cm, 30 g) with CH_2Cl_2–MeOH gradients, to give 5 frs. Frs I–V were subjected to prep. TLC [silica gel F_{254}, CH_2Cl_2–MeOH–NH_4OH (25%), 85:15:2] yielding alkaloids **1–5** with R_fs 0.38, 0.30, 0.10, 0.54 and 0.43, respectively.

Analysis. A fused silica capillary column (DB1) was directly coupled to a quadrupole mass spectrometer. EI-mass spectra were recorded at 40 eV. Conditions: injector 250°; temp. prog. 150–300°, 6°C min^{-1}; split ratio 1:20; carrier gas He, 0.5 bar. Routine FID-GC measurements were performed under the following conditions: DB1-30W fused silica capillary column 30 m × 0.317 mm film thickness; carrier gas He; detector temp. 300°; injector temp. 250°; oven temp. prog.; initial temp. 170°, 5 min isothermal, 170–300°, 10°C min^{-1}, 300°, 15 min isothermal. Kovats retention indices [24] were calculated with respect to a set of co-injected even-numbered hydrocarbons (C_{14}–C_{28}). Each RI was subjected to a library search by comparison with the reference RI stored in a data base of the Institute of Pharmaceutical Biology. EI-mass spectra were recorded at 80 eV by direct inlet. ^1H and ^{13}C NMR were recorded in $CDCl_3$, at 400 and 100 MHz, respectively.

Echihumiline (**1**). Oily. $[\alpha]_D = +10°$ (EtOH, *c* 0.1). RI 2578, GC-EIMS *m/z* (rel. int.): $[M]^+$ 397 (0.1), 382 (0.2), 352 (0.1), 338 (0.1), 321 (0.1), 297 (2), 238 (2), 221 (20), 220 (100), 219 (4), 138 (6), 137 (6), 136 (40), 121 (15), 120 (39), 119 (16), 106 (4), 94 (20), 93 (30), 83 (36), 80 (6), 59 (5), 55 (6).

Pycnanthine (**2**). Oily. $[\alpha]_D = +4°$ (EtOH, *c* 0.1). RI 2793 EIMS, 80 eV, *m/z* (rel. int.): $[M]^+$, 397 (0.8), 382 (0.4), 354 (2), 352 (2), 281 (3), 254 (12), 237 (32), 236 (100), 235 (7), 138 (26), 137 (14), 136 (74), 121 (46), 120 (77), 119 (20), 99 (20), 94 (50), 93 (58), 80 (20), 71 (20), 58 (15), 45 (20), 44 (22), 43 (20).

Echihumiline N-oxide (**3**). Gum. FABMS (glycerol matrix; positive mode), *m/z* (rel. int.): $[M + 1]^+$, 414 (100), $[M + 1 - 16]^+$ 398 (12), $[M - 16]^+$ 397 (4), 254 (10), 221 (4), 220 (7), 219 (5), 138 (6), 137 (8), 136 (10), 121 (4), 120 (10), 119 (8), 93 (6), 83 (18), 80 (4), 59 (5), 55 (4).

Carnegine (**4**). Oil. $[\alpha]_D = -4°$ (EtOH, *c* 0.1). RI 1727 GC–EIMS, *m/z* (rel. int.): $[M]^+$ 221 (1), $[M - H]^+$ 220 (2), $[M - CH_2]^+$ 207 (14), $[M - CH_3]^+$ 206 (100), $[206 - CH_3]^+$ 191 (4), $[M - OCH_3]^+$ 190 (13), $[M - CH_2{=}N{-}CH_3]^+$ 178 (3), 162 (5), 145 (3), 132 (2), 115 (1), 103 (4), 91 (3), 73 (1), 65 (1), 56 (1). ^1H NMR ($CDCl_3$, 400 MHz), δ 3.65 (*q*, H-1), 2.93 (*m*, H-3), 3.75 (*m*, H-4), 6.56 (*s*, H-5), 6.59 (*s*, H8), 1.55 (*d*, C-1, $J = 6.6$ Hz), 2.64 (*s*, *N*-Me), 3.85 (6-OMe, 7-OMe).

6 or 7-N orcarnegine (**5**). Oil. Optically inactive. RI 1715 GC–EIMS, *m/z* (rel. int.): $[M]^+$ 207 (7), $[M - H]^+$ 206 (8), 193 (20), $[M - CH_3]^+$ 192 (100), 190 (5), 177 (19), $[M - OCH_3]^+$ 176 (11), $[M - CH_2{=}N{-}CH_3]^+$ 164 (3), 148 (8), 131 (3), 118 (2), 103 (3), 96 (5), 91 (5), 77 (4), 65 (2), 56 (2). ^1H NMR ($CDCl_3$, 400 MHz), δ 3.65 (*q*, H-1), 2.9 (*m*, H-3), 3.4 (*m*, H-4),

Table 3. GC–mass spectral analysis of alkaloidal extract of *Echium humile*

Peak	Alkaloid	Area (%)
1	6- or 7-Norcarnegine	16.9
2	Carnegine	2.0
3	7-(2-Methylbutyryl)retronecine	Trace
4	7-Senecioylretronecine	0.3
5	9-Senecioylretronecine	Trace
6	Lycopsamine	3.8
7	7-Acetyl-lycopsamine	2.8
8	7-(2-Methylbutyryl)-9-(2,3-dihydroxybutyryl)retronecine	0.3
9	7-Senecioyl-lycopsamine	13.4
10	7-(2-Methylbutyryl)-9-echimidinylretronecine	35.4

6.57, 6.58, 6.59 and 6.66 (*s*, H-5 and H-8), 1.54 and 1.70 (*d*, 3H, C-1, $J = 6.6$ Hz), 2.65 (*s*, *N*-Me), 3.85 (7-OMe).

7-(2-Methylbutyryl)-retronecine or its isomer (**6**). RI 1738. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 239 (6), 178 (1), 154 (3), 137 (27), 136 (16), 124 (20), 111 (70), 106 (59), 93 (6), 85 (5), 80 (100), 68 (10), 57 (19).

7-Senecioylretronecine (**7**). RI 1816. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 237 (4), 154 (4), 137 (30), 136 (16), 124 (25), 111 (37), 106 (40), 94 (21), 83 (23), 80 (100), 68 (8), 55 (15) [14, 20].

9-Senecioylretronecine (**8**). RI 1833. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 237 (1), 193 (2), 155 (11), 154 (10), 138 (23), 137 (28), 136 (15), 126 (9), 109 (5), 94 (25), 93 (100), 83 (20), 80 (16), 67 (6), 55 (11) [20].

Lycopsamine (**9**). RI 2145. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 299 (0.5), 254 (1), 156 (8), 139 (31), 138 (100), 137 (12), 136 (12), 120 (10), 108 (4), 95 (15), 94 (55), 93 (84), 80 (14), 67 (10), 45 (8), 43 (20) [20, 21].

7-Acetyl-lycopsamine (**10**). RI 2230. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 341 (1), 296 (1), 281 (3), 198 (10), 181 (33), 180 (100), 179 (8), 136 (17), 121 (20), 120 (60), 119 (10), 101 (12), 95 (7), 94 (23), 93 (50), 80 (9), 67 (5), 45 (7), 43 (33) [18].

7-(2-Methylbutyryl)-9-(2,3-dihydroxybutyryl) retronecine (**11**). RI 2285. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 341 (1), 239 (10), 223 (20), 222 (100), 143 (18), 138 (11), 137 (7), 136 (50), 121 (17), 120 (80), 119 (30), 106 (8), 94 (40), 93 (83), 85 (15), 80 (15), 57 (30), 45 (9).

7-Senecioyl-lycopsamine (**12**). RI 2497. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 381 (0), 336 (0.5), 281 (1), 238 (5), 221 (28), 220 (100), 219 (4), 141 (15), 138 (14), 137 (12), 136 (80), 121 (38), 120 (70), 119 (20), 94 (38), 93 (61), 83 (76), 80 (11), 55 (15), 45 (8), 43 (28).

7-(2-Methylbutyryl)-9-echimidinylretronecine (**13**). RI 2512. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 399 (0.1), 384 (0.5), 297 (1), 223 (20), 222 (100), 221 (5), 143 (15), 138 (8), 137 (8), 136 (40), 121 (30), 120 (95), 119 (30), 106 (9), 94 (31), 93 (70), 85 (15), 80 (15), 67 (8), 59 (15), 57 (28), 45 (8), 43 (24).

Echimidine (**14**). RI 2560. GC–EIMS, *m/z* (rel. int.): $[M]^+$ 397 (70.1), 297 (2), 221 (26), 220 (100), 219 (4), 138 (6), 137 (6), 136 (42), 121 (25), 120 (67), 119 (21), 106 (5), 94 (23), 93 (42), 83 (33), 80 (10), 59 (8), 55 (19), 45 (4), 43 (13) [23].

Acknowledgements—We thank Dr A. El-Gadi, Department of Botany, Faculty of Science, Al Faateh University, Tripoli, Libya, for plant identification.

REFERENCES

1 Jafri, S. M. A. and El-Gadi, A. (1979) *Flora of Libya*, Al Faateh University **68**, 33.
2. Bull, L. B., Culvenor, C. C. J. and Dick, A. T. (1968) *The Pyrrolizidine Alkaloids*. North-Holland Publishing Co., Amsterdam.
3. Mattocks, A. R. (1986) *Chemistry and Toxicology of Pyrrolizidine Alkaloids*, Academic Press, London.
4. Robins, D. J. (1982) *Fortschr. Chem. Org. Naturstoffe* **41**, 115.
5. Pedersen, E. and Larsen, L. (1970) *Org. Mass Spectrom.* **4**, 249.
6. Roeder, E., Liu, K. and Bourauel, T. (1971) *Phytochemistry* **30**, 3107.
7. Asibal, C. F., Glinski, J. A., Gelbaum, L. T. and Zalkow, L. H. (1989) *J. Nat. Prod.* **52**, 109.
8. Culvenor, C. C. J. and Woods, W. G. (1965) *Aust. J. Chem.* **18**, 1652.
9. Culvenor, C. C. J., Hoffernan, M. L. and Woods, W. G. (1965) *Aust. J. Chem.* **18**, 1605.
10. Roeder, E. (1990) *Phytochemistry* **29**, 11.
11. Cheng, D. L., Niu, J. K. and Roeder, E. (1992) *Phytochemistry* **31**, 3671.
12. Roeder, E., Bourauel, T. and Neuberger, V. (1992) *Phytochemistry* **31**, 4041.
13. Roeder, E., Wiedenfeld, H. and Pfitzer, A. (1988) *Phytochemistry* **27**, 4000.
14. Roeder, E., Wiedenfeld, H. and Kirstgen, R. B. (1984) *Phytochemistry* **23**, 1761.
15. Culvenor, C. C. J., Edgar, J. A., Frahn, J. L. and Smith, L. W. (1980) *Aust. J. Chem.* **33**, 1105.
16. Hodgkins, J. E., Brown, S. D. and Massingill, J. L. (1967) *Tetrahedron Letters* **14**, 1321.
17. Brown, S. D., Hodgkins, J. E., Massingill, J. L. and Reinecke, M. G. (1972) *J. Org. Chem.* **37**, 1825.
18. Kelley, R. B. and Seiber, J. N. (1992) *Phytochemistry* **31**, 2369.
19. Roeder, E., Sarg, T., El-Dahmy, S. and Abdel Ghani, A. (1992) *Fitoterapia* **63**, 405.
20. Witte, L., Rubiolo, P., Bicchi, C. and Hartmann, T. (1993) *Phytochemistry* **32**, 187.
21. Roeder, E. and Bourauel, T. (1992) *Phytochemistry* **31**, 3613.
22. Pedersen, E. (1975) *Arch. Pharm. Chem. Sci. Ed.* **3**, 55.
23. Sarg, T., El-Dahmy, S., Abdel Aziz, E., Abdel Ghani, A. and Roeder, E. (1992) *Fitoterapia* **63**, 466.
24. Kovats, E. (1958) *Helv. Chem. Acta* **41**, 1915.