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PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM ROTUNDIFOLIUM*

CLARITA F. ASIBAL,[1] LESLIE T. GELBAUM,[2] and LEON H. ZALKOW*

School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

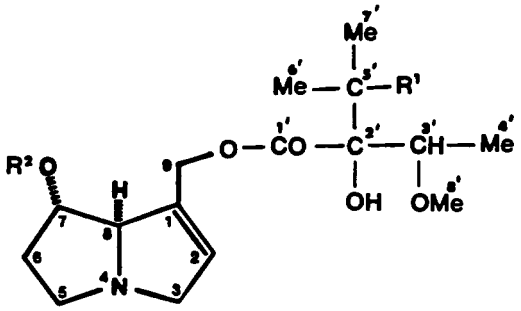
ABSTRACT.—*Heliotropium rotundifolium* was shown to contain, in addition to the previously isolated europine [**1**], three other alkaloids: heliotrine [**2**], lasiocarpine [**3**], and a new alkaloid identified as 5′-acetyleuropine [**4**]. The alkaloids were isolated by dccc and the structures established by spectroscopic means (^{1}H and ^{1}H-^{13}C HETCOR nmr and ms), physical properties (melting points and/or optical rotations), comparison with authentic samples, or by semisynthesis.

The hepatotoxic pyrrolizidine alkaloids (PAs) are of increasing concern as possible causes of human poisoning (1). Besides their presence in some traditional herbal medicines, they are also low-level contaminants in some foodstuffs. The broad range of pharmacological activity associated with pyrrolizidine alkaloids has continued to generate extensive studies on these compounds.

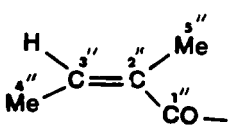
The genus *Heliotropium* (Boraginaceae) is among the genera of plants known to be rich in pyrrolizidine alkaloids (1). In line with our continuing objective (2–4) to identify and isolate PAs due to their potential as antitumor agents, we have reinvestigated *Heliotropium rotundifolium* Sieber ex Lehm. from which europine *N*-oxide [**1a**] was previously obtained (3). We report here the isolation of three other pyrrolizidine alkaloids, one of which is new.

RESULTS AND DISCUSSION

The MeOH extract of *H. rotundifolium* obtained from Jerusalem, Israel, was directly reduced with Zn dust and dilute H_2SO_4 in the usual manner. The resulting crude alkaloid fraction was separated by dccc. Monitoring of fractions by tlc and ^{1}H-nmr spec-



1 R^1=OH, R^2=H
1a *N*-oxide of **1**
2 $R^1=R^2$=H
2a *N*-oxide of **2**
3 R^1=OH, R^2=



3a *N*-oxide of **3**
4 R^1= $-OCO^{9'}CH_3^{10'}$, R^2=H
5 R^1=OH, R^2=Ac
5a *N*-oxide of **5**
6 R^1= $-OCO^{9'}CH_3^{10'}$, R^2= $CH_3^{2''}CO^{1''}-$

[1]Taken from the Ph.D. Dissertation of Clarita Florendo Asibal, School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia, 1987.

[2]Research Center for Biotechnology, Georgia Institute of Technology, Atlanta, Georgia 30332.

troscopy revealed the following heliotridine-based pyrrolizidine alkaloids in the order of their elution by dccc: europine [**1**], heliotrine [**2**], lasiocarpine [**3**], and a new alkaloid identified as 5′-acetyleuropine [**4**]. Europine, heliotrine, and lasiocarpine were identified by analysis of their ^{1}H-nmr, ^{13}C-nmr, and mass spectra and comparison of these spectral properties with those reported in the literature (3–8) as well as with those of authentic samples available in our laboratory from previous isolation work (4). Conversion of these free bases to their *N*-oxides provided additional support for their structures. ^{1}H- and ^{13}C-nmr data were obtained for both free bases and *N*-oxides. ^{1}H-^{13}C HECTOR nmr was used to assign unambiguously the ^{13}C chemical shifts for all H-containing carbons. Based on this, we have revised the previous ^{13}C chemical shift assignments for carbons 3, 5, 7, 8, and 9 in **1a** (3,5). Tables 1 and 3 give the ^{1}H-nmr data for the free bases and *N*-oxides, respectively, and Tables 2 and 4 give the ^{13}C-nmr data.

TABLE 1. 400-MHz ^{1}H nmr in $CDCl_3$ of Alkaloids and Derivatives from *Heliotropium rotundifolium*.

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-2	5.64 s	5.64 s	5.77 bs	5.70 s	5.75 s	5.78 bs
H-3u	3.26 d	3.28 m	3.31 m	3.30 dd	3.27 m	3.33 d
H-3d	3.80 d	3.80 d	3.89 d	3.86 d	3.84 d	3.91 d
H-5u	2.52 m	2.52 m	2.78 m	2.58 m	2.73 m	2.79 m
H-5d	3.19 m	3.22 m	3.14 m	3.3 m	3.11 m	3.19 m
H-6u	1.78 m	1.83 m	1.85 m	1.86 m	1.80 m	1.85 m
H-6d	1.90	1.97 m	1.85 m	1.99 m	1.80 m	1.85 m
H-7	4.07 m	4.01 m	5.09 m	4.13 m	4.99	5.02 bs
H-8	3.92 bs	3.81 s	4.06 bs	3.94 bs	3.99 bs	4.04 bs
H-9u	4.70 AB	4.60 AB	4.88 s	4.66 AB	4.82 ABq	4.86 s
H-9d	4.92 AB	4.98 AB	4.88 s	4.98 AB	4.82 ABq	4.86 s
H-3′	3.74 q	3.54 q	3.75 q	3.81 q	3.74 q	3.79 q
H-4′	1.17 d	1.07 d	1.21 d	1.20 d	1.20 d	1.22 d
H-6′	1.22 s	0.87 d	1.24 s	1.61 s	1.23 s	1.61 s
H-7′	1.17 s	0.82 d	1.11 s	1.61 s	1.11 s	1.62 s
H-8′	3.24 s	3.27 s	3.20	3.25 s	3.19 s	3.23 s
H-5′	—	2.07 hept	—	—	—	—
H-3″	—	—	6.03 q of q	—	1.98 s	—
H-4″	—	—	1.92 dd	—	—	—
H-5″	—	—	1.81 s	—	—	—
H-10′	—	—	—	1.96 s	—	1.96 s
H-2″	—	—	—	—	—	2.03 s

The structure of 5′-acetyleuropine [**4**] was elucidated from its mass and nmr spectra. Although eims showed no molecular ion peak, cims gave a strong $[MH]^{+}$ peak at *m/z* 372. This mass was higher than that of **1** by 42 mass units, suggesting the presence of an acetyl group. This was verified in the ^{1}H-nmr spectrum by a sharp singlet (integrating for three protons) at δ 1.96. The common diester arrangement (7-acetyleuropine [**5**]) was ruled out by the H-7 multiplet at δ 4.13 which is typical for the presence of an unesterified C-7 hydroxyl. Evidence was obtained by acetylating **4** to give 5′,7-diacetyleuropine [**6**] whose ^{1}H-nmr spectrum showed a broad singlet for H-7 at δ 5.02. Furthermore, **4** showed a singlet at δ 1.61 integrating for six protons, whereas **1** exhibited two singlets, at δ 1.17 and 1.22, each integrating for three protons, accounting for the 6′ and 7′ methyl protons. This downfield shift of 0.39–0.44 ppm in **4** is consistent with acylation of the hydroxyl group at C-5′. This is also sup-

TABLE 2. 100-MHz ^{13}C nmr in $CDCl_3$ of Pyrrolizidine Alkaloids from *Heliotropium rotundifolium*.

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	135.55	136.11	134.68	136.00	134.59
C-2	125.61	126.86	128.32	126.79	128.19
C-3	(61.39)[a]	(61.89)[a,c]	62.21	61.85	62.00
C-5	53.81	54.11	54.22	54.20	53.94
C-6	33.57	34.26	30.44	34.151	30.39
C-7	(74.11)[b]	75.31	(76.71)[d]	75.07	76.95
C-8	(79.26)[b]	(79.90)[b]	(78.73)[a,c,d]	79.97	78.40
C-9	(61.81)[a]	(62.51)[a,c]	62.21	62.46	62.00
C-1′	173.40	174.64	173.60	172.67	173.53
C-2′	83.88	82.52	83.58	(85.77)	83.57
C-3′	78.69	(78.49)[b]	(78.65)[a,c]	78.51	78.66
C-4′	12.74	12.39	12.98	12.97	12.96
C-5′	72.99	31.94	72.80	(85.13)	72.80
C-6′	25.92	17.10	26.47	22.47	26.36
C-7′	24.73	16.56	24.53	22.22	24.51
C-8′	56.23	56.97	56.38	56.74	56.36
C-9′			—	170.23	—
C-10′			—	22.56	—
C-1″			167.50		170.69
C-2″			127.47		21.13
C-3″			138.26		
C-4″			15.87		
C-5″			20.53		
C-11′					
C-12′					

[a]Reported as interchangeable in Jones *et al.* (6).
[b]Reported as interchanged in Jones *et al.* (6).
[c]Reported as interchangeable in Mody *et al.* (7).
[d]Reported as interchangeable in Zalkow *et al.* (4).

ported by the ^{13}C nmr data (see Table 2), which shows a C-5′ signal at 85.13 (or 85.77) in **4** compared to 72.99 ppm in **1**.

The fragmentation pattern in the mass spectrum of **4** likewise supports a free C-7 hydroxyl as shown by the base peak at *m/z* 138 resulting from allylic cleavage. Diesters with an acetylated C-7 hydroxyl exhibit typical intense fragment ions at *m/z* 180, 136, and 120 (9). In the case of 7-acetyleuropine [**5**] obtained from the acetylation of europine, these peaks have intensities of 100, 19, and 61, respectively. The ^1H nmr spectrum of **5** showed the H-7 multiplet at δ 4.99. It also showed two methyl singlets at δ 1.11 and 1.23 (for 6′ and 7′ methyl protons) analogous to those in **1**.

The above data are consistent with structure **4**. Precedents for this structure are found in 5′-acetylheliosupine (1) and in acetyllasiocarpine (1,10) which is just 7-angelyl-5′-acetyleuropine.

Europine, lasiocarpine, and heliotrine have been reported to occur singly or in combination in several other species of *Heliotropium* (1, 10–14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All ^1H- and ^{13}C-nmr spectra were obtained using a Varian XL-400 spectrometer. Chemical shifts are reported relative to residual $CHCl_3$ (7.24 ppm) for ^1H and to $CDCl_3$ (77.0 ppm) for ^{13}C. ^1H-^{13}C heteronuclear shift correlated nmr spectra were done as previously described (2). Melting points were taken on a Kofler hot stage and are corrected. Optical rotations were taken

TABLE 3. 400-MHz ^{1}H nmr in $CDCl_3$ of Pyrrolizidine *N*-Oxides.

Proton	Compound			
	1a	**2a**	**3a**	**5a**
H-2	5.69 s	5.61 s	5.91 bs	5.87 s
H-3u	4.32 ABq	4.28 AB	4.42 d	4.36 AB
H-3d	4.32 ABq	4.42 AB	4.58 d	4.51 AB
H-5u	3.51 m	3.56 m	3.79 m	3.70 m
H-5d	3.81 m	3.96 m	3.92 m	3.86 m
H-6u	2.01 m	1.96 m	2.20 m	2.11 m
H-6d	2.24 m	2.30 m	2.52 m	2.40 m
H-7	4.21 m	4.20 m	5.14 m	5.05 m
H-8	4.52 bs	4.70 s	4.67 bs	4.63 s
H-9u	4.68 AB	4.68 AB	4.95 ABq	4.79 AB
H-9d	4.81 AB	4.78 AB	4.95 ABq	4.91 AB
H-3′	3.70 q	3.59 q	3.79 q	3.73 q
H-4′	1.11 d	1.03 d	1.22 d	1.18 d
H-6′	1.19 s	0.83 d	1.25 s	1.21 s
H-7′	1.14 s	0.80 d	1.18 s	1.15 s
H-8′	3.18 s	3.17 s	3.25 s	3.20 s
H-5′	—	1.85 hept	—	—
H-2″	—	—	—	2.60 s
H-3″	—	—	6.17	—
H-4″	—	—	1.99 dd	—
H-5″	—	—	1.88 s	—

TABLE 4. 100-MHz ^{13}C nmr in $CDCl_3$ of Pyrrolizidine *N*-Oxides.

Carbon	Compound			
	1a	**2a**	**3a**	**5a**
C-1	133.58	134.09	132.92	132.82
C-2	121.19	120.15	123.13	122.86
C-3	(78.89)[a]	77.29	77.28	76.99
C-5	(68.03)[a]	68.34	67.92	67.74
C-6	32.82	33.23	30.66	30.42
C-7	(71.52)[a]	71.76	73.19	73.25
C-8	(96.05)[a]	96.23	94.93	94.53
C-9	(60.76)[a]	60.77	61.18	60.80
C-1′	173.30	173.87	173.49	173.36
C-2′	84.45	83.06	83.88	84.22
C-3′	78.87	78.77	78.80	78.78
C-4′	12.70	11.60	12.95	12.81
C-5′	73.34	33.00	70.98	73.25
C-6′	25.82	17.07	26.36	26.17
C-7′	24.87	17.07	24.84	24.95
C-8′	56.29	56.58	56.34	56.31
C-1″	—	—	167.26	170.71
C-2″	—	—	126.52	20.92
C-3″	—	—	140.76	—
C-4″	—	—	16.15	—
C-5″	—	—	20.48	—

[a]Assignment revised from that given in Zalkow *et al.* (3) and Broadbent and Paul (5).

with a JASCO DIP 360 digital polarimeter. Mass spectra were obtained on a Varian MAT 112S spectrometer interfaced with an SS 200 data system. Tlc was performed either on EM aluminum oxide 150 F-254 or Si gel 60 F_{254} (Merck) plates developed in MeOH/$CHCl_3$ mixtures. Centrifugal tlc was carried out on a Chromatotron model 7924 T (Harrison Research, Palo Alto, CA) using rotors coated with either Si gel 60 PF_{254} (Merck) or Al_2O_3 60 PF_{254} (Merck). For the dccc separation, a Büchi 670 dcc chromatograph, equipped with 500 tubes of 2.7 mm i.d. and attached to a Gilson FC-220 fraction collector, was used.

PLANT MATERIAL.—*H. rotundifolium* was collected, identified by Dr. A. Danin, and extracted at the Chemistry Department of Ben Gurion University of the Negev in Beer-Sheva, Israel, under the direction of Prof. A. Shani, and the extracts were shipped to the Georgia Institute of Technology (4). A voucher specimen is deposited at the herbarium in The Hebrew University of Jerusalem, Israel.

EXTRACTION AND SEPARATION OF THE ALKALOIDS.—MeOH extract (31.1 g; extracted in December 1977) of *H. rotundifolium* was dissolved in 200 ml of 2 N H_2SO_4 and stirred overnight with excess Zn dust. After filtration, the solution was extracted with $CHCl_3$ (3 × 200 ml). The acid solution was basified with NH_4OH (pH > 9) and reextracted with $CHCl_3$ (3 × 350 ml). After drying over $MgSO_4$, the solvent was removed in vacuo to give 4.23 g of crude alkaloid fraction (13.89%).

Separation of the alkaloid fraction was accomplished by dccc using the solvent system $CHCl_3$-C_6H_6-MeOH-H_2O (5:5:7:2) in ascending mode. Fractions (20 ml volume) were collected and monitored by a combination of tlc and ^{1}H-nmr analyses. The alkaloids eluted as follows: europine (fractions 41–53), heliotrine (fractions 60–64), 5′-acetyleuropine (fractions 70–80), and lasiocarpine (from the recovered stationary phase). Intervening fractions contained mixtures of the alkaloids from which additional pure samples were obtained by centrifugal tlc.

5′-ACETYLEUROPINE [**4**].—Non-crystallizable gum; $[\alpha]^{24}D$ +27.2° (*c* = 1.58, $CHCl_3$); ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 59 (74), 93 (75), 94 (36), 138 (100), 156 (18); cims $[MH]^+$ 372 (55), 312 (96), 254 (100); exact mass calcd for $C_{18}H_{30}NO_7$ $[MH]^+$ 372.2013, found 372.2007; ir ($CHCl_3$) 3660, 3650–3300, 2970, 2940, 2830, 1705, 1610, 1412, 1360, 1280–1180, 1148, 1090 cm^{-1}.

7-ACETYLEUROPINE [**5**].—To 100 mg of **1** dissolved in 5 ml pyridine was added 5 ml Ac_2O, and the mixture was left overnight at room temperature. Excess reagents were removed in vacuo, and the residue was taken up in $NaHCO_3$ solution and extracted with $CHCl_3$. The dried $CHCl_3$ extract was concentrated in vacuo to give a gummy material with the following properties: $[\alpha]^{24}D$ +0.44° (*c* = 4.06, $CHCl_3$); ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 93 (46), 94 (24), 119 (40), 120 (61), 121 (21), 136 (19), 180 (100); cims $[MH]^+$ 372 (49), 81 (100); exact mass calcd for $C_{18}H_{29}O_7N$, 371.1936, found 371.1949; ir ($CHCl_3$) 3660, 3500, 2980, 2940, 2890, 2830, 1720, 1450, 1375, 1270–1195 cm^{-1}.

5′,7-DIACETYLEUROPINE [**6**].—In the same manner as the acetylation of **1**, **4** was acetylated to give compound **6**, which has the following properties: ^{1}H nmr see Table 1; eims *m/z* (%) 43 (96), 59 (90), 93 (49), 94 (25), 119 (57), 120 (64), 136 (24), 178 (27), 180 (100), 181 (31), 236 (0.12), 295 (15); cims $[MH]^+$ 414 (9), 131 (100).

PREPARATION OF *N*-OXIDES **1a**, **2a**, **3a**, AND **5a**.—The *N*-oxides were prepared according to the following procedure. One meq of the base was dissolved in 10 ml of $CHCl_3$, *m*-chloroperbenzoic acid (1 meq) was added, and the solution was allowed to stand at room temperature for 15–30 min. The solvent was removed in vacuo and the residue dissolved in 10 ml H_2O and extracted with 4 × 10 ml Et_2O. The *N*-oxide was obtained by evaporating the aqueous solution in vacuo.

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LITERATURE CITED

1. A.R. Mattocks, "Chemistry and Toxicology of Pyrrolizidine Alkaloids," Academic Press, Orlando, Florida, 1986, pp. 1–16, 250–271.
2. L.H. Zalkow, C.F. Asibal, J.A. Glinski, S.J. Bonetti, L.T. Gelbaum, D. Van Derveer, and G. Powis, *J. Nat. Prod.*, **51**, 690 (1988).
3. L.H. Zalkow, L. Gelbaum, and E. Keinan, *Phytochemistry*, **17**, 172 (1978).
4. L.H. Zalkow, S. Bonetti, L. Gelbaum, M.M. Gordon, B.B. Patil, A. Shani, and D. Van Derveer, *J. Nat. Prod.*, **42**, 603 (1979).
5. T.A. Broadbent and E.G. Paul, *Heterocycles*, **20**, 863 (1983).

6. A.J. Jones, C.C.J. Culvenor, and L.W. Smith, *Aust. J. Chem.*, **35**, 1173 (1982).
7. N.V. Mody, R.S. Sawhney, and S.W. Pelletier, *J. Nat. Prod.*, **42**, 417 (1979).
8. E. Pedersen and E. Larsen, *Org. Mass Spectrom.*, **4**, 249 (1970).
9. C.C.J. Culvenor, J.A. Edgar, J.L. Frahn, L.W. Smith, A. Ulubelen, and S. Doganca, *Aust. J. Chem.*, **28**, 173 (1975).
10. C.C.J. Culvenor, S.R. Johns, and L.W. Smith, *Aust. J. Chem.*, **28**, 2319 (1975).
11. S.T. Akramov, Z. Shadmanov, A. Samatov, and S.Y. Yunusov, *Khim. Prir. Soedin.*, 258 (1968); *Chem. Abstr.*, **68**, 893 (1968).
12. M. Kugelman, W.C. Liu, M. Axelrod, T.J. McBride, and K.V. Rao, *Lloydia*, **39**, 125 (1976).
13. F. Kiyamitdinova, S.T. Akramov, and S.Y. Yunusov, *Khim. Prir. Soedin.*, 411 (1967); *Chem. Abstr.*, **68**, 75730 (1968).
14. V.B. Pandey, J.P. Singh, A.R. Mattocks, and E. Bailey, *Planta Med.*, **49**, 254 (1983).

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