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PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM BOVEI*

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Key Word Index—*Heliotropium bovei*; Boraginaceae; pyrrolizidine alkaloids; lasiocarpine; europine; 5′-acetyllasiocarpine; 7-acetyleuropine; lasiocarpine *N*-oxide; 5′-acetyllasiocarpine *N*-oxide; activity.

Abstract—*Heliotropium bovei* was shown to contain lasiocarpine, europine, 5′-acetyllasiocarpine and a new alkaloid 7-acetyleuropine. Lasiocarpine *N*-oxide and 5′-acetyllasiocarpine *N*-oxide are also present in this plant species. These structures were established from spectral and chemical studies including 2D NMR. Europine showed both antifungal and insect antifeedant activity, while 7-acetyleuropine was inactive.

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are widely distributed in genera of the Boraginaceae, Compositae and Leguminosae and are of great pharmacological and biological interest [1, 2]. This has prompted us to carry out a phytochemical study of *Heliotropium bovei*. Here we describe the separation and structural elucidation of PAs present in this plant species, and also their biological activity.

RESULTS AND DISCUSSION

The crude alkaloidal extract obtained from the plant was subjected to column chromatography and preparative TLC on silica gel to give six alkaloids. Their structures were established by IR, mass (EIMS and HRMS), ^{1}H and ^{13}C NMR spectroscopic methods. DEPT and 2D NMR experiments (H-COSY, HMQC [3], HMBC [4] and ROESY [5]) were performed to assign unambiguously the ^{1}H and ^{13}C chemical shifts of all the signals to the new alkaloid (**4**) (Table 1). The alkaloids **1**–**3**, obtained as gums, were identified by comparison of their spectral data (EIMS, IR, ^{1}H and ^{13}C NMR) with those reported in the literature. Therefore, **1** is lasiocarpine [6], **2** is europine [6] and **3** is 5′-acetyllasiocarpine [7].

The new alkaloid **4** was isolated as an oil, $[\alpha]_D + 6^\circ$ (EtOH; *c* 0.7) and its HRMS presented the molecular ion at *m/z* 371.1912 (2.7%) corresponding to the molecular formula $C_{18}H_{29}NO_7$ (calcd 371.1944). Characteristic peaks appear at *m/z* 311.1740 (3.2%), $C_{16}H_{26}NO_5$ (calcd 311.1732) and 180.1021 (100%), $C_{10}H_{14}NO_2$ (calcd 180.1024), typical fragmentation of a diester with an acetylated C-7 hydroxyl for heliotrine [8]. The ^{13}C NMR spectrum (DEPT experiments) showed 18 carbon atoms (five methyl, four methylene, four methine and five quaternary carbon atoms). The ^{1}H and ^{13}C NMR spectra exhibited the presence of an acetyl group, lasiocarpic acid moiety, and the shift positions of the ring protons and carbon correspond with published data for heliotridine ester [9]. In the ROESY spectrum no NOE between the H-7 and H-8 protons was detected; therefore heliotridine is the necine [10]. The mode of the ester attachment at two linkage sites, C-7 or C-9, was determined by a long-range spectrum with inverse detection using the technique of heteronuclear multiple bond connectivity (HMBC, Table 1). The H-9 protons 4.93 and 4.87 (doublets) gave connectivities with carbonyl carbon at 174.2 (C-1′), the quaternary carbon at 135.0 (C-1), the methine carbon at 128.3 (C-2) and the methine carbon at 78.8 (C-8), indicating the attachment at C-9 of lasiocarpic acid. Therefore, the acyl group is linked at C-7. The structure of the diester is confirmed as 7-acetyleuropine. Chemical evidence was obtained by acetylation of europine with Ac_2O–pyridine. The 7-acetyleuropine has not been reported as a natural product.

The *N*-oxide alkaloids **5** and **6** were identified by comparison of their spectral data (IR, ^{1}H, ^{13}C NMR, mass spectra) with those of lasiocarpine *N*-oxide [9] and 5′-acetyllasiocarpine *N*-oxide [11].

We tested the major alkaloids of *H. bovei* for antifungal and antifeedant activity. Europine showed a significant antifungal activity against *Fusarium moniliforme* (EC_{50} value of 0.74 mg ml^{-1}), while 7-acetyleuropine at the same concentration was inactive. This result suggests that the presence of the acetyl group results in the loss of activity. Antifeedant insect bioassays with europine showed mild activity against *Spodoptera littoralis* when tested at 5 μg cm^{-2} in choice tests, giving an average feeding reduction of 67% ($\pm$4.7 s.e.).

PAs isolated from *Heliotropium* sp., e.g. lasiocarpine and europine, have been described as antimicrobial and antifungal agents [12, 13], but there are few reports of this

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Table 1. ^1H, ^{13}C, HMQC and HMBC NMR data of alkaloid **4** (400 MHz, $CDCl_3$)

Proton		Correlated	Carbon
		HMQC	HMBC
		135.0 *s* (C-1)	
H-2	5.81 *br s*	128.3 *d*	C-9, C-8, C-1
H-3d	4.02 *d* (15.0)		C-1
		62.3 *t*	
H-3u	3.38 *br d* (14, 8)		
H-5d	3.28 *m*		C-8
		54.4 *t*	
H-5u	2.86 *m*		C-3, C-7, C-8
H-6d			
	1.93 *m*	30.6 *t*	
H-6u			
H-7β	5.09 *m*	76.9 *d*	
H-8	4.16 *m*	78.8 *d*	C-3, C-7
H-9d	4.93 *d* (12.5)		C-1, C-2, C-8, C-1′
		62.6 *t*	
H-9u	4.87 *d* (12.5)		C-1, C-2, C-8, C-1′
		174.2 *s* (C-1′)	
		84.0 *s* (C-2′)	
H-3′	3.81 *q* (6.3)	79.2 *d*	C-8′, C-2′, C-1′
H-4′	1.27 *d* (6.3)	13.3 *q*	C-2′, C-5′
		73.4 *s* (C-5′)	
H-6′	1.19 *s*	26.7 *q*	C-7′
H-7′	1.31 *s*	24.9 *q*	C-6′
H-8′	3.27 *s*	56.8 *q*	C-3′
C=O		171.3 *s*	
Me	2.06	21.5 *q*	C-1″

Coupling constants (Hz) are shown in parentheses.

class of compounds functioning as deterrents to insect feeding. Bentley *et al.* [14] isolated senkirkine as a feeding deterrent against the lepidopteran *Choristoneura fumiferana* from *Tussilago farfara* (Compositae). These authors also tested additional PAs, showing that most were inactive against this insect. They also showed that the most active compounds, senkirkine and lasiocarpine, bore a, b unsaturation in the side chain. Europine and europine *N*-oxide did not show any significant feeding reduction under their experimental conditions. Dreyer *et al.* [15] also found mild feeding deterrency of PAs against the pea aphid *Acyrthosiphon pisum*.

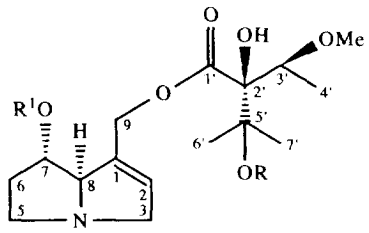
EXPERIMENTAL

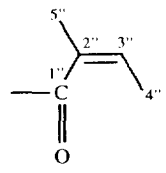
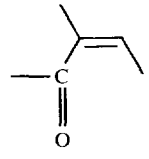
General. ^1H and ^{13}C NMR spectra were measured on a Bruker AMX 400 MHz spectrometer (chemical shifts reported are relative to residual $CDCl_3$, 7.26 ppm, for ^1H and 77.0 ppm for ^{13}C). The programs used for DEPT, H-COSY, HMQC, HMBC and ROESY experiments were those furnished in the Bruker manual. Mass spectra were obtained at 70 eV on a VG Micromass ZAB-2F instrument. Sepns were performed on flash CC (silica gel, 63–200 mesh) and prep. TLC (silica gel GF-254, 0.2 mm cards).

Plant material. Heliotropium bovei Boiss was collected in August 1992 from Honaz Mountain-Denizli, Turkey and identified by Prof. Dr Ertan Tuzlaci from the University of Marmara, Fac. of Pharmacy, Dept of Pharmaceutical Botany, Istanbul. A voucher specimen is deposited in the Herbarium of this University, with the number MARE 3791.

Isolation of the alkaloids. A portion (1.25 kg) of aerial parts of *Heliotropium bovei* was exhaustively extracted as described in ref. [16]. The crude alkaloids fr. (2.6 g) was chromatographed on a silica gel column. Elution was carried out with increasing order of polarity gradients using $CHCl_3$ and MeOH. The eluate obtained with $CHCl_3$–MeOH (19:1) gave lasiocarpine (208 mg), with $CHCl_3$–MeOH (9:1), 7-acetyleuropine (35 mg), europine (70 mg) with $CHCl_3$–MeOH (17:3), and a mixt. of *N*-oxides (200 mg) with the solvent system $CHCl_3$–MeOH (3:1). Further prep. TLC of either the mother liquid of lasiocarpine or the *N*-oxides fr., eluted with $CHCl_3$–MeOH–NH_3 (95:4:1) and $CHCl_3$–MeOH–NH_3 (85:14:1) gave lasiocarpine, 5′-acetyllasiocarpine (3 mg) and lasiocarpine *N*-oxide (70 mg).

Antifungal assays. The antifungal activity of europine and 7-acetyleuropine was tested against the following species of phytopathogenic fungi: *Fusarium moniliforme*,



	R	R¹
1	H	
2	H	H
3	Ac	
4	H	Ac
5	N-oxide from **1**	
6	N-oxide from **3**	

F. oxysporum, *F. solani*, *F. avenaceum*, *Botrytis cinerea*, *Phytophthora syringae* and *Ascochyta lentis*, and estimated as mycelial growth inhibition [17]. The test substances were dissolved in acetone and incorporated in PDA culture media (5% final concn of solvent). The dose series used was: 0.01, 0.025, 0.05, 0.1 and 0.25 mg ml^{-1}. For each experiment 8 replicates of 2 cm diameter culture media disks, inoculated with the appropriate fungal sp., were incubated at 27° in darkness and the colony diameters measured after 48 hr. Control experiments consisted of solvent-treated media. The effective concn (EC_{50}) was calcd from log-probit analysis [18].

Insect bioassays. Leaf disk feeding assays were conducted with third-instar (L3) *Spodoptera littoralis* larvae according to Escoubas *et al.* [19]. Lettuce (*Lactuca sativa*) leaf disks (1 cm^2) were treated on the upper surface with 5 μg cm^{-2} of the test substance or solvent alone (10 μl). The uneaten leaf disk surfaces were measured with a computer interfaced scanner [Escoubas, P., personal communication]. Per cent feeding reduction (% FR) was calcd according to Bentley *et al.* [14].

Lasiocarpine (**1**). Oil, $[\alpha]_D$ −2° (EtOH; *c* 0.102) [lit. [20] $[\alpha]_D$ − 3° (EtOH)]. EIMS *m/z* (rel. int.): 411.2243 for $C_{21}H_{33}NO_7$ (calcd 411.2257), IR and MS data identical to lit. values.

Europine (**2**). Non-crystalline gum, $[\alpha]_D$ +8.1° (EtOH; *c* 1.2); [lit. [20], $[\alpha]_D$ +10.9° (EtOH)]; EIMS *m/z* (rel. int.): 329.1846 for $C_{16}H_{27}NO_6$ (calcd 329.1838); IR and MS data identical to lit. values.

5′-Acetyllasiocarpine (**3**). A resin, $[\alpha]_D$ −3° (EtOH; *c* 0.03), [lit. [7], $[\alpha]_D$ − 0.9° (EtOH; *c* 2.0)]; EIMS *m/z* (rel. int.): 454.2429 [M + 1]$^+$ for $C_{23}H_{36}NO_8$ (calcd 454.2440), 335.1734 for $C_{18}H_{25}NO_5$ (calcd 335.1732), 236 for $C_{13}H_{18}NO_3$ (calcd 236.1286), 220.1339 for $C_{13}H_{18}NO_2$ (calcd 220.1337); IR and ^{1}H NMR data identical to lit. values.

7-Acetyleuropine (**4**). A gum, $[\alpha]_D$ +5.9° (EtOH; *c* 0.67); IR (CH_2Cl_2) cm^{-1}: 3670, 3424, 2936, 1736, 1458, 1377, 1246, 1092; EIMS *m/z* (rel. int.): 371.1912 (2.7) for $C_{18}H_{29}NO_7$ (calcd 371.1944), 356.1715 (1) for $C_{17}H_{26}NO_7$ (calcd 356.1709), 311.1740 (3.2) for $C_{16}H_{26}NO_5$ (calcd 311.1732), 282.1344 (4.5) for $C_{14}H_{20}NO_5$ (calcd 282.1341), 180.1021 (100) for $C_{10}H_{14}NO_2$ (calcd 180.1024), 120.0806 (72) for $C_8H_{10}N$ (calcd 120.0813), 93.0577 (65) for C_6H_7N (calcd 93.0578), 80 (17), 59 (95).

Lasiocarpine N-*oxide* (**5**). Oil, $[\alpha]_D$ + 5° (EtOH; *c* 0.1) [lit. [20] $[\alpha_D$ + 13.1° (EtOH)]; IR and MS data identical to lit. values.

5′-Acetyllasiocarpine N-*oxide* (**6**). Oil, $[\alpha]_D$ + 10° (EtOH; *c* 0.08) [lit. [11] $[\alpha]_D$ + 10° (EtOH)]; ^{13}C NMR data identical to lit. values.

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