elliptica were extracted with EtOH at room temp. Evapn of the EtOH in *vacuo* gave a residue which was partitioned between 0.5 M HCl and C_6H_6–Et_2O (1:1). After addition of $KHCO_3$ to the aq. layer, the latter was extracted with $CHCl_3$–EtOH (2:1). Evapn of the solvents gave raw material, which was chromatographed over silica gel with $CHCl_3$–MeOH (19:1) and later over the same adsorbent with toluene–MeOH–15 N NH_3 (88:11:0.6). Crystallization from MeOH afforded **1**; yield 0.03%; needles, mp 198–200°, $[\alpha]_D^{22}$ −101.0° [$CHCl_3$–MeOH (1:1); *c* 0.38]. IR ν_{max}^{KBr} cm^{-1}: 1606, 1593, 1516. UV λ_{max}^{MeOH} nm (log ε): 282 (3.51), 225 (sh, 4.22). ORD (MeOH): $[\phi]_{290}$ −1450° (trough), $[\phi]_{275}$ −450° (peak), $[\phi]_{235}$ −6600° (trough). ^1H NMR (200 MHz, DMSO-d_6, TMS): δ 2.27 (*s*, 3H, NMe), 3.68 (*s*, 6H, OMe), 6.28 (*s*, 1H, 8-H), 6.60 (*d*, *J* = 8 Hz, 2H, H-3′, H-5′), 6.93 (*d*, *J* = 8 Hz, 2H, H-2′, H-6′), 8.90 (*s*, 1H, OH), 9.06 (*s*, 1H, OH). EIMS (6–16 eV) *m/z* (rel. int.): 222 [M − hydroxybenzyl]$^+$ (100), 107 [hydroxybenzyl]$^+$ (20). EAMS (2–4 eV) *m/z*: 328 [M − H]$^-$. (Found: C, 69.6; H, 7.5; N, 4.2. $C_{19}H_{23}NO_4$ requires: C, 69.3; H, 7.0; N, 4.2%.)

The NOE difference expts were carried out at 200 MHz using an automated sequence. The concn of **1** in DMSO-d_6 was < 5% (w/v) and the sample was examined without degassing.

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PYRROLIZIDINE ALKALOIDS FROM SEEDS OF *CROTALARIA SCASSELLATII*

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Key Word Index—*Crotalaria scassellatii*; Leguminosae; seeds; pyrrolizidine alkaloids; axillaridine; axillarine; desoxyaxillarine.

Abstract—Three pyrrolizidine alkaloids were isolated from the seeds of *Crotalaria scassellatii*. Axillaridine and axillarine were the two major alkaloids whereas the third minor alkaloid was a new compound. Its structure was determined as desoxyaxillarine.

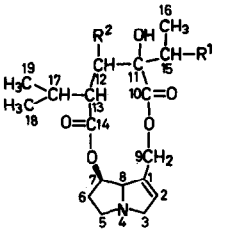
INTRODUCTION

Crotalaria species are known as a rich source of pyrrolizidine alkaloids. Therefore it was presumed that *C. scassellatii*, an East African species, also contained these compounds. After extraction and purification of the seeds of the plant, four alkaloids could be detected by TLC. We succeeded in the isolation of one minor and two major alkaloids. The two major alkaloids were identified as axillarine (**2**) and axillaridine (**1**), which were previously isolated from *C. axillaris* [1]. The minor alkaloid is new and the name desoxyaxillarine (**3**) is proposed.

RESULTS AND DISCUSSION

Methanol extraction of the seeds was followed by purification as previously described [2]. Two alkaloids were separated by low-pressure column chromatography (CC) from the resulting mixture. The mass spectra gave the formulae $C_{18}H_{27}NO_6$ (**1**) and $C_{18}H_{27}NO_7$ (**2**). The fragmentation patterns of both alkaloids and the agreement of the melting points with those given in an earlier paper [1] proved the structures to be axillaridine (**1**) and axillarine (**2**). This was also confirmed by the NMR data (Tables 1 and 2).

By investigation of the mother liquors of the CC fractions we succeeded in isolating a third, minor, alkaloid



R^1 = H; R^2 = OH : **1**
R^1 = R^2 = OH : **2**
R^1 = OH; R^2 = H : **3**

Table 1. ^{1}H NMR data of axillaridine (1), axillarine (2) and desoxyaxillarine (3) (1, 2 = $CDCl_3$; 3 = DMSO-d_6; TMS; δ in ppm; J in Hz)

	1	Lit.[1]	2	Lit.[1]	3
C2-H	6.18, *m*, 1H	6.24	6.16, *m*, 1H	—	6.11, *m*, 1H
C3-H_a	3.99, *d*, 1H	—	3.90, *d*, 1H	—	4.02, *d*, 1H
	J=5.5		J=5		J=5
C3-H_b	3.36, *d*, 1H	—	3.40, *d*, 1H	—	3.30, *d*, 1H
	J=5.5		J=5		J=5
C5-H_a	3.57, *m*, 1H	—	3.71, *m*, 1H	—	3.61, *m*, 1H
C5-H_b	2.66, *m*, 1H	—	2.71, *m*, 1H	—	2.45, *m*, 1H
C6-H_2	2.03, *m*, 2H	—	2.01, *m*, 2H	—	2.02, *m*, 2H
C7-H	5.56, *m*, 1H	5.68	5.60, *m*, 1H	—	5.35, *m*, 1H
C8-H	4.36, *m*, 1H	—	4.53, *m*, 1H	—	4.52, *m*, 1H
C9-H_a	5.03, *d*, 1H	5.12, *d*	4.93, *d*, 1H	—	4.82, *d*, 1H
	J=12	J=12	J=14		J=12
C9-H_b	4.46, *d*, 1H	4.52, *d*	4.47, *d*, 1H	—	4.48, *d*, 1H
	J=12	J=12	J=14		J=12
C11-OH	3.42, *m*, 1H	—	3.35, *m*, 1H	—	—
C12-H	4.12, *d*, 1H	—	4.04, *d*, 1H	—	—
	J=7		J=6		
C12-H_2	—	—	—	—	1.99, *d*, 2H
					J=7
C12-OH	4.06, *m*, 1H	—	2.98, *m*, 1H	—	—
C13-H	2.32, *t*, 1H	—	2.49, *t*, 1H	—	2.67, *m*, 1H
	J=6		J=5		
C15-H	—	—	4.08, *q*, 1H	—	3.81, *q*, 1H
			J=7		J=6
C15-H_2	2.13, *q*, 2H	—	—	—	—
	J=7				
C15-OH	—	—	2.98, *m*, 1H	—	—
C16-H_3	0.79, *t*, 3H	0.85, *t*	1.22, *d*, 3H	1.02, *d*	1.03, *d*, 3H
	J=7	J=8	J=7	J=7	J=6.5
C17-H	1.75, *m*, 1H	—	2.18, *m*, 1H	—	1.22, *m*, 1H
C18-H_3	0.89, *d*, 3H	0.94, *d*	0.89, *d*, 3H	1.26, *d*	0.86, *d*, 3H
	J=7	J=6	J=6.5	J=6	J=6
C19-H_3	0.96, *d*, 3H	1.04, *d*	0.95, *d*, 3H	1.62, *d*	0.81, *d*, 3H
	J=7	J=6	J=6.5	J=6	J=6

which was obtained in very small amounts. Therefore its structure could be proved only by mass spectrometry and ^{1}H NMR analysis. The mass spectrum shows the same molecular formula as 1: $C_{18}H_{27}NO_6$. The fragmentation pattern indicates that 3 must be a 12-desoxyaxillarine. This becomes clear from a peak at m/z 236 and the fact that there is no peak at m/z 250. Ion m/z 236 indicates that C-12 has no oxygen function. It is generated by the loss of $C_3H_5O_2$ from m/z 309. The fragmentation pattern would show an intense ion at m/z 250 if there was a hydroxyl group at C-12 as in 1 and 2. The ^{1}H NMR data (Table 1) also establish the structure as 3. The CHOH–Me signal at C-11 is established by the presence of a doublet for C-16 H_3 at δ1.03 with a coupling constant of 6 Hz. The corresponding proton at C-15 is found at δ3.81. The high-field shift of 1.99 ppm for C-12 H_2 confirms that this position shows no hydroxyl function. The other data are similar to those of 1 and 2. All NMR values were verified by decoupling experiments and by evaluation of coupled and noise-decoupled spectra. The alkaloidal content in the seeds was measured by densitometric methods. The contents were 0.47% for 2, 0.26% for 1 and 0.09% for 3 on a dry wt basis.

EXPERIMENTAL

Seeds of *C. scassellatii* Chiov. were collected in the region of Mtito Andei, near Mombasa, Kenya, East Africa. Dried and pulverized material was defatted with petrol and then extracted with MeOH in a Soxhlet apparatus for 1 week. After evapn to dryness, the resulting residue was purified as described previously [2]. The three alkaloids were isolated with MeOH–CH_2Cl_2 (4:1) on silica gel 60 by low-pressure CC. They were recrystallized from EtOH–petrol.

Axillaridine (1). Mp 150° (lit. 148–152° [1]); EIMS, 70 eV, m/z (rel. int.): 353.1847 $[M]^+$ (6.7), 338 $[M-Me]^+$ (1.9), 324 $[M-C_2H_5]^+$ (1.37), 309 $[M-CO_2]^+$ (6.3), 250 $[309-C_3H_7O]^+$ (92.4), 223 $[309-C_4H_6O_2]^+$ (5.3), 208 $[223-Me]^+$ (14.8), 138 $[250-C_6H_8O_2]^+$ (33.3), 137 $[M-C_{10}H_{16}O_5]^+$ (49.7), 136 $[M-C_{10}H_{17}O_5]^+$ (85.2), 121 $[250-C_6H_9O_3]^+$ (48.8), 120 $[137-OH]^+$ (100), 119 $[M-C_{10}H_{18}O_6]^+$ (90.9), 95 $[121-C_2H_2]^+$ (27.5), 94 $[120-C_2H_2]^+$ (49.8), 93 $[119-C_2H_2]^+$ (71.3), 80 $[95-Me]^+$ (24.8).

Axillarine (2). Mp 195–197° (lit. 205° [1]); EIMS, 70 eV, m/z (rel. int.): 369.1799 $[M]^+$ (3.3), 354 $[M-Me]^+$ (1.2), 325 $[M-C_2H_4O]^+$ (6.3), 307 $[325-H_2O]^+$ (4.8), 264 $[307-C_3H_7]^+$ (10.8), 250 $[354-C_3H_4O_4]^+$ (91.9), 206 $[250-C_2H_4O]^+$ (18.2),

Table 2. ^{13}C NMR data of axillaridine (1) and axillarine (2) (MeOH-d_4; TMS: δ in ppm)

Carbon No.	1	2	
1	133.5	132.1	=C
2	136.4	135.7	=CH
3	62.2	61.4	CH_2
5	55.0	54.1	CH_2
6	34.9	34.0	CH_2
7	74.7	72.4	CH
8	78.9	77.9	CH
9	59.9	59.5	CH_2
10	175.2	173.1	C=O
11	82.3	82.2	C
12	74.3	69.9	CH
13	52.7	51.4	CH
14	174.6	172.5	C=O
15	31.7	—	CH_2
15	—	69.8	CH
16	8.2	17.8	Me
17	28.9	27.5	CH
18	22.2	21.7	Me
19	18.1	17.7	Me

138 $[250-C_6H_8O_2]^+$ (30.8), 137 $[307-C_8H_{10}O_4]^+$ (45.0), 136 $[M-C_{10}H_{17}O_6]^+$ (86.8), 121 (49.5), 120 (100), 119 (93.6), 95 (24.8), 94 (43.8), 93 (70.7), 80 (20.9).

Desoxyaxillarine (3). $[\alpha]_D^{20}$ −5° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3390 (OH); 2840–2940 (C–H); 1720, 1730 (ester); 1630 (C=C); EIMS, 70 eV, *m/z* (rel. int.): 353.1831 $[M]^+$ (5.1), 338 $[M-Me]^+$ (0.4), 309 $[M-CO_2]^+$ (13.6), 236 $[309-C_3H_5O_2]^+$ (0.8), 222 $[309-C_4H_7O_2]^+$ (1.8), 208 $[309-C_5H_9O_2]^+$ (11.3), 138 $[222-C_5H_8O]^+$ (25.4), 137 $[M-C_{10}H_{16}O_5]^+$ (26.1), 136 $[M-C_{10}H_{17}O_5]^+$ (61.8), 121 (45.8), 120 (100), 119 (99.4), 95 (40.1), 94 (31.9), 93 (51.3), 80 (18.2).

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