211 (4.40), 249 (4.26), 271 (4.18), 308 (3.76), 349 (3.77), 388 (3.66), 433 (3.57); IR ν_{max}^{nujol} cm^{-1}: 1635 (s), 1590 (s), 1570 (s), 1510 (s), 1300 (s), 1270 (m), 1210 (m), 1130 (m), 1050 (s), 1020 (w), 1000 (m), 960 (s), 900 (w), 880 (m), 850 (m), 815 (w), 775 (m); ^{1}H NMR (CF_3COOH): δ4.16 (3H, *s*, OMe), 4.22 (3H, *s*, OMe), 6.71 (2H, *s*, OCH_2O), 7.56 (1H, *s*, H-3), 8.03 (1H, *s*, H-8), 8.29 (1H, *s*, H-11), 8.47 (1H, *d*, J = 6.4 Hz, H-4), 8.78 (1H, *d*, J = 6.4 Hz, H-5).

Dasymachaline (2). Amorphous; $C_{20}H_{21}O_3N$; $[\alpha]_D^{26}$ −47° ($CHCl_3$; *c* 0.34); MS *m/z*: 355 $[M]^+$ (100), 340 (33), 326 (16), 312 (44), 296 (14), 281 (9), 266 (9), 254 (13), 190 (53); UV λ_{max}^{EtOH} nm (log ε): 221 (4.37), 285 (4.12), 297 (4.07); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (br OH), 1605 (m), 1515 (s), 1460 (s), 1400 (m), 1380 (m), 1340 (m), 1300 (w), 1270 (s), 1245 (s), 1220 (s), 1090 (vs), 1050 (m), 1035 (w), 970 (w), 940 (m), 865 (m), 820 (w); ^{1}H NMR: δ2.17 (*br s*, exch. D_2O), 2.63 (3H, *s*, N–Me), 2.67–3.17 (4H, *m*, 2H-4, 2H-5), 3.23 (1H, *d*, J = 2.6 Hz, H-6a), 3.94 (6H, *s*, 2 × OMe), 4.83 (1H, *d*, J = 2.6 Hz, H-7, *s* on irradiation at 3.23), 5.94 and 6.09 (each 1H, *d*, J = 1.5 Hz, OCH_2O), 6.52 (1H, *s*, H-3), 6.96 (1H, *s*, H-8), 7.74 (1H, *s*, H-11).

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(+)-11-OXOCYTISINE, A LUPIN ALKALOID FROM LEAVES OF *SOPHORA SECUNDIFLORA*

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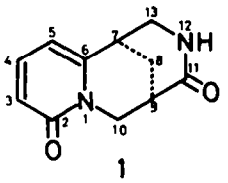
Key Word Index—*Sophora secundiflora*; Leguminosae; leaves; lupin alkaloid; quinolizidine alkaloid; (+)-11-oxocytisine; (−)-cytisine; (−)-*N*-methylcytisine; (−)-anagyrine; (−)-baptifoline.

Abstract—A new lupin alkaloid, (+)-11-oxocytisine (1), was isolated from the leaves of *Sophora secundiflora* together with (−)-anagyrine, (−)-*N*-methylcytisine, (−)-baptifoline, (−)-*N*-formylcytisine, (−)-*N*-acetylcytisine and (−)-cytisine. The structure of the new alkaloid (1) was presumed to be (+)-11-oxocytisine on the basis of its spectroscopic data.

INTRODUCTION

The seeds of *Sophora secundiflora* are commonly referred to as 'mescal beans', 'red beans' or 'dry whisky', and have been utilized as a divinatory medium for various ceremonial purposes by Indians of the Southwest United States and adjacent Mexico because of their purported hallucinogenic activity [1–3]. Previous phytochemical investigations on lupin alkaloids reveal that seeds harvested in America contain seven quinolizidine alkaloids, cytisine, *N*-methylcytisine, anagyrine, thermopsine, sparteine, Δ^5-dehydrolupanine and epilupinine [4–8]. Bourn *et al.* reported recently that the major alkaloids of the seeds, cytisine, *N*-methylcytisine and sparteine produced responses similar to those of the known hallucinogenic



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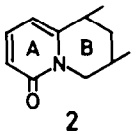

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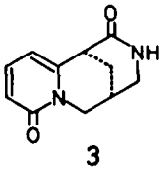

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drugs, mescaline, *N,N*-dimethyltryptamine and psilocybin in rats [9]. However, there is insufficient evidence that the hallucinogenic activity of the seeds is due only to these lupin alkaloids.

As a continuation of our screening for lupin alkaloids in leguminous plants, we have studied the basic constituents of fresh leaves of *S. secundiflora* native to Pakistan. A new alkaloid (**1**) was found together with six cytisine-type lupin alkaloids, (−)-anagyrine, (−)-baptifoline, (−)-*N*-methylcytisine, (−)-*N*-acetylcytisine, (−)-*N*-formylcytisine and (−)-cytisine. Thus, the constituents of the leaves native to Pakistan are different from those of the seeds native to America which contain sparteine- and lupinine-type lupin alkaloids in addition to cytisine-type alkaloids. The present report describes the isolation of the new alkaloid (**1**) from the leaves of *S. secundiflora* and the structural elucidation of the new base (**1**) as (+)-11-oxocytisine.

RESULTS AND DISCUSSION

The new alkaloid (**1**) was isolated from the alkaloid mixture which was extracted from an ethanol extract of the leaves of *S. secundiflora* harvested at Peshawar, Pakistan. Compound **1** gave colourless needles from ether–methanol, $[\alpha]_D^{20}$ + 6.7° (*c* 0.45; EtOH), mp 272–275°. The molecular formula, $C_{11}H_{12}N_2O_2$, was established by a high resolution mass spectrum ($[M]^+$ *m/z* 204.088, calc. 204.090). The UV spectrum of **1** in ethanol revealed absorption peaks at 234 nm (log ε = 3.40) and 304 nm (log ε = 3.45), indicating the presence of an α-pyridone ring in the molecule [10]. The mass spectrum of **1** showed prominent fragment ions at *m/z* 147 (62%) and 146 (100%) which are attributable to the AB ring system of cytisine-type alkaloids [11, 12]. Thus, the presence of a partial structure **2** in the molecule of **1** was presumed from the above results.

The ^{13}C NMR spectrum of **1** showed two signals at δ 172.0 (*s*) and δ 50.6 (*t*) assigned to an amide carbonyl carbon and a methylene carbon next to a nitrogen, respectively, in addition to the nine carbon signals corresponding to C-2–C-10 of the partial structure **2**: δ 163.0 (*s*, C-2), 118.2 (*d*, C-3), 139.0 (*d*, C-4), 106.3 (*d*, C-5), 148.3 (*s*, C-6), 35.9 (*d*, C-7), 23.4 (*t*, C-8), 32.3 (*d*, C-9), 48.3 (*t*, C-10).

The amide group in the molecule was assumed to be secondary from the ^{1}H NMR signal at δ 6.39 (1H, *br*) assigned to –CONH–. Consideration of the above data allowed for two possible molecular structures, **1** and **3**.

All the ^{1}H NMR signals of **1** were assigned as shown in Table 1 from analysis of decoupling experiments and comparison with the spectral data of cytisine-type alkaloids such as (−)-cytisine and (−)-*N*-methylcytisine. The spectrum revealed two sets of AB parts of ABX spin systems. One set was a doublet centred at δ 4.56 (1H, *J* = 15.5 Hz) and a double doublet centred at δ 3.72 (1H, *J* = 15.5 and 5.5 Hz), the other being a double doublet centred at δ 3.74 (1H, *J* = 12 and 4.5 Hz) and a double multiplet centred at δ 3.40 (1H, *J* = 12 Hz). The signals of the former set could be assigned to H-10β and H-10α, respectively, because of the larger geminal coupling constant (15.5 Hz) and the lower chemical shifts [13, 14], and hence the later set should be ascribed to methylene protons at either the 13-position of the possible structure **1** or the 11-position of the structure **3**. Irradiation at δ 3.10 (1H, *m*) caused the double doublet at δ 3.72 due to H-10α to become a doublet (*J* = 15.5 Hz) and did not cause any change to the signals of the latter AB set. On the other hand, on irradiation of δ 3.30 (1H, *m*) the double doublet at δ 3.74 of the latter AB set was collapsed to a doublet (*J* = 12 Hz), but the signals of the former AB set did not show any change. These decoupling experiments indicate that the 1H multiplets at δ 3.10 and 3.30 are assigned to the methine protons at the 9- and 7-positions, respectively, and hence the methylene protons corresponding to the later AB set should be situated at the 13-position adjacent to the methine group at the 7-position. Therefore, the structure of the new base was presumed to be (+)-11-oxocytisine (**1**).

EXPERIMENTAL

General. Mps are uncorr. High and low resolution MS were measured at 70 eV using a direct inlet system. ^{1}H NMR (100 and 270 MHz) and ^{13}C NMR (25 MHz) spectra were recorded using TMS as int. standard. Analytical TLC was carried out on silica gel in the following solvent systems: (1) CH_2Cl_2–MeOH–28% NH_4OH (43:6:1); (2) CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); (3) CH_2Cl_2–MeOH (4:1); (4) Et_2O–MeOH–28% NH_4OH (40:2:1). Analytical HPLC were performed with the following solvent systems: (5) 15% MeOH in Et_2O–2.5% NH_4OH (50:1); (6) 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3);

Table 1. ^{1}H NMR data for (+)-11-oxocytisine (**1**)

Chemical shifts (δ, ppm)	No. of protons	Multiplicity (*J*, Hz)	Assignment
6.48	1	*d*, *J* = 9	3
7.31	1	*dd*, *J* = 9 and 7	4
6.10	1	*d*, *J* = 7	5
3.30	1	*m*	7
2.23	1	*dm*, *J* = 13	8
2.11	1	*dm*, *J* = 13	8
3.10	1	*m*	9
3.72	1	*dd*, *J* = 15.5 and 5.5	10α
4.56	1	*d*, *J* = 15.5	10β
6.39	1	*br*	–CONH–
3.40	1	*dm*, *J* = 12	13
3.74	1	*dd*, *J* = 12 and 4.5	13

(7) 25% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15), using a LiChrosorb SI 100 (Merck, 10 μm, 3 × 500 mm) column employing a monitoring flow system (220 and 310 nm) at a flow rate of 1 ml/min. Analytical GC was performed with a glass column (2 m × 3 mm i.d.) packed with 2%OV-17 on Gas Chrom Q, using N_2 as carrier (40 ml/min). The column was programmed from 220° to 280° at 5°C/min and afterwards isothermally. For GC/MS the same chromatographic system was used but using He as carrier. The known alkaloids involved in this study were identified by comparison with authentic samples (co-TLC, co-HPLC, GC/MS, $[\alpha]_D$) as described previously [13–16].

Plant material. Sophora secundiflora was identified by Dr. M. Ikram, Pakistan Council of Scientific and Industrial Research Laboratories, Peshawar. Voucher specimens have been deposited in the Herbarium of P.C.S.I. Res. Laboratories, Peshawar, Pakistan.

Isolation of (+)-*11-oxocytisine*. Leaves (85 g) of *S. secundiflora* collected at Peshawar, Pakistan, were homogenized in EtOH. The EtOH extract obtained was treated as described previously [13–16] to give the crude alkaloid mixture (86.5 mg, 0.1%/fr. wt). This mixture (78 mg) was applied to a silica gel (Merck, type 60, 230–400 mesh, 30 g) column and eluted with 5% MeOH in CH_2Cl_2–28% NH_4OH (500:1), 30 ml fractions being collected. (−)-Anagyrine (1.5 mg, oil, $[\alpha]_D^{25}$ −160° (*c* 0.38; EtOH)) and (−)-*N*-methylcytisine [4.5 mg, mp 137°, $[\alpha]_D^{25}$ −220° (*c* 1.0; EtOH)] appeared in fractions 4 and 5, respectively, in an almost pure form. Fraction 6 (3 mg) was a mixed fraction of (−)-*N*-formylcytisine and (−)-*N*-acetylcytisine, which was characterized by GC/MS without isolation. (+)-11-Oxocytisine (**1**) appeared in fractions 11–14, which were purified by silica gel CC using the same solvent system as described above and crystallized from Et_2O–MeOH to give 9 mg of colourless crystals of **1**, $[\alpha]_D^{12}$ +6.7° (*c* 0.45; EtOH), mp 272–275°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (lactam and α-pyridone C=O). ^{13}C NMR ($CDCl_3$): δ 163.0 (*s*, C-2), 148.3 (*s*, C-6), 139.0 (*d*, C-4), 118.2 (*d*, C-3), 106.3 (*d*, C-5), 48.3 (*t*, C-10), 35.9 (*d*, C-7), 32.3 (*d*, C-9), 23.4 (*t*, C-8), 172.0 (*s*, C-11), 50.6 (*t*, C-13). MS *m/z* (rel. int.): 204.088 ($[M]^+$, calc. for $C_{11}H_{12}N_2O_2$ 204.090, 86), 147 (62), 146 (100), 118 (19). UV λ_{max}^{EtOH} nm (log ε): 234 (3.40), 304 (3.45). (−)-Cytisine [10 mg, mp 154°, $[\alpha]_D^{25}$ −114° (*c* 0.95; EtOH)] and (−)-baptifoline [5 mg, mp 208°, $[\alpha]_D^{25}$ −135° (*c* 0.82; EtOH)] were eluted in fractions 15–20 and fractions 31–41, respectively, in an almost pure form.

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