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ALKALOIDS OF *SARCOCOCCA SALIGNA*

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Key Word Index—*Sarcococca saligna*; Buxaceae; saracocinaene (3α-dimethylamino-20α-*N*-methyl-*N*-acylamino-pregna-5,16-diene); saracodine (3α-dimethylamino-20α-*N*-methyl-*N*-acylamino-pregnane); pachyaximine-A (3β-methoxy-20α-dimethylamino-pregn-5-ene); spectroscopic studies.

Abstract—A new alkaloid, saracocinaene (3α-dimethylamino-20α-*N*-methyl-*N*-acylamino-pregna-5,16-diene), and two known alkaloids, saracodine and pachyaximine-A, were isolated from *Sarcococca saligna*. The structures of these steroidal bases were established on the basis of detailed spectroscopic techniques. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Sarcococca saligna Muel (syn. *Sarcococca pruniformis* Lindl.) is an evergreen shrub found widely distributed in the northwest region of Pakistan [1]. The leaves of the herb enjoy considerable reputation as a remedy for different diseases and for the treatment of fever and rheumatism in the indigenous system of medicines. Our studies of the crude ethanolic extract of the aerial parts of *S. saligna* also showed good antibacterial activity against *Pseudomonas pseudomalliae*, *Shigella boydii* and *Carnebacterium diphtheria* bacterial strains.

A number of steroidal alkaloids which induce a non-recoverable fall in the blood pressure in dogs and are toxic to *Paramoecia* as well as exhibiting other activities have been isolated from the leaves of the plant [2–8]. The leaves of the plant also contain betulin [9, 10].

The present investigation has resulted in the isolation of a new alkaloid, saracocinaene (**1**), along with the known bases saracodine (**2**) and pachyaximine-A (**3**). Compound **3** was isolated for the first time from this plant. Compound **2** is the major constituent of this plant, which was first isolated by Kohli *et al.* [7].

RESULTS AND DISCUSSION

An ethanolic extract of the aerial parts of *S. saligna* was evaporated and partitioned between chloroform and aqueous acid solution at various pH values. The acidic fraction was subjected to repeated column chromatography to afford compounds **1–3**.

Compound **1** was isolated as a white solid. The HREI mass spectrum of **1** exhibited the $[M]^+$ peak at *m/z* 393.3260 analysing for $C_{26}H_{42}N_2O$ (calc. 398.3296). Hence, **1** ($C_{26}H_{42}N_2O$) possessed seven degrees of unsaturation. Four of these were accounted for by the tetracyclic structure of a pregnane type steroid, two were due to endocyclic double bonds and one due to a carbonyl function. The UV spectrum showed only terminal absorption. The IR spectrum ($CHCl_3$) showed an intense absorption at 1620 cm^{-1} characteristic of an amide function.

The HREI mass spectrum of **1** showed the molecular ion peak at *m/z* 398.3260. A peak at *m/z* 383.3020 was due to the loss of a methyl group from the $[M]^+$ ion. The compound showed the base peak at *m/z* 84.0830 ($C_5H_{10}N$), representing the cleavage of ring A along with the $N(CH_3)_2$ substituent in the ring A [4].

The ^1H NMR spectrum of **1** showed doubling of signals for various protons due to the hindered rotation of the C-20 amide function. The methyl signals at δ 0.71/0.77 and 1.04/1.06 were due to quaternary methyl groups [11]. Another three-proton doublet at δ 1.14/1.26 was due to the secondary C-21 methyl group. A three-proton singlet at δ 2.66/2.69 was due to *N*-methyl and another one-proton singlet at δ 5.27 was characteristic of a Δ^5-double bond. A one-proton triplet at δ 5.66 was assigned to the C-16 olefinic proton. The small coupling constant indicated a *gauche* conformation of the olefinic proton with respect to the C-15 methylenic protons. Similar doubling of the *N*-acetyl (δ 2.05/2.17) signal and various other neighbouring proton signals were also observed due to the restricted rotation. A one-proton quartet at δ 4.40/5.40 showed COSY-45° interaction with the C-21 methyl group at δ 1.14/1.26, confirming it to be the C-20 methine proton.

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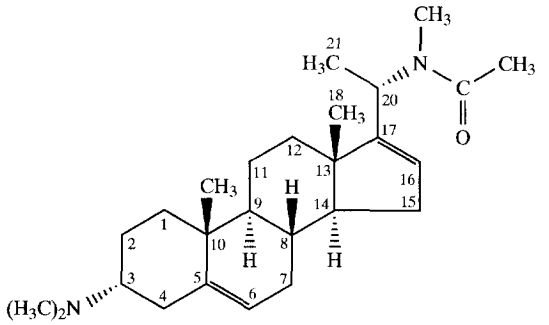
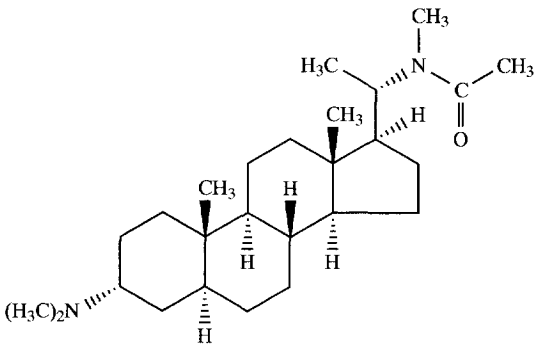
Extensive 2D-NMR experiments (COSY 45°, NOESY, HOHAHA, HMQC and HMBC) [12] further confirmed the above mentioned assignments. Thus, compound **1**, called saracosinaene, was identified as 3α-dimethylamino-20α-*N*-methyl-*N*-acylamino-pregna-5,16-diene.

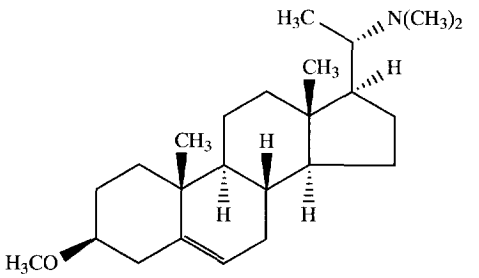
Compound **2** was found to be identical to saracodine, which was synthesized by Goutarel *et al.* [13]. The HREI mass spectrum of **2** exhibited the $[M]^+$ peak at *m/z* 402.3579 analysing for $C_{26}H_{46}N_2O$ (calc. 402.3609) with five degrees of unsaturation. Four of these were accounted for by the tetracyclic structure of a pregnane-type steroid, while one was due to a carbonyl function. The UV spectrum was inconclusive. The IR spectrum ($CHCl_3$) showed intense absorption at 1620 cm^{-1} characteristic of an amide function. The ^1H NMR spectrum of the compound also showed doubling of peaks due to the restricted rotation of the amide function. Two quarternary methyl signals at δ 0.69/0.72 and 0.79/0.80 were due to the C-18 and C-19 methyl groups, respectively. A three-proton doublet at δ 1.05/1.15 ($J = 7.2$ Hz) was due to the C-21 methyl group. Two three-proton singlets at δ 2.01/2.68 and 2.71/2.76 were due to $COCH_3$ and NCH_3 groups, respectively. A one-proton double quartet at δ 3.60/4.70 was due to the C-20 methine proton, which showed coupling with the C-21 methyl group. A six-proton broad singlet at δ 2.20 was due to the *N*-dimethyl group. These chemical shifts were further confirmed by HMQC, HMBC and HOHAHA NMR spectroscopic techniques. Thus, compound **2** was identified as saracodine (3α-dimethylamino-20α-*N*-methyl-*N*-acylamino-pregnane).



1



2



3

Compound **3** was obtained as a white solid. The HREI mass spectrum of **3** exhibited the $[M]^+$ peak at *m/z* 359.32065, corresponding to the molecular composition $C_{24}H_{41}NO$ (calc. 359.3188). Hence, compound **3** possessed five degrees of unsaturation. Four of these were accounted for by the tetracyclic pregnane-type structure and one by a double bond. The IR spectrum showed peaks at 2902, 2810, 1665, 1590, 1455 and 1090 cm^{-1}. The ^1H and ^{13}C NMR assignments were in agreement with the known compound 3β-methoxy-20α-dimethylamino-pregn-5-ene earlier isolated from *Pachysandra axillaris* [8] and given the trivial name pachyaximine-A.

EXPERIMENTAL

General experimental procedure. IR spectra: JASCO 302-A spectrophotometer. UV spectra: Hitachi U 3200 spectrophotometer. EI, FD and HREI MS: JMS HX100 (with data system) and JMS-DA 500 mass spectrometers. ^1H and ^{13}C NMR spectra: Bruker NMR spectrometer at 500 and 125 MHz, respectively, at room temp. Chemical shift values (δ) in ppm and coupling constants (J) in Hz. Standard pulse sequences were used for COSY, HOHAHA, DEPT, HMQC and HMBC experiments.

Chromatographic conditions. TLC (precoated silica G-25), UV254 plates; CC, silica gel, 230–400 mesh. Visualization of TLC plates was at 250 and 336 nm and Dragendorff's spray reagent was used for detection.

Plant material. Aerial parts of *S. saligna* (40 kg) were collected from Kuldana, Murree Hills, Pakistan, in October 1992.

Extraction and isolation. The EtOH extract of plant aerial parts (40 kg) was evapd to a gum (2 kg). Total alkaloids (700 g) were obtained by extraction into 10% AcOH. Partial sepn of the alkaloids was achieved by extraction with $CHCl_3$ at different pH values (3.5, 8.5). The fr. obtained at pH 3.5 (560 g) was subjected to CC on silica gel. Elution with $CHCl_3$ and then with $CHCl_3$–MeOH yielded several frs. A fr. obtained by CC on elution with $CHCl_3$–MeOH (41:9) (1.5 g) was purified by prep. TLC with *n*-hexane–EtOAC–Et_2NH (85:13:2) to afford **1** (5.5 mg). A fr. obtained by CC on elution with $CHCl_3$–MeOH (47:3) (3.0 g) was

Table 1. ^{13}C NMR chemical shifts (in ppm) of compounds **1–3**

	δ	Multiplicity*	δ	Multiplicity*	δ	Multiplicity
No.	**1**		**2**		**3**	
1	35.7	CH_2	33.0	CH_2	37.2	CH_2
2	25.3	CH_2	28.7	CH_2	28.0	CH_2
3	62.7	CH	61.8	CH	80.4	CH
4	29.7	CH_2	31.9	CH_2	38.7	CH_2
5	141.0	C	39.5	CH	140.9	C
6	121.0	CH	24.9	CH_2	121.6	CH
7	30.1	CH_2	31.8	CH_2	31.9	CH_2
8	33.5	CH	35.4	CH	31.9	CH
9	49.8	CH	54.0	CH	50.2	CH
10	37.3	C	36.1	C	36.9	C
11	20.2	CH_2	20.7	CH_2	21.0	CH_2
12	34.5	CH_2	39.7	CH_2	39.6	CH_2
13	46.1	C	41.7	C	41.4	C
14	58.0	CH	56.6	CH	56.9	CH
15	31.2	CH_2	23.7/23.8†	CH_2	24.1	CH_2
16	127.4	CH	26.01†	CH_2	27.7	CH_2
17	154.3	C	53.7/54.2	CH	54.5	CH_2
18	15.9	Me	12.6/12.8	Me	12.1	Me
19	19.8	Me	12.1	Me	19.4	Me
20	44.6/51.1	CH	49.4/55.4	CH	61.1	CH
21	16.1/17.5	Me	18.2/19.0	Me	9.9	Me
NMe	27.8/29.8	Me	26.5/29.4	Me	—	—
NCOMe	22.2/22.6	Me	22.0/22.2	Me	—	—
$N(Me_2)$	43.7	Me	43.8	Me	39.9	Me
(OMe)	—		—		55.6	Me
NCOMe	170.0/170.1	C	169/170.0	C	—	—

*Multiplicities were determined by DEPT techniques.
†Assignments may be interchangeable.

again purified by CC using $CHCl_3$–MeOH (9:1) to yield a crystalline solid which was recrystallized using CH_2Cl_2 and *i*-octane mixt. to give a crystalline compound **2** (32 mg) (1.49×10^{-3}%). A fr. obtained on elution with $CHCl_3$–MeOH (43:7) was again purified, on a small column, using *n*-hexane–EtOAc–Et_2NH (85:13:2) as eluent to give a pure crystalline solid compound **3** (206 mg) (0.51×10^{-3}%).

Saracocinaene (**1**). Solid, mp 129–130°; $[\alpha]_D^{25}$ 170.0 (*c* 0.05, $CHCl_3$); IR $\nu_{max}^{[CHCl_3]}$ cm^{-1}, 1620 (amide): MS *m/z* (rel. int.): 398 $[M]^+$ (20), 383 $[M - Me]^+$ (4), 84.1 (100). ^1H NMR ($CDCl_3$, 500 MHz): δ 0.71/0.77 (3H, *s*, Me-18), 1.04/1.06 (3H, *s*, Me-19), 2.05/2.17 (3H, *s*, Ac), 2.20 (6H, *s*, NMe_2), 2.66/2.69 (3H, *s*, NMe_3), 1.14/1.26 (3H, *d*, $J = 6.8$ Hz, Me-21), 5.27 (1H, *m*, H-6), 5.66 (1H, *t*, H-16), 4.40/5.40 (1H, *q*, H-20). ^{13}C NMR ($CDCl_3$, 125 MHz); δ: see Table 1.

Saracodine (**2**). Crystals mp 246–248°, $[\alpha]_D^{25}$ −11.9 ($CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}, 1620 (amide); MS *m/z* (rel. int.): 402 $[M]^+$ (26), 302 (4), 110 (35), 100 (7), 84 (100), 58 (18). ^1H NMR ($CDCl_3$ 500 MHz at room temp.); δ 0.69/0.70 (3H, *s*, Me-18), 0.79/0.80 (3H, *s*, Me-19); δ 1.05/1.15 (3H, *d*, $J = 6.6/6.7$ Hz, Me-21), 2.01/2.68 (3H, *s*, Ac), 2.71/2.76 (3H, *s*, NMe), 2.20 (6H, *br s*, NMe_2), 3.60/4.70 (1H, *dq*, H-20). ^{13}C NMR ($CDCl_3$, 125 MHz); δ: see Table 1.

Pachyaximine-A (**3**). Crystalline solid mp 141–142°, $[\alpha]_D^{25}$ 11.90 ($CHCl_3$: IR ν_{max}^{KBr} cm^{-1}, 2902, 2810, 1665, 1590, 1455, 1090; MS *m/z* (rel. int.): 359 $[M]^+$ (2.4), 344 (4), 84 (7), 72 (100), 55 (3). ^1H NMR ($CDCl_3$, 500 MHz): δ 1.85/1.03 (2H, *m*, H-1), 1.41/1.90 (2H, *m*, H-2) 3.05 (1H, *ddd*, $J = 15.0$, 15.0, 9.0, 4.5 Hz, H-3), 2.38/2.50 (2H, *m*, H-4) 5.35 (1H, *t*, H-6) 1.53/1.97 (2H, *m*, H-7), 1.47 (1H, *m*, H-8), 0.92 (1H, *m*, H-9), 1.42/1.50 (2H, *m*, H-11), 1.14/1.91 (2H, *m*, H-12), 1.04 (1H, *m*, H-14), 1.06/1.59 (2H, *m*, H-15), 1.47/1.83 (2H, *m*, H-16), 1.35 (1H, *m*, H-17), 0.66 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19), 2.41 (1H, *m*, H-20), 0.86 (3H, *d*, $J = 4.5$ Hz, Me-21), 2.15 (6H, *br s*, NMe_2,), 3.34 (3H, *s*, OMe). ^{13}C NMR ($CDCl_3$, 125 MHz); δ: see Table 1.

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