Pergamon

PII: S0031-9422(97)00069-1

Phytochemistry, Vol. 45, No. 4, pp. 861–864, 1997

0031-9422/97 $17.00 + 0.00

STEROIDAL ALKALOIDS FROM *SARCOCOCCA SALIGNA*

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(*Received in revised form* 2 *December* 1995)

Key Word Index—*Sarcococca saligna*; Buxaceae; sarconidine; sarcorine; epipachysandrine-A; saracocine.

Abstract—In addition to epipachysandrine-A and saracocine, two new steroidal alkaloids, sarconidine and sarcorine were isolated from the aerial parts of *Sarcococca saligna*. Their structures were established as (20*S*)-20-dimethylamino-3β-methylamino-pregnane-5,16-diene and (20*S*)-3β-acetylamino-20-dimethylamino-5α-pregnane, respectively, by detailed spectroscopic studies.

INTRODUCTION

Sarcococca saligna is an evergreen shrub widely distributed in the north west regions of Pakistan and Kashmir. Its aroma is intense and can be detected many yards away. Leaves and shoots of the plant are used as a remedy for different diseases and for the treatment of pain and rheumatism in folk medicine [1]. Some steroidal alkaloids isolated from this species can induce a fall in blood pressure in experimental animals, such as rats and guinea-pigs, and are toxic to *Paramoecium*; steroidal alkaloids exhibiting other activities have also been isolated [2–5]. Salignine, a major alkaloid isolated from *S. saligna*, has also exhibited ganglion-blocking activity, decreasing or abolishing the effects of nicotine on blood pressure and smooth muscles of isolated guinea-pig ileum, as well as by partially blocking the responses in cats [2]. Previous phytochemical investigations on the genus *Sarcococca* have resulted in the isolation of several steroidal alkaloids [3–12].

The present investigation has resulted in the isolation of two new steroidal alkaloids, sarconidine (**1**) and sarcorine (**2**), along with two known steroidal bases, epipachysandrine-A (**3**) [13] and saracocine (**4**) [4–6], which were initially isolated from *Pachysandra terminalis* and *S. pruniformis*, respectively. Their structures were confirmed by extensive one- and two-dimensional NMR experiments. Compound (**3**) was isolated for the first time from *S. saligna*, while the ^{3}C NMR chemical shift assignments of compound **4** [14–15] are also reported for the first time.

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RESULTS AND DISCUSSION

An ethanolic extract of *S. saligna* yielded compounds **1–4** after solvent–solvent extraction and repeated column and thin-layer chromatography.

Compound **1** was isolated as an amorphous solid. The HR EI mass spectrum exhibited a $[M]^+$ at *m/z* 356.3193, analysing for $C_{24}H_{40}N_2$ (calcd 356.3191), thus, showing six degrees of unsaturation. Four of these were accounted for by the tetracyclic structure of a pregnane-type skeleton and two were due to endocyclic double bonds. A peak at *m/z* 341.2958 was ascribed to the loss of a methyl group from the $[M]^+$. Compound **1** showed a base peak at *m/z* 72.0808 ($C_4H_{10}N$), representing a trimethylimminium ion. Its UV spectrum showed only terminal absorption, while the IR spectrum ($CHCl_3$) exhibited absorptions at 3651 (NH), 3325 (CN) and 1620 (C=C) cm^{-1} [5].

The ^1H NMR ($CDCl_3$, 500 MHz) spectrum of compound **1** showed two one-proton broad singlets at δ 5.42 and 5.56 due, to the H-6 and H-16 olefinic protons, respectively. A three-proton singlet at δ 2.20 was ascribed to the 3-*N*-methyl group. A one-proton multiplet at δ 3.63 was assigned to H-3α. A three-proton doublet at δ 1.09 was due to H_3-21, while the one-proton quartet at δ 2.87 was due to H-20, which also showed vicinal coupling with H_3-21 in the COSY 45° spectrum. A six-proton singlet at δ 2.23 was ascribed to the 20-*N*,*N*-dimethyl group. Two three-proton singlets at δ 0.84 and 1.02 were assigned to H_3-18 and H_3-19, respectively. A HMQC spectrum was used to establish the direct $^1H/^{13}C$ one-bond shift correlations of protonated carbons. The H-3 methine proton (δ 3.63) showed direct $^1H/^{13}C$ connectivity with C-3 (δ 55.4). The H-6 (δ 5.42) showed HMQC interaction with the C-6 (δ 124.5). Similarly, H-16 (δ 5.56) also showed shift correlation with the C-16 (δ 125.3).

Table 1. ^1H-^{13}C Chemical shift correlations* of compound **1**

Carbon	^1H (δ)	^{13}C (δ)	Carbon	^1H (δ)	^{13}C (δ)
1	1.90, 1.35	34.3	13	—	46.5
2	1.80, 1.75	30.5	14	2.45	31.3
3	3.63	55.4	15	1.85/2.06	31.2
4	2.60, 2.30	35.0	16	5.56	125.3
5	—	136.0	17	—	156.0
6	5.42	124.5	18	0.84	18.3
7	2.61, 2.00	32.7	19	1.02	19.6
8	2.40	31.7	20	2.87	59.3
9	1.38	49.7	21	1.09	15.8
10	—	35.4	N_aCH_3	2.20	30.3
11	1.99, 1.79	20.4	$N_b(CH_3)_2$	2.23	42.3
12	1.58, 1.50	23.6	—	—	—

* Deduced from HMQC spectrum.

Complete $^1H/^{13}C$ one-bond shift correlations of the protonated carbons of **1**, as determined from a HMQC spectrum, are shown in Table 1. The HMBC spectrum was very informative for accurate ^{13}C NMR chemical shifts assignments of the quaternary carbons and for locating the exact position of double bonds. H_2-2 (δ 1.75 and 1.80) showed HMBC interactions with C-3 (δ 55.4), whilst the H-3 (δ 3.63) methine proton exhibited long-range interaction with C-5 (δ 136.0). The olefinic H-6 (δ 5.42) showed cross-peaks with C-10 (δ 35.4) and C-7 (δ 32.7). The olefinic H-16 (δ 5.56) showed cross-peaks with C-13 (δ 46.5), while H-20 (δ 2.87) exhibited interactions with C-16 (δ 125.3), C-17 (δ 156.0) and C-21 (δ 15.8). Important HMBC interactions are shown in Fig. 1(a). Therefore, sarconidine was (20*S*)-20-dimethylamino-3β-methylaminopregna-5,16-diene (**1**).

Compound **2** was isolated as an oily liquid. Its HR EI mass spectrum, showed the $[M]^+$ at *m/z* 388.3446, analysing for $C_{25}H_{44}N_2O$ (calcd 388.3453), indicating five degrees of unsaturation in the molecule, four of which were accounted for by a tetracyclic pregnane-type skeleton and one was due to an acetamide functionality at the C-3 position. The peaks at *m/z* 377.3217 and 72.0819 were due to the loss of a methyl group from the $[M]^+$ and formation of the trimethyliminium ion through cleavage of the C-17/C-20 bond, respectively. Another peak at *m/z* 84.0820 was due to $C_5H_{10}N$, resulting from the cleavage of the C-13/C-17 and C-16/C-17 bonds, while a peak at *m/z* 58.0616 was due to C_2H_4NO. The UV spectrum showed only terminal absorption, while the IR spectrum showed absorption at 1658 (amide carbonyl) and 3650 (NH) cm^{-1}.

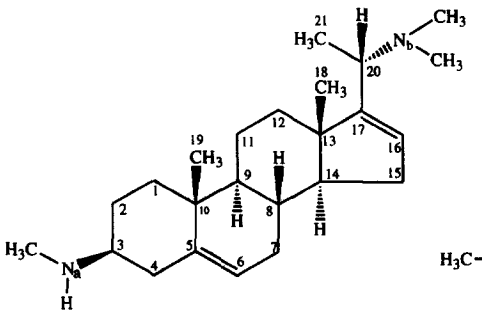
The ^1H NMR spectrum of **2** included two three-proton singlets at δ 0.63 and 0.73, corresponding with H_3-18 and H_3-19, respectively. The H_3-21 resonated at δ 0.90 as a doublet ($J_{21,20}$ = 6.5 Hz), while the vicinal H-20 appeared as a multiplet at δ 2.69. A six-proton singlet at δ 2.23 was assigned to the protons of the 20-dimethylamino functionality. A three-proton singlet at δ 1.96 was due to the acetamide methyl protons, while a one-proton doublet at δ 5.74 ($J_{NH,3}$ = 6.5 Hz) was ascribed to the NH proton. A one-proton multiplet at δ 4.09 was assigned to H-3α, which showed vicinal coupling with the amidic NH in a COSY-45° spectrum. Based on these spectroscopic studies, sarcorine is (20*S*)-3β-acetylamino-20-dimethylamino-5α-pregnane (**2**).

EXPERIMENTAL

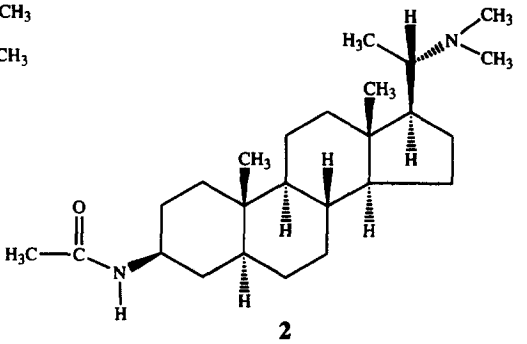
General. ^1H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers operating at 500, 400, 125 and 100 MHz at room temp. Chemical shift values are given in δ from TMS and coupling constants (*J*) are in Hz. Standard pulse sequences were used for COSY, DEPT, HMQC and HMBC exps. Precoated thin-layer silica gel F_{254} (Merck) glass plates were used for purification. Visualization of TLC plates was achieved at 254 and 366 nm and Dragendorff's reagent was used for detection of steroidal alkaloids.

Plant material. Aerial parts of *S. saligna* Muell. (syn. *S. pruniformis* Lindl.) were collected in the Distt. Bagh, Azad Kashmir, at altitudes between 3000–45000 ft in March, 1995. The species, was identified by Mr Tahir Ali, Plant Taxonomist, Department of Botany, University of Karachi; a voucher specimen is deposited in the Herbarium of the Department of Botany, University of Karachi.

Extraction and isolation. Air-dried material (15.5 kg) was extracted with EtOH (40 l) and the extract concd under red. press. to yield a gum (1.5 kg), which was dissolved in H_2O (2 l) and defatted with *n*-hexane. The aq. soln was extracted with $CHCl_3$ at pH 3.5 (220 g) and pH 10 (7.3 g); HOAc and NH_4OH were used to adjust pH values. The residue from $CHCl_3$ extract obtained at pH 10 was chromatographed on silica gel and eluted with *n*-hexane $CHCl_3$ and $CHCl_3$–MeOH mixts of increasing polarities to afford several frs. Frs



1



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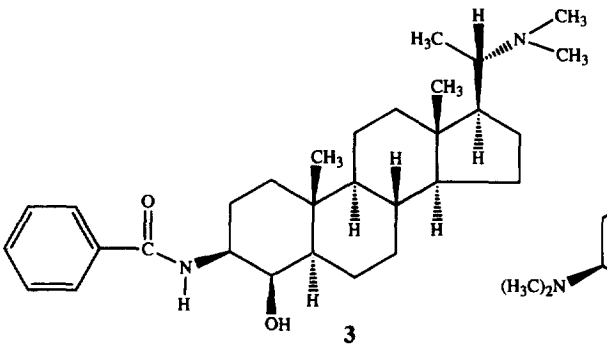

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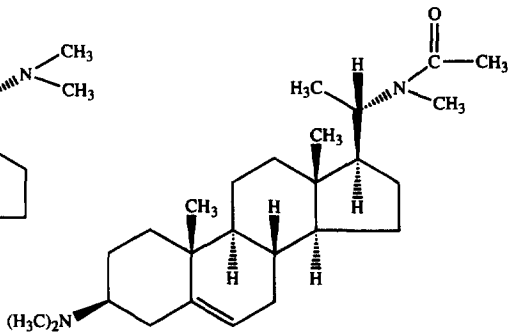
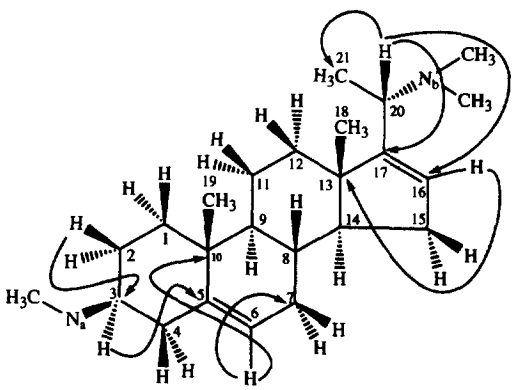


4



1a

eluted with MeOH–$CHCl_3$ (1:4) were combined and subjected to prep. TLC [silica gel F_{254}, *n*-hexane–$CHCl_3$–Et_2NH (14:5:1)] yielding alkaloid **1** (17.1 mg) (11.0×10^{-5}%). The $CHCl_3$ extract obtained by extraction at pH 3.5 was subjected to CC and eluted with *n*-hexane-Me_2CO and Me_2CO MeOH mixts of increasing polarities, to afford several frs. Precipitates appearing in the fr. eluted with MeOH–Me_2CO (1:4) were filtered off and washed with petrol (40–60°) and Me_2CO to obtain compound **4** (36.7 mg) (23.6×10^{-1}%). Another fr. (5.2 g), which was obtained by elution with MeOH–Me_2CO (3:1) was further subjected to CC on silica gel (F_{254}) and elution with *n*-hexane–Me_2CO and Me_2CO–MeOH mixts of increasing polarities, to afford 33 sub-frs. Sub-frs eluted with *n*-hexane–Me_2CO (7:3) were combined and subjected to prep. TLC using *n*-hexane–Me_2CO–Et_2NH (8:1:1) as mobile phase, to yield compounds **2** (16.3 mg, 10.5×10^{-5}%) and **3** (12.8 mg, 8.25×10^{-5}%).

Sarconidine (**1**). Yellow amorphous solid. $[\alpha]_D^{20} = -66°$ (*c* 0.02, $CHCl_3$). $R_f = 0.53$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3651 (NH), 3325 (CN), 1620 (C=C). ^1H NMR ($CDCl_3$, 500 MHz): δ 0.84 (3H, *s*, H_3-18), 1.02 (3H, *s* H_3-19), 1.09 (3H, *d*, $J_{21,20} = 6.6$ Hz, H_3-21), 2.23 (6H, *s*, $N_b(CH_3)_2$, 2.20 (3H, *s*, N_aCH_3), 2.87 (1H, *dd*, $J_{20,21} = 6.6$ Hz, H-20), 3.63 (1H, *m*, H-3α), 5.42 (1H, *br s*, H-6), 5.56 (1H, *br s*, H-16). HREI-MS *m/z* (rel. int., calcd): 356.3193 ($C_{24}H_{40}N_2$, 10, 356.3191), 341.2958 ($C_{23}H_{37}N_2$, 86, 341.2957), 72.0808 ($C_4H_{10}N$, 29, 72.0813). ^{13}C NMR ($CDCl_3$, 125 MHz): Table 1.

Sarcorine (**2**). Oily liquid $[\alpha]_D^{25} = +49°$ (*c* 0.815, $CHCl_3$). $R_f = 0.20$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1658 (amide), 3650 (NH). ^1H NMR ($CDCl_3$, 500 MHz): δ 0.63 (3H, *s*, H_3-18), 0.73 (3H, *s*, H_3-19), 0.90 (3H, *d*, $J_{21,20} = 6.5$ Hz, H_3-21), 1.96 (3H, *s*, $COCH_3$), 2.23 (6H, *br s*, $N_a(CH_3)_2$), 2.69 (1H, *m*, $W_{1/2} = 10.1$, H-20), 4.09 (1H, *m*, $W_{1/2} = 7.5$ Hz, H-3α), 5.74 (1H, *d*, $J_{NH,3} = 6.5$ Hz, NH). HREI-MS *m/z* (rel. int., calcd): 388.3446 ($C_{25}H_{44}N_2O$, 0.2, 388.3454), 373.3217 ($C_{24}H_{41}N_2O$, 0.7, 373.3219), 84.0820 ($C_5H_{10}N$, 18.7, 84.0813), 72.0819 ($C_4H_{10}N$, 100, 72.0813), 58.0613 (C_3H_8N, 19, 58.0657). ^{13}C NMR ($CDCl_3$, 125 MHz): Table 2.

Epipachysandrine-A (**3**). Amorphous solid. $[\alpha]_D^{23} - 58°$ (*c* 0.192, $CHCl_3$). $R_f = 0.17$. UV λ_{max}^{MeOH} (log ε) 203 (2.8). IR ν_{max} cm^{-1} ($CHCl_3$, 3625 (NH), 3420 (OH), 1659 (benzamide). ^1H-NMR ($CDCl_3$, 500

Table 2. ^{13}C NMR chemical shift assignments of compounds **2** and **4***

Carbon	**2**	**4**
1	32.0 (CH_2)	33.5 (CH_2)
2	28.5 (CH_2)	31.4 (CH_2)
3	62.1 (CH)	49.4 (CH)
4	39.9 (CH_2)	39.2 (CH_2)
5	41.1 (CH)	139.8 (-C-)
6	27.6 (CH_2)	121.2 (CH)
7	31.3 (CH_2)	31.5 (CH_2)
8	35.4 (CH)	49.0 (CH)
9	44.8 (CH)	53.6† (CH)
10	36.0 (-C-)	37.0 (-C-)
11	20.8 (CH_2)	20.6 (CH_2)
12	42.3 (CH_2)	39.0 (CH_2)
13	42.1 (-C-)	41.5 (-C-)
14	54.4 (CH)	56.7 (CH)
15	24.1‡ (CH_2)	26.0‡ (CH_2)
16	26.1‡ (CH_2)	24.9‡ (CH_2)
17	54.6† (CH)	56.9† (CH)
18	10.6 (CH_3)	18.3 (CH_3)
19	11.5 (CH_3)	19.6 (CH_3)
20	56.7† (CH)	62.8 (CH)
21	12.4 (CH_3)	12.4/12.5 (CH_3)
$N_a(CH_3)_n$	—	43.5 ($n = 2$)
$N_b(CH_3)_n$	44.8 $n = 2$	31.5/31.4 ($n = 1$)
N_aCOCH_3	23.7	—
N_b $COCH_3$	—	22.0/22.2
N_b $\underline{C}OCH_3$	169.2	170.1

* Multiplicities determined by DEPT.
†,‡ Assignments interchangeable.

MHz): δ 0.63 (3H, *s*, H_3-18), 1.05 (3H, *s*, H_3-19), 0.86 (3H, *d*, $J_{21,20} = 6.3$ Hz, H_3-21), 7.44–7.75 (5H, *m*, Ar-H), 2.16 (6H, *br s*, $N\alpha$-($\underline{CH}_3)_2$, 2.39 (1H, *m*, $W_{1/2} = 6.0$, H-20), 4.09 (1H, *m*, $W_{1/2} = 6.0$ Hz, H-3α), 6.22 (1H, *d*, $J = 7.28$ Hz, NH), 4.19 (1H, *dd*, $J = 3.0, 6.3$, H-4α). HREI-MS *m/z* (rel. int., calcd): 466.3519 ($C_{30}H_{46}N_2O_2$, 0.8, 466.3559), 451.3341 ($C_{29}H_{43}N_2O_2$, 1.2, 451.3325), 105.0336 (C_7H_5O, 3, 105.0340), 72.0797 ($C_4H_{10}N$, 100, 72.0813).

Saracocine (**4**). Crystals, mp 228–229°. $[\alpha]_D^{23} -26°$; (*c* 0.124, $CHCl_3$). $R_f = 0.32$. UV λ_{max} (MeOH) 202 ($\log \varepsilon = 3.2$). IR ν_{max} cm^{-1} ($CHCl_3$): 1620 (amide). ^1H NMR ($CDCl_3$), 500 MHz): δ 0.98/0.99 (3H, *s*, H_3-18), 1.02/1.04 (3H, *s*, H_3-19), 1.13/1.15 (3H, *d*, $J_{21,20} = 6.6$ Hz, H_3-21), 1.99/2.06 (3H, *s*, $COC\underline{H}_3$), 2.69/2.74 (3H, *s*, N_bCH_3), 2.20 (6H, *br s*, $N_a(CH_3)_2$), 4.72 (1H, *q*, $J_{20,21} = 6.6$ Hz, H-20), 5.32 (1H, *br s*, H-6). HREI-MS *m/z* (rel. int., calcd), 400.3471, $C_{26}H_{44}N_2O$, 1, 400.3454). ^{13}C NMR ($CDCl_3$, 125 MHz): Table 2.

Acknowledgements—We are grateful to Glaxo, Pakistan, for providing financial assistance to M.R.K. We are also thankful to Mr Tahir Ali, Plant Taxonomist, Department of Botany, University of Karachi, for identification of plant material.

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