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STEROIDAL ALKALOIDS FROM ROOTS OF *SARCOCOCCA VAGANS*

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Key Word Index—*Sarcococca vagans*; Buxaceae; Steroidal alkaloids.

Abstract—Three new steroidal alkaloids, named sarcovagenine A–C, were isolated from roots of *Sarcococca vagans*. Their structures were established on the basis of spectral evidence and biogenetic considerations as (20*S*)-20-(dimethylamino)-3β-(tigloylamino)-5α-pregn-16-en-2β,4β-diol, (20*S*)-4β-acetoxy-20-(dimethylamino)-3β-(tigloylamino)-5α-pregn-16-en-2α-ol, and (20*S*)-20-(dimethylamino)-3-(tigloylamino)-5α-pregn-2,16-dien-4-one, respectively.

INTRODUCTION

Sarcococca vagans Stapf. is a Chinese folk medicine distributed over southern China. It has been used as an antitumour agent. The occurrence of steroidal alkaloids in Buxaceae is well documented [1], but little phytochemical work has been carried out on *Sarcococca vagans* [2]. In this paper we describe the isolation and structural elucidation of three new steroidal alkaloids from this species.

RESULTS AND DISCUSSION

The crude alkaloids were isolated from the concentrated ethanol extracts of *Sarcococca vagans* roots by extraction at pH 3.5 with $CHCl_3$. Separation of the mixture by repeated column chromatography on silica gel and further purification by preparative TLC resulted in the isolation of compounds **1**, **2** and **3**.

Sarcovagenine A (**1**) was isolated as colourless crystals. Its molecular formula, $C_{28}H_{46}N_2O_3$, was deduced from the quasi-molecular ion at *m/z* 459 in CI-MS and elemental analysis. The IR spectrum showed intense absorptions at 3444 (NH and OH), 1664 (C=O) and 1619 (C=O) cm^{-1}. The ^1H NMR spectrum of **1** exhibited the signals for two tertiary methyl groups at δ 0.85 and 1.27 as well as one secondary methyl group at δ 1.08, corresponding to H_3-18, H_3-19 and H_3-21, respectively. The singlet of six protons at δ 2.22 was ascribed to the NMe_2 moiety. These features, associated with the ^{13}C NMR data (see Table 1) suggested that **1** had a 20-dimethylamino pregnane skeleton [2, 3], which was further confirmed by the characteristic fragment peak at *m/z* 72 [4] in its EI-mass spectrum. This fragment ion, however, had a lower abundance in comparison with the mass spectrum of common steroidal alkaloids with 20-NMe_2, in which the ion at *m/z* 72 was always the base peak. This was attributed to the presence of a double bond between C-16 and C-17, which was confirmed by the olefinic proton signal at δ 5.52 (*br. s*) in the ^1H NMR spectrum and a pair of olefinic carbon signals at δ 157.23 and 123.58 in the ^{13}C NMR spectrum.

In addition, the ^1H NMR spectrum of **1** showed characteristic resonances of a tigloyl group at δ 6.50 (1H, *q*, *J* = 6.9 Hz), 1.87 (*s*) and 1.76 (3H, *d*, *J* = 6 Hz). The ^{13}C NMR spectrum also confirmed the presence of a tigloyl group. The amidic NH proton afforded a doublet at δ 6.73 (*J* = 7.6 Hz), while a doublet of double doublet at δ 3.90 (*J* = 7.6, 3.3, 2.9 Hz) was assigned to the H-3α proton. The presence of two hydroxy groups was demonstrated by their ^1H NMR signals at δ 4.14 (1H, *br. s*) and 3.89 (1H, *br s*)

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Table 1. The ^{13}C NMR data of compounds **1**–**3** (in $CDCl_3$)

	1	2	3		1	2	3
1	44.27	45.43	38.94	16	123.58	123.75	125.65
2	71.39	70.00	126.09	17	157.23	157.25	156.46
3	53.19	57.38	131.48	18	15.94	16.02	16.00
4	75.77	75.49	196.44	19	17.38	18.93	13.25
5	50.13	44.71	54.57	20	59.00	59.14	59.34
6	25.74	23.09	20.44	21	16.08	16.02	16.00
7	31.02	29.69	30.45	NMe_2	42.45	42.44	42.28
8	33.49	31.29	34.35	C=O	169.03	171.39	167.68
9	57.38	57.21	55.08	2′	131.74	132.33	132.22
10	35.12	35.80	40.17	3′	131.17	131.00	131.77
11	20.29	20.72	20.81	4′	13.93	14.06	14.07
12	31.96	31.00	31.08	5′	12.36	14.25	12.16
13	46.77	46.82	46.29	OAc		171.39	
14	57.38	56.86	57.09			20.96	
15	34.43	34.53	34.35				

All assignments were confirmed by ^1H-^1H COSY, ^{13}C-^1H COSY and DEPT spectra.

and their ^{13}C NMR signals at δ 71.39 and 75.77, the location of which should be restricted to C-2 and C-4, respectively. The ^1H-^1H COSY experiment revealed a spin system [CH2 (1)—CH (2)—CH (3)—CH (4)—CH (5)] confirming the above assignments, while the coupling constants for H-2 and H-4 required the β-configuration of the two hydroxy groups. The stereochemistry of the other chiral centres was deduced by comparison of the ^1H NMR and ^{13}C NMR signals with those reported for axillarine [2] and from biogenetic reasons [5]. Therefore, the structure of sarcovagenine A was elucidated as (20*S*)-20-(dimethylamino)-3β-(tigloylamino)-5α-pregn-16-en-2β,4β-diol (**1**).

Sarcovagenine B (**2**) was obtained as colourless needles. Its molecular formula $C_{30}H_{48}N_2O_4$ was deduced by the quasi-molecular ion at *m/z* 501 in CI-mass spectral and elemental analysis. The IR spectrum displayed absorptions at 3444 (NH), 1724 (OAc), 1662 (C=O) and 1612 (C=O) cm^{-1}. The ^1H NMR and ^{13}C NMR data of **2** were similar to those of **1**, especially the signals due to ring C and D as well as the side chain, suggesting that **2** was a 20-dimethylamino pregn-16-ene alkaloid. Similarly, a 3β-tigloylamino group followed from the ^1H and ^{13}C NMR signals. The presence of an acetyl group was deduced by IR (1724), ^1H NMR (δ 2.06) and ^{13}C NMR (δ 171.39, 20.96). The position of this acetyl group was clearly assigned at C-4β from the chemical shift (δ 5.10) and coupling constant (*dd*, J = 6.1, 5.9 Hz) for the H-4α. In addition, a ^1H NMR signal at δ 3.77 (1H, *ddd*, J = 9.7, 7.4, 7.4 Hz) and ^{13}C NMR resonances at δ 70.00 revealed a hydroxy group attached to C-2. The observed coupling of H-2 required an α-orientation of the hydroxy group. According to the detailed analyses of ^1H, ^{13}C, ^1H-^1H COSY and ^{13}C-^1H COSY NMR spectral data, the structure of sarcovagenine B was determined as (20*S*)-4β-acetoxy-20-(dimethylamino)-3β-(tigloylamino)-5α-pregn-16-en-2α-ol (**2**).

Sarcovagenine C (**3**) had the molecular formula $C_{28}H_{42}N_2O_2$ confirmed by the CI-mass spectral (*m/z* 439) and elemental analysis. Its IR spectrum showed absorptions at 3392 (NH), 1657 (C=O), 1630 (C=O) cm^{-1}. The ^1H NMR and ^{13}C NMR spectra also displayed characteristics of a 20-dimethylamino pregn-16-ene alkaloid similar to **1** and **2**. However, the signals for the A-ring were significantly different, although a tigloylamino group also attached to C-3. The ^{13}C NMR resonances at δ 196.44, 126.09 and 131.48 indicated the presence of an α,β-unsaturated carbonyl moiety in ring A. The amidic NH resonated as a singlet at δ 8.19, shifted downfield due to the proximity of the α,β-unsaturated carbonyl moiety. The C-2 olefinic proton appeared as a double doublet at δ 7.67 (J = 6.8, 2.6 Hz). The downfield chemical shift of this proton was apparently due to the proximity of the carbonyl group of the tigloylamide and β-position of the α,β-unsaturated carbonyl system. The splitting pattern of this proton supported the fact that the α,β-unsaturated carbonyl moiety was at C-4 and not at C-1. Therefore, the structure of sarcovagenine C was elucidated as (20*S*)-20-(dimethylamino)-3-(tigloylamino)-5α-pregn-2,16-dien-4-one (**3**).

EXPERIMENTAL

General. Mps were determined with a Boetius micro melting point apparatus and are uncorr. EI MS were recorded on a Zab-2f mass spectrometer. All NMR spectra were measured on a Bruker AM-500 spectrometer with $CDCl_3$ as solvent and int. standard. IR spectra were recorded on a Perkin–Elmer 683 spectrometer.

Plant material. Roots of *S. vagans* were collected in Guangxi province, Peoples Republic of China. The plant material was identified by Prof. S. Y. Liu, Guangxi College of Traditional Chinese Medicine. A

voucher specimen is deposited in Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and isolation. Air-dried roots (300 g) of *S. vagans* were extracted with 95% EtOH, and the extract was evapd to afford a gum, which was treated with 0.5% HCl. Partial sepn of an alkaloid mixt. was carried out by extraction into $CHCl_3$ at different pH values. The fr. obtained at pH 3.5 was repeatedly subjected to silica gel and elution with cyclohexane containing various proportions of Me_2CO and Et_2NH. Further purification by prep. TLC resulted in the isolation of three steroidal alkaloids, 68 mg (0.023%) sarcovagenine A (**1**), 36 mg (0.012%) sarcovagenine B (**2**) and 31 mg (0.01%) sarcovagenine C (**3**).

Sarcovagenine A (**1**). Colourless crystals, mp 260–261°, $[\alpha]_D^{25}$ +9.43° ($CHCl_3$, *c* 0.053). Found C 72.79, H 9.95, N 6.14; $C_{28}H_{46}N_2O_3$ requires C 73.32, H 10.11, N 6.11%. IR ν_{max}^{KBr} cm^{-1}: 3444, 2930, 2849, 1664 (C=O), 1619 (C=C), 1519, 1384, 1152, 1080. CI-MS *m/z*: 459 $[M+H]^+$, 443. EI-MS *m/z* (rel. int.): 458 $[M]^+$ (4), 433 $[M-CH_3]^+$ (100), 100 (8), 83 (8), 72 (10), 55 (2). ^1H NMR ($CDCl_3$): δ 6.73 (1H, *d*, *J* = 7.6 Hz, NH), 6.50 (1H, *q*, *J* = 6.9 Hz, C=CH), 5.52 (1H, *br. s*, H-16), 4.14 (1H, *br. s*, H-2α), 3.90 (1H, *ddd*, *J* = 7.6, 3.3, 2.9 Hz, H-3α), 3.89 (1H, *br. s*, H-4α), 2.82 (1H, *q*, *J* = 6.6 Hz, H-20), 2.22 (6H, *s*, NMe_2), 2.17 (1H, *dd*, *J* = 14.5, 3.1 Hz, H-1), 2.06 (1H, *ddd*, *J* = 15.9, 6.5, 3.2 Hz, H-15), 1.87 (3H, *s*, 2′-CH_3), 1.86 (1H, overlap, H-15), 1.76 (3H, *d*, *J* = 6.9 Hz, 3′-CH_3), 1.27 (3H, *s*, H_3-19), 1.08 (3H, *d*, *J* = 6.6 Hz, H_3-21), 0.85 (3H, *s*, H_3-18). ^{13}C NMR see Table 1.

Sarcovagenine B (**2**). Colourless crystals, mp 283–284°, $[\alpha]_D^{25}$ +5.92° ($CHCl_3$, *c* 0.152). Found C 72.58, H 9.59, N 5.55. $C_{30}H_{48}N_2O_4$ requires C 71.96, H 9.66, N 5.59%. IR ν_{max}^{KBr} cm^{-1}: 3444 (NH and OH), 2939, 1724, (OAc), 1662 (C=O), 1612 (C=C), 1554, 1384, 1239. CI-MS *m/z*: 501 $[M+H]^+$, 485, 72. EI-MS *m/z* (rel. int.): 500 $[M]^+$ (2), 485 $[M-CH_3]^+$ (100), 425 (15), 100 (7), 83 (25), 72 (58), 55 (15). ^1H NMR ($CDCl_3$): δ 6.48 (1H, *q*, *J* = 6.9 Hz, H-3′), 6.03 (1H, *d*, *J* = 6.6 Hz, NH), 5.54 (1H, *br. s*, H-16), 5.10 (1H, *dd*, *J* = 6.1, 5.9 Hz, H-4α), 4.17 (1H, *ddd*, *J* = 9.7, 6, 6.6 Hz, H-3α), 3.77 (1H, *ddd*, *J* = 9.7, 7.4, 7.4 Hz, H-2β), 2.86 (1H, *q*, *J* = 6.5 Hz, H-20), 2.25 (6H, *s*, NMe_2), 2.06 (3H, *s*, OAc), 2.05 (1H, *ddd*, *J* = 13.7, 6.4, 3.1 Hz, H-15), 1.91 (1H, *dd*, *J* = 14.3, 7.4 Hz, H-1), 1.83 (3H, *s*, 2′-CH_3), 1.76 (3H, *d*, *J* = 6.9 Hz, 3′-CH_3), 1.61 (1H, *dd*, *J* = 14.3, 7.4 Hz, H-1), 1.17 (3H, *s*, H_3-19), 1.11 (3H, *d*, *J* = 6.5 Hz, H_3-21), 0.84 (3H, *s*, H_3-18). ^{13}C NMR see Table 1.

Sarcovagenine C (**3**). Colourless crystals, mp 160–161°, $[\alpha]_D^{25}$ +5.43° ($CHCl_3$, *c* 0.056). Found C 75.85, H 9.49, N 6.64; $C_{28}H_{42}N_2O_2$ requires C 76.67, H 9.65, N 6.39%. IR ν_{max}^{KBr} cm^{-1}: 3392 (NH), 2933, 2850 1657 (C=O), 1630 (C=C), 1519, 1367. CI-MS: *m/z* 439 $[M+H]^+$. EI-MS *m/z* (rel. int.): 438 $[M]^+$ (5), 423 $[M-CH_3]^+$ (100), 83 (57), 72 (20), 55 (22). ^1H NMR ($CDCl_3$): δ 8.19 (1H, *s*, NH), 7.67 (1H, *dd*, *J* = 6.8, 2.6 Hz, H-2), 6.51 (1H, *q*, *J* = 6.9 Hz, H-3′), 5.66 (1H, *br s*, H-16), 2.82 (1H, *q*, *J* = 6.6 Hz, H-20), 2.53 (1H, *dd*, *J* = 18.5, 6.9 Hz, H-1), 2.20 (6H, *s*, NMe_2), 2.07 (1H, *ddd*, *J* = 14.5, 7.3, 3.2 Hz, H-15), 1.89 (3H, *s*, 2′-CH_3), 1.78 (3H, *d*, *J* = 6.9 Hz, 3′-CH_3), 1.08 (3H, *d*, *J* = 6.6 Hz, H_3-21), 0.91 (3H, *s*, H_3-19), 0.85 (3H, *s*, H_3-18). ^{13}C NMR see Table 1.

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