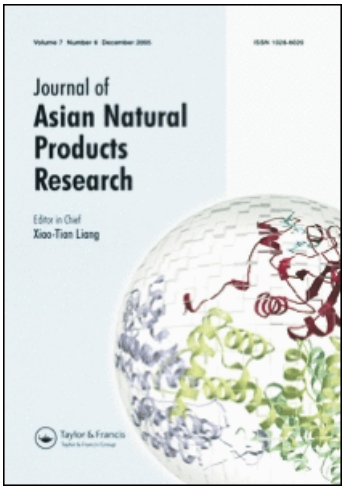

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Two new steroidal alkaloids from the roots of *Sarcococca ruscifolia*

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ORIGINAL ARTICLE

Two new steroidal alkaloids from the roots of *Sarcococca ruscifolia*

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Two new steroidal alkaloids, 20α-dimethylamino-2α-hydroxyl-3β-tigloylamino-5α-pregnane (**1**) and Δ^{16}-20α-dimethylamino-3β,4α-diol-5α-pregnane (**2**), along with five known alkaloids, sarcovagine D (**3**), pachysamine G (**4**), pachysamine H (**5**), pachysamine A (**6**), and terminaline (20*S*)-20-(*N*,*N*-dimethylamino)-5α-pregn-3β,4α-diol (**7**), were isolated from the roots of *Sarcococca ruscifolia*. Their structures were elucidated on the basis of NMR and MS data, and the relative stereochemistry of **1** was finally determined by X-ray crystallographic analysis.

Keywords: *Sarcococca ruscifolia*; steroidal alkaloids; 20α-dimethylamino-2α-hydroxyl-3β-tigloylamino-5α-pregnane; Δ^{16}-20α-dimethylamino-3β,4α-diol-5α-pregnane

1. Introduction

Sarcococca ruscifolia belongs to the genus *Sarcococca* of the family Buxaceae. It is regionally distributed in Shanxi, Gansu, Hubei, Hunan Provinces, Guangxi Zhuang Autonomous Region, and other places in China. The root of *S. ruscifolia*, a Chinese traditional medicine named 'Wei You', has been commonly used to treat stomach pain, rheumatism, and bruises [1]. Previous studies showed that steroidal alkaloids are the major components of *S. ruscifolia* [2–17]. Our phytochemical research led to the isolation of two new steroidal alkaloids, 20α-dimethylamino-2α-hydroxyl-3β-tigloylamino-5α-pregnane (**1**) and Δ^{16}-20α-dimethylamino-3β,4α-diol-5α-pregnane (**2**), together with five known steroidal alkaloids. This paper deals with the isolation and structural elucidation of compounds **1**–**7** (Figure 1) by 1D and 2D NMR techniques, HR-ESI-MS analysis, as well as X-ray crystallographic analysis.

2. Results and discussion

Compound **1** was isolated as a colorless cubic crystal, positive to Dragendorff's reagent. The IR spectrum displayed absorptions of the hydroxyl group (3286 cm^{-1}), carbonyl group (1663 cm^{-1}), and double bond (1614 cm^{-1}). Its HR-ESI-MS displayed $[M+H]^+$ at *m/z* 445.3783, indicating the molecular formula of $C_{28}H_{48}N_2O_2$. The GC–MS showed $[M]^+$ at *m/z* 444 and $[M-Me]^+$ at *m/z* 429. The characteristic mass fragments at *m/z* 72 and 83 led to the establishment of the $-CH_2(CH_3)N(CH_3)_2$ and tigloyl moieties, which were located at C-20 and C-3 of the pregnane skeleton, respectively [18,19]. The ^1H NMR ($CDCl_3$) spectrum of **1** displayed two methyl signals at δ 0.64 (3H, s) and 0.82 (3H, s) for the C-18 and C-19

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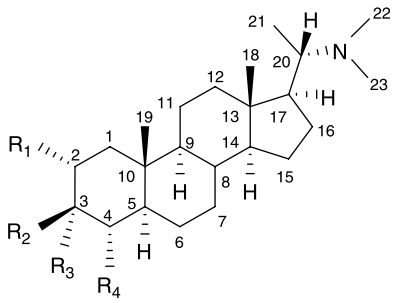

	R_1	R_2	R_3	R_4	Unsaturation
1	OH	$-NHCOCCH_3{=}CHCH_3$	H	H	
2	H	OH	H	OH	$\triangle^{16,17}$
3	H	$-NHCOCCH_3{=}CHCH_3$	H	=O	$\triangle^{2,3}$
4	H	$-NHCOCCH_3{=}CHCH_3$	H	H	
5	H	H	–NHCOPh	H	
6	H	$-NHCH_3$	H	H	
7	H	OH	H	OH	

Figure 1. Structures of compounds **1**–**7**.

methyl protons. A methyl doublet at δ 0.93 (3H, d, $J = 6.5$ Hz) was due to the C-21 methyl protons. The *N*,*N*-dimethyl protons resonated as a 6H singlet at δ 2.13 (6H, s). Two methyl signals at δ 1.83 (3H, s) and 1.77 (3H, d, $J = 6.5$ Hz) were assigned to C-5′ and C-4′ methyl protons. A methine quartet at δ 6.46 ($J = 6.5$ Hz) was due to H-3′. The ^{13}C NMR and DEPT spectra of **1** exhibited 28 carbon resonances due to seven methyl, eight methylene, eight methine, and five quaternary carbons. Comparison of ^{13}C NMR spectral data between **1** and **4** revealed great similarity, and the only difference was the downfield chemical shift of C-2 at δ 71.3, which suggested that compound **1** possessed a hydroxyl group at C-2. In the HMBC spectrum of **1**, H-1 at δ 1.02, H-3 at δ 3.66, and H-4 at δ 1.65 established correlation signals with C-2 at δ 71.3, which further confirmed that the hydroxyl group was located at the C-2 position. The correlations from H-4′ at δ 1.77 with C-2′ at δ 131.0, and H-5′ at δ 1.83 with C-1′ at δ 171.3 and C-3′ at δ 131.6, showed the presence of a tigloylamide moiety in **1** (Figure 2). The relative configuration of the hydroxyl group at C-2 was deduced from the NOESY interaction of H-2 at δ 3.56 with H-19 at δ 0.82 (Figure 3). The relative stereochemistry of **1** was determined by X-ray crystallographic analysis as shown in Figure 4.[1] According to the IUPAC nomenclature rule, the relative stereocenters of C-2, -3, -10, -13, -17, and -20 were deduced as *R**, *S**, *S**, *S**, *R**, and *S**, respectively. Therefore, compound **1** was determined as 20α-dimethylamino-2α-hydroxyl-3β-tigloylamino-5α-pregnane (**1**) (Figure 1).

Compound **2** was obtained as a colorless schistic crystal, positive to Dragendorff's reagent. The IR spectrum displayed absorption of hydroxyl groups (3454 cm^{-1}) and olefinic function (1641 cm^{-1}). Its molecular formula was determined as $C_{23}H_{39}NO_2$ by the positive HR-ESI-MS at *m/z* 362.3056 $[M+H]^+$, indicating five degrees of unsaturation.

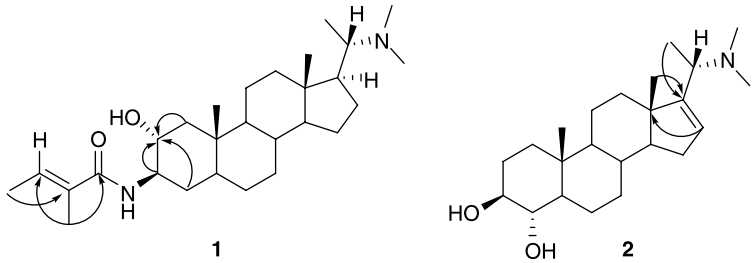

Figure 2. Selected HMBC correlations of compounds **1** and **2**.

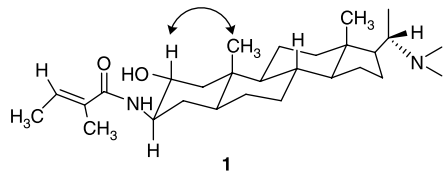

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Figure 3. Selected NOESY correlations of compound **1**.

The GC–MS showed $[M]^+$ at *m/z* 361 and $[M-Me]^+$ at *m/z* 346, respectively. The characteristic mass fragments at *m/z* 72 revealed the establishment of $-CH_2(CH_3)N(CH_3)_2$. The ^1H NMR spectrum of **1** displayed two methyl signals at δ 0.81 (3H, s) and 0.88 (3H, s) for the C-18 and C-19 methyl protons. A methyl doublet at δ 1.20 (3H, d, $J = 6.5$ Hz) was due to the C-21 methyl protons. The *N*,*N*-dimethyl protons resonated as a 6H singlet was present at δ 2.13 (6H, s). An olefinic proton singlet at δ 5.61 was due to the C-16 proton. The ^{13}C NMR spectrum of **2** exhibited 23 carbon resonances due to five methyl, seven methylene, eight methine, and three quaternary carbons. Comparison of its ^1H and ^{13}C NMR spectra with those of **7** suggested that **2** possessed an olefinic function at C-16/C-17, with chemical shifts of C-16 at δ 123.3, C-17 at δ 155.9 and the appearance of a H singlet at δ 5.61 in the ^1H NMR spectrum. In the HMBC spectrum, $^{13}C-^1H$ long-range correlations were observed at H-19/C-17 and H-21/C-17, which confirmed the existence of the double bond at C-16/C-17 (Figure 2). Further comparison of the NMR spectra of **2** with the known compound Δ^{16}-3α-dimethylamino-20α-dimethylamino-pregnane [20], possessing the same C and D rings, also confirmed the above deduction. These evidences led to the establishment of **2** to be Δ^{16}-20α-dimethylamino-3β,4α-diol-5α-pregnane (**2**) (Figure 1).

The known compounds sarcovagine D (**3**) [10], pachysamine G (**4**) [21], pachysamine H (**5**) [21], pachysamine A (**6**) [10], and terminaline (20*S*)-20-(*N*,*N*-dimethylamino)-5α-pregn-3β,4α-diol (**7**) [22] were identified by comparison of their ^1H, ^{13}C NMR, and MS data with those reported in the literature.

3. Experimental

3.1 General experimental procedures

The melting points were determined on an XT-4 melting point apparatus and are uncorrected. A WZZ-2A digital polarimeter was used for measuring the optical rotations. The UV spectra were recorded on an HP8453 spectrophotometer, and IR spectra were recorded on a VECTOR22 Fourier transform IR spectrophotometer. The NMR spectra were recorded on an INOVA 400 MHz instrument. The chemical shift (δ) values are reported in ppm units, and coupling constants (J) are given

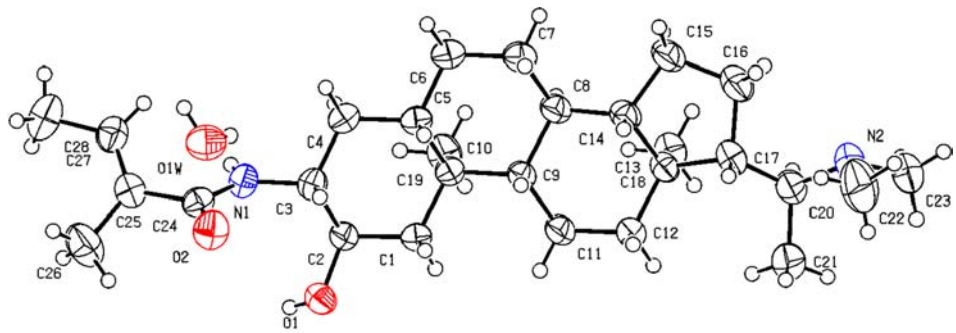

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Figure 4. X-ray crystal structure of compound **1**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** ($CDCl_3$, δ in ppm).

	1		**2**	
Position	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	46.3	1.02, 2.05 m	36.0	1.06, 1.68 m
2	71.3	3.56 m	31.3	1.80, 1.85 m
3	54.3	3.66 m	76.4	3.26 m
4	33.8	1.65, 1.31 m	75.6	3.22 dd (10.3, 9.2)
5	44.9	1.29 m	50.9	1.06 m
6	27.2	1.42, 1.85 m	22.5	1.92, 1.97 m
7	31.5	0.93, 1.72 m	28.3	1.58, 1.86 m
8	34.4	1.30 m	33.5	2.33 m
9	56.0	1.06 m	55.0	0.81 m
10	36.7	–	37.4	–
11	20.9	1.52, 1.61 m	20.7	1.61, 1.23 m
12	39.4	1.88, 1.92 m	31.0	1.85, 1.13 m
13	41.7	–	46.5	–
14	56.1	1.07 m	57.8	1.31 m
15	23.8	1.08, 1.60 m	34.5	1.38, 1.75 m
16	27.5	1.35, 1.81 m	123.3	5.61 m
17	53.9	0.76 m	155.9	–
18	12.0	0.64 s	15.9	0.81 s
19	12.9	0.82 s	13.5	0.88 s
20	61.2	2.44 m	58.9	2.83 m
21	9.8	0.93 d (6.5)	18.0	1.20 d (6.5)
NMe_2	39.4	2.13 s	42.5	2.13 s
1′	171.3	–		
2′	131.0	–		
3′	131.6	6.46 q (6.5)		
4′	13.7	1.77 d (6.5)		
5′	12.0	1.83 s		

in Hz. The GC–MS were recorded on a 5973 MSD mass spectrometer, while positive HR-ESI-MS were carried out on an API Qstar Pulsar spectrometer. The X-ray crystallographic analysis was recorded on a SMART APEX CCD SYSTEM single crystal X-ray diffractometer. The purity of the samples was checked by TLC (silica gel precoated plates). Dragendorff's reagent was used to detect the alkaloids.

3.2 Plant material

The plant material was collected in July 2006 from the Baihuahu District, Guizhou Province, and was identified by Prof. QianHai Chen (Guizhou Institute of Biology). A voucher specimen (GZ 060712) has been deposited in the herbarium of the Guiyang College of Traditional Chinese Medicine.

3.3 Extraction and isolation

The roots of *S. ruscifolia* (10 kg) were air-dried and extracted with 90% EtOH (3 h × 4, 5 liters each) under reflux. The crude residue (620 g), obtained after evaporation of the solvent on a rotary evaporator, was dissolved in H_2O (3 liters) and defatted with petroleum ether (6 liters). The aqueous layer was extracted with $CHCl_3$ (pH 10, 8 liters) to obtain the crude alkaloidal fraction (107.8 g). The pH was adjusted with the addition of 10% ammonium hydroxide. The crude alkaloidal fraction (425 g) obtained on removal of $CHCl_3$ *in vacuo* was adsorbed on an equal quantity of silica gel and purified on a column prepacked with silica gel (200–300 mesh) and eluted with increasing polarities of petroleum ether–$CHCl_3$ and $CHCl_3$–MeOH to obtain eight fractions

(A–H). Fraction C (110 g) was repeatedly isolated and purified by silica gel column chromatography, with elution by increasing polarities of petroleum ether–acetoacetate–diethylamine to yield compounds **3** (9 g), **4** (10 g), **5** (87 mg), and **6** (130 mg). Fraction F (5.3 g) was subjected to repeated column chromatography to afford subfractions (1–11). Subfraction 4 (0.56 g) was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH (8:2) to obtain compounds **1** (1 g), **2** (9 mg), and **7** (8 mg).

*3.3.1 20α-Dimethylamino-2α-hydroxyl-3β-tigloylamino-5α-pregnane (**1**)*

Colorless cubic crystals; mp 237–239°C; $[\alpha]_D^{25}$ −26.6 ($c = 0.58$, $CHCl_3$); UV (MeOH) λ_{max}: 244 nm; IR (KBr) ν_{max}: 3286, 1663, 1614 cm^{-1}; ^1H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS: *m/z* 445.3783 $[M+H]^+$ (calcd for $C_{28}H_{49}N_2O_2$, 445.3794).

*3.3.2 Δ[16]-20α-Dimethylamino-3β,4α-diol-5α-pregnane (**2**)*

Colorless schistic crystals; mp 208–210°C; $[\alpha]_D^{25}$ −23.4 ($c = 0.31$, $CHCl_3$); UV (MeOH) λ_{max}: 211, 229, 275 nm; IR (KBr) ν_{max}: 3454, 1641 cm^{-1}; ^1H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS: *m/z* 362.3056 $[M+H]^+$ (calcd for $C_{23}H_{39}NO_2$, 362.3059).

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Note

1. Crystallographic data of compound **1**: $C_{28}H_{48}N_2O_2$ (H_2O), MW = 462.69; monoclinic, space group $P2_1$; $a = 6.5514$ (6) Å, $b = 11.2971$ (10) Å, $c = 37.401$ (3) Å, $\alpha = 90.00$, $\beta = 90.00$, $\gamma = 90.00$, $V = 2768.1$ (4) $Å^3$, $Z = 4$, $d = 1.108$ g/cm^3. The total number of reflections measured was 6027, of which 3650 were observed, $I > 2\sigma(I)$. Final indices: $R_1 = 0.0549$, $wR_2 = 0.1380$. The crystal structure of **1** was solved by direct method SHELXS-97 [23] and expanded using difference Fourier technique, refined by the program SHELXL-97 [24] and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 751548). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or Email: desposit@ccdc.cam.ac.uk).

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