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TWO NEW STEROIDAL SAPONINS WITH ANTIFUNGAL ACTIVITY FROM *Hosta plantaginea* RHIZOMES

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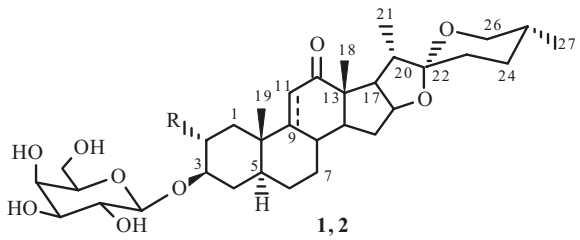
*Two new steroidal saponins, (25*R*)-3*β*-hydroxy-5*α*-spirost-9-en-12-one 3*-O-β-*D-galactopyranoside (hostasaponin A, **1**), and (25*R*)-2*α*,3*β*-dihydroxy-5*α*-spirost-12-one 3*-O-β-*D-galactopyranoside (hostasaponin B, **2**), together with a new natural product, 2*α*,3*β*-dihydroxy-5*α*-pregn-16-en-20-one (**3**), were isolated from the rhizomes of* Hosta plantaginea*, a perennial herb used mainly as an ornamental plant and folk medicine for fungal infection and various inflammations. The active evaluation results showed that **1** and **2** possess potent inhibition activity against* Candida albicans*, with MIC values less than 20 μg/mL.*

Keywords: *Hosta plantaginea*, steroidal saponin, antifungal.

Hosta plantaginea (Lam.) Asch., belonging to the family Liliaceae, is widely cultivated as an ornamental plant worldwide. Its rhizomes (Yu-zan-gen in Chinese) are commonly used as a traditional folk medicine in China and Japan for the treatment of fungal infection, sore throat, mastitis, and otitis media [1–3]. Previous phytochemical investigations of this plant focused on the flowers and led to the isolation of some steroidal saponins [4–6], flavonoid glycosides [7], and a cerebroside [8]. Recently, 18 alkaloids were also isolated from the whole plant [9, 10]. Various steroidal saponins and benzylphenethylamine alkaloids isolated from *H. plantaginea* showed obvious antitumor and antiviral activities. Nevertheless, the chemical constituents of *H. plantaginea* rhizomes are not clear yet. As a continuation of the chemical investigation on Hosta plants [11, 12], the isolation and structural elucidation of new steroidal glycosides from *H. plantaginea* rhizomes are reported herein.

Compound **1** was obtained as white needles (MeOH) with the molecular formula $C_{33}H_{50}O_9$ which was deduced from the positive-ion HR-ESI-MS. The ^{1}H NMR spectrum indicated the presence of two tertiary methyl groups [δ 0.97 and 0.91 (each 3H, s)], two secondary methyl groups [δ 1.33 (3H, d, J = 6.8 Hz) and 0.69 (3H, d, J = 5.8 Hz)], typical spirostanolmethyls, and an anomeric proton signal [δ 4.98 (1H, d, J = 7.7 Hz)] showing HSQC correlation with an anomeric carbon signal (δ 102.7) (Table 1). The orientation of C-5 is α because of the chemical shift of C-5 (δ 43.6). For 5α steroidal compounds, the chemical shifts of C-5 appear at 43–46, while for 5β steroids, the chemical shifts are observed at 35–36.5 [13].



1, 2

1: R = H, Δ9; **2:** R = OH

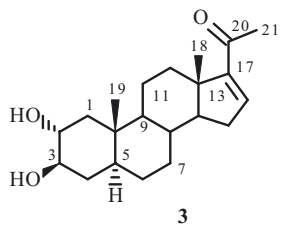


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TABLE 1. ^{13}C (150 MHz) and ^{1}H NMR (600 MHz) Data of Compounds **1–3** (C_5D_5N, TMS, δ, ppm, J/Hz)*

C atom	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	35.3	0.76 (ddd, J = 12.0, 11.2, 3.6)	45.2	1.10[a]	46.0	1.25 (dd, J = 12.5, 10.0)
		1.39 (dd, J = 12.0, 3.6)		2.14 (dd, J = 12.6, 4.3)		2.24 (dd, J = 12.5, 4.7)
2	29.9	1.76[a], 2.04 (br.d, J = 11.2)	70.4	3.95 (ddd, J = 12.6, 9.3, 4.3)	72.8	4.03 (ddd, J = 12.5, 8.9, 4.7)
3	77.5	3.88 m	84.2	3.83 (ddd, J = 12.7, 9.3, 4.2)	76.4	3.84 (ddd, J = 12.6, 9.0, 5.2)
4	34.9	1.78[a]	34.0	1.45 (dd, J = 13.2, 10.2)	36.9	1.55 (dd, J = 12.3, 9.0)
		1.90 (br.d, J = 12.8)		1.84 (dd, J = 13.2, 4.2)		1.89 (dd, J = 12.3, 1.6)
5	43.6	0.86 m	44.4	0.93 m	45.2	1.03 m
6	28.0	1.16[a], 1.15[a]	27.8	1.03 m; 1.12[a]	28.0	1.08 m; 1.13m
7	32.6	0.78 m	31.5	0.76 (dd, J = 12.6, 4.6)	32.0	0.72 (dd, J = 15.0, 4.7)
		1.63 (br.d, J = 12.0)		1.50 m		1.51 m
8	36.9	2.42 m	33.6	1.65[a]	33.0	1.63 m
9	171.4	–	55.2	0.96 m	54.9	0.87 m
10	39.6	–	38.0	–	37.4	–
11	120.1	5.87 s	37.2	2.32 (dd, J = 14.2, 5.2)	21.3	1.20 m; 1.38 m
				2.38 (dd, J = 14.2, 13.6)		
12	204.4	–	212.6	–	35.0	1.44 (br.d, J = 14.5)
						2.56 (dd, J = 14.5, 3.0)
13	51.4	–	55.3	–	46.3	–
14	52.8	1.75[a]	55.7	1.35 m	56.1	1.40 m
15	31.9	1.57 m	31.4	1.55 m	31.8	1.91 (dd, J = 12.0, 1.7)
		2.17 (ddd, J = 11.5, 6.6, 5.7)		2.07 (ddd, J = 11.2, 6.3, 5.4)		2.08 m
16	80.3	4.49 m	79.6	4.47 m	144.4	6.59 (dd, J = 3.1, 1.7)
17	54.6	2.64 (dd, J = 7.4, 6.8)	54.2	2.73 (dd, J = 8.4, 6.3)	155.1	–
18	15.3	0.91 s	16.0	1.04 s	16.0	0.91 s
19	18.4	0.97 s	12.8	0.71 s	13.3	0.85 s
20	43.0	1.98 (d, J = 6.8)	42.6	1.90 (d, J = 6.8)	196.0	–
21	13.8	1.33 (d, J = 6.8)	13.9	1.32 (d, J = 6.8)	26.8	2.24 s
22	109.4	–	109.3	–		
23	31.8	1.60 m; 1.71 m	31.7	1.60 m; 1.66[a]		
24	29.2	1.53[a]; 1.55[a]	29.2	1.52[a]; 1.55[a]		
25	30.5	1.54[a]	30.5	1.53[a]		
26	67.0	3.46 (dd, J = 11.2, 9.6)	66.9	3.45 (dd, J = 11.2, 9.6)		
		3.57 (dd, J = 11.2, 3.6)		3.57 (dd, J = 11.2, 3.6)		
27	17.3	0.69 (d, J = 5.8)	17.3	0.68 (d, J = 5.8)		
1′	102.7	4.98 (d, J = 7.7)	103.8	5.00 (d, J = 7.7)		
2′	72.5	4.46[a]	72.3	4.47[a]		
3′	75.3	4.19 (dd, J = 9.6, 3.0)	75.2	4.19 (dd, J = 9.6, 3.0)		
4′	70.3	4.53 (d, J = 3.0)	70.2	4.53 (d, J = 3.0)		
5′	77.0	4.11 (dd, J = 6.6, 4.8)	77.2	4.12 (dd, J = 6.6, 4.8)		
6′	62.4	4.45[a]	62.3	4.46[a]		
		4.40 (dd, J = 10.8, 4.8)		4.40 (dd, J = 10.8, 4.8)		

*Assignments were based on ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY experiments. [a]overlapped.

The NOESY correlation between H-3 and H-5 indicated the α-equatorial configuration of H-3. The two protons at C-26 of the aglycone appearing at 3.46 (dd) and 3.57 (dd) indicated that the configuration of C-25 was *R*. The IR spectra (intensity 899 > 918 cm^{-1}) also confirmed this conclusion. Acid hydrolysis of **1** gave **1a** and D-galactose. The ^{1}H and ^{13}C NMR spectra of **1a** and the data of aglycone of **1** allowed the identification of **1a** as (25*R*)-3β-hydroxy-5α-spirost-9-en-12-one [14]. So, the aglycone moiety of **1** was confirmed to be (25*R*)-3β-hydroxy-5α-spirost-9-en-12-one. The β-anomeric configuration for galactose was judged from their coupling constant (J > 7.0 Hz). The HMBC experiment showed the correlation between the proton signal at δ 4.98 (H-1′, galactosyl group) and the carbon signal at δ 77.5 (C-3, aglycone). Thus, the structure of **1** was established as (25*R*)-3β-hydroxy-5α-spirost-9-en-12-one 3-*O*-β-D-galactopyranoside, named hostasaponin A.

TABLE 2. The Antifungal Activities of Compounds **1–3** against *Candida albicans*

Sample	Inhibition zone diameter, mm[a]	MIC, μg/mL
1	11.3 ± 0.7	8
2	9.7 ± 1.2	16
3	N.a	N.t
Nystatin	14.8 ± 0.9	4
Solvent vehicle[b]	N.a	N.t

[a]Concentration of each sample was 100 μg/mL in methanol, the data are presented as mean ± SD (n = 3); [b]methanol. N.a: not active. N.t: not tested.

Compound **2** was obtained as white needles (MeOH). Its molecular formula was determined to be $C_{33}H_{52}O_{10}$ by HR-ESI-MS. The ^{1}H and ^{13}C NMR data (Table 1) revealed that **2** was a steroidal monoglycoside, and the aglycone was essentially analogous to the aglycone of the previously reported (25*R*)-2α,3β-dihydroxy-5α-spirost-12-one (manogenin) [15]. The chemical shift of C-5 appearing at 44.4 indicated that the orientation of C-5 is α. The NOESY correlations between H-2 and H-19 and H-3 and H-5 indicated the β-equatorial configuration of H-2 and the α-equatorial configuration of H-3, respectively. The two protons at C-26 of the aglycone appearing at 3.45 (dd) and 3.57 (dd) indicated that the configuration of C-25 was *R*. The IR spectra (intensity 900 > 920 cm^{-1}) also confirmed this conclusion. Acid hydrolysis of **2** gave **2a** and D-galactose. The ^{1}H and ^{13}C NMR spectra of **2a** and the data of aglycone of **2** allowed the identification of **2a** as manogenin. Thus, the aglycone of **2** was confirmed to be (25*R*)-2α,3β-dihydroxy-5α-spirost-12-one. The β-anomeric configuration for galactose was judged from its coupling constant. The HMBC experiment showed the correlation between the proton signal at δ 5.00 (H-1′, galactosyl group) and the carbon signal at δ 84.2 (C-3, aglycone). So, the structure of **2** was elucidated as (25*R*)-2α,3β-dihydroxy-5α-spirost-12-one 3-*O*-β-D-galactopyranoside, named hostasaponin B.

Compound **3** was obtained as white needles (MeOH). It was identified by NMR and MS as 2α,3β-dihydroxy-5α-pregn-16-en-20-one, a new natural product. Although its structure was reported [16], its spectral data have never reported. In the present study, we attempted to assign all of the chemical shifts with the aid of the 2D NMR spectra (^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY). The NMR spectral data assigned are listed in Table 1.

The primary antimicrobial screening was carried out using the agar disc-diffusion method. Compounds **1** and **2** were both observed to be active against fungal *Candida albicans*, with an inhibition zone greater than 9 mm at a concentration of 100 μg/mL. However, compound **3** did not show the obvious activity (Table 2). Further antifungal evaluation showed that compounds **1** and **2** both displayed dominant antimicrobial activity, with MIC values of 8 and 16 μg/mL for *Candida albicans*, respectively.

EXPERIMENTAL

General Methods and Instruments. Silica gel G (200–300 mesh, Qingdao Haiyang Chemical Group Co., Ltd.) was used for column chromatography. TLC was carried out using precoated silica gel aluminum sheets (Silica gel 60 F_{254}, Merck & Co., Inc.) and was visualized by dipping into 10% H_2SO_4 in alcohol followed by heating. Melting point was determined on an XT4A micro melting point apparatus (uncorrected). UV spectra were measured on a UV1102 spectrophotometer. Optical rotations were measured on a JASCO P-2000 polarimeter. IR spectra were recorded on an Equinox 55 FT-IR spectrophotometer. ESI-MS analyses were achieved on an Agilent 6410 triple-quad mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX-600 spectrometer using TMS as internal standard. Semi-preparative HPLC chromatography was performed on a Shimadzu LC 2010 AHT liquid chromatography system, equipped with a quaternary solvent delivery system, an auto-sampler, a UV-Vis detector, and a Nova-Pak HR C_{18} column (7.9 × 300 mm, 6 μm). GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30 m × 0.25 mm × 0.25 μm). *Candida albicans* was provided by Shanghai Institute of Pharmaceutical Industry.

Plant Material. The rhizomes of *Hosta plantaginea* were collected in May 2010 from Chongqing, China, and authenticated by one of the authors (Mengyue Wang). A voucher specimen (CQ20100517) has been deposited at the Herbarium at the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

Extraction and Isolation. The rhizomes of *H. plantaginea* (1.2 kg) were powdered and extracted with 8 L 95% alcohol by reflux three times (2 h each). The combined alcohol extract was concentrated (204 g), suspended in water (750 mL), and partitioned successively with CH_2Cl_2 and EtOAc three times (500 mL each). The EtOAc extracts were combined and concentrated under vacuum to afford the EtOAc portion (24.8 g).

Part of the EtOAc portion extract (24 g) was subjected to silica gel column chromatography (5.5 × 60 cm, eluted with CH_2Cl_2–MeOH with increasing methanol ratio from 1% to 15%) to obtain fractions 1–42 (250 mL each).

The fractions obtained were concentrated under vacuum and monitored by TLC. Fractions 7–9, showing the same R_f on TLC, were combined (1.2 g in total) and further purified on a silica gel column (3.5 × 60 cm, eluted with CH_2Cl_2–MeOH 97:3, v/v) to obtain fractions 7-1–7-11 (100 mL each). Fraction 7-6 (52 mg) was further purified by semi-preparative HPLC (column temperature 30°C; detection wave length 210 nm; eluent acetonitrile–H_2O, 85:15; flowrate 3.0 mL/min) to afford compound **3** (19 mg).

Fractions 25 and 26 showing the same R_f on TLC were combined (920 mg) and further purified on a silica gel column chromatography (3.5 × 60 cm, eluted with CH_2Cl_2–H_2O with methanol ratio from 8% to 12%) to obtain fractions 25-1–25-17 (150 mL each). Fraction 25-10 (71 mg) was further purified by semi-preparative HPLC (column temperature 30°C; detection wavelength 210 nm; eluent acetonitrile–H_2O, 55:45; flowrate 2.0 mL/min) to afford compound **1** (32 mg) and **2** (15 mg).

Compound 1, white amorphous powder, mp 246–248°C. UV (MeOH, λ_{max}, nm) (log ε): 240 (4.26). $[\alpha]_D^{20}$–19.0° (*c* 0.10, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2927 (CH), 1665 (C=O), 1595 (C=C), 1455, 1377, 1240, 1157, 1083 (C–O), 981, 918, 899, 865 (intensity: 899 > 918). HR-ESI-MS *m/z* 613.3350 $[M + Na]^+$ (calcd for $C_{33}H_{50}O_9Na$, 613.3352). For ^1H and ^{13}C NMR, see Table 1.

Compound 2, white amorphous powder, mp 265–267°C. $[\alpha]_D^{20}$–7.0° (*c* 0.10, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 285 (2.52). IR (KBr, ν_{max}, cm^{-1}): 3404 (OH), 2931 (CH), 1706 (C=O), 1375, 1154, 1080 (C–O), 980, 920, 900, 866 (intensity: 900 > 920). HR-ESI-MS *m/z* 631.3461 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_{10}Na$, 631.3458). For ^1H and ^{13}C NMR, see Table 1.

Compound 3, white needles (MeOH), mp 231–232°C. $[\alpha]_D^{20}$+1.7° (*c* 0.05, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 239 (4.17). IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2925 (CH), 2840 (CH), 1655 (C=O), 1589 (C=C), 1375, 1230, 920, 892. HR-ESI-MS *m/z* 355.2245 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_3Na$, 355.2249). For ^1H and ^{13}C NMR, see Table 1.

Acid Hydrolysis of 1. A solution of **1** (8 mg) in 1 M HCl–MeOH (4:1, 10 mL) was refluxed at 90°C for 2 h. Then, the reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The CH_2Cl_2 layer was washed with water to neutrality, concentrated under vacuum, and then subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH (95:5, v/v) to give (25*R*)-3β-hydroxy-5α-spirost-9-en-12-one (2.0 mg). The water layer was neutralized with 10% NaOH solution and concentrated *in vacuo* to dryness to give the sugar fraction. The sugar fraction was dissolved in pyridine (1.5 mL), and then L-cysteine methyl ester hydrochloride (3 mg) was added to the solution. The mixture was heated at 60°C for 2 h, followed by an equal volume of acetic anhydride at 90°C for another 2 h. The solution was concentrated *in vacuo* to dryness and dissolved in MeOH (0.5 mL), which was analyzed by GC (column temperature 100–280°C, programmed increase 10°C/min; carrier gas N_2, 1.5 mL/min; injector and detector temperature 280°C; injection volume 0.5 μL; split ratio 10:1). The derivatives of D-galactose were detected (retention time 26.84 min). The standard D-galactose was subjected to the same reaction and GC analysis under the same conditions.

Acid Hydrolysis of 2. A solution of 2 (5 mg) was subjected to acid hydrolysis as described for **1** to give manogenin (l.5 mg) and D-galactose.

Antifungal Activity Assay. 1. The primary antimicrobial screening was carried out using the agar disc-diffusion method [17]. The media were inoculated with cell suspension (10^4 CFU/mL), and the discs were loaded with the tested compounds dissolved in methanol (100 μg/mL). After evaporation of the loading solvent, each disc was placed at the center of a Petri dish containing potato dextrose agar (PDA) previously inoculated with the specific microorganism. Incubation was continued for 72 h at 28°C. After these periods, the plates were examined for the inhibition zones. To rule out the influence of the loading solvent, solvent-treated plates were maintained and served as a negative control.

2. Minimal Inhibitory Concentration (MIC). MIC was determined by the microdilution method [18]. Sample was dissolved in methanol at an initial concentration of 1000 μg/mL and then mixed with PDA media. The final concentrations of sample in the media were 0.782, 1.563, 3.125, 6.25, 12.5, 25.0, 50.0, 100.0, and 200 μg/mL. All Petri dishes, including those containing nystatin (positive control), were inoculated with the test organism by streaking the medium with a calibrated loop (0.05 mL). After incubation for 72 h at 28°C, the Petri dishes were examined for microbial growth, and the minimum concentration of each treatment that inhibits microbial growth was measured.

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