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Steroidal aglycones from acid-hydrolyzed products of *Ophiopogon japonicus*

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ABSTRACT

Four steroidal aglycones were isolated from acid-hydrolyzed products of *Ophiopogon japonicus*. The spectral analysis and chemical evidence revealed their chemical structures to be prazerigenin A (**1**), 1-O-sulfate-ruscogenin (**2**), ophiogenin (**3**) and rugcogenin (**4**). Among them compound 1 was isolated from *O. japonicus* for the first timeÿand compounds 1 and 4 exerted significant anti-inflammatory activity. In this paper, we first focus on the acid-hydrolyzed products of fibrous roots in *O. japonicus*. These steroidal aglycones in fibrous roots of *O. japonicus* would be promising sources to explore and utilize. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Ophiopogon japonicus;
Steroidal aglycones;
Fibrous roots;
Acid-hydrolyzed products.

INTRODUCTION

Ophiopogon japonicus (Chinese name: Maidong) is one of the most frequently used Chinese herbs, whose tuberous roots are considered effective to treat a wide range of disorders, mainly including thrombosis, myocardial ischemia, arrhythmias, respiratory disease and hyperglycemia[1-3]. In China, the major cultivation regions of *O. japonicus* are Sichuan and Zhejiang province, and related herbs are popularly known as Chuan maidong (CMD) and Zhe maidong (ZMD), respectively. It is well documented that the quantity and quality of herbs grown in different regions are significantly influenced by environmental and growing conditions[4,5]. After the medicinal part of tuberous roots is obtained, the fibrous roots (nearly 40% weight of total roots) are usually discarded. Recent studies have indicated that a serious of steroidal saponins were found in the fibrous roots of *O. japonicus*[6-8]. And as we all known, steroids of spirostane and furostane series and their glycosides are attracting the attention of researchers not only as economically important raw materials convertible into various steroidal hormonal drugs, but also as biologically active materials with independent value[9]. Therefore, the steroidal compounds in fibrous roots of *O. japonicus* would be promising sources to explore and utilize. ZMD is one famous herbs in our Zhejiang provine, and various steroidal glycosides were isolated from ZMD. However, the main steroidal

aglycones of ZMD, especially in fibrous roots, are still unclear. So in the paper it is introduced about the isolation, structural elucidation and anti-inflammatory activity of various steroidal aglycones from acid-hydrolyzed products of the fibrous roots in ZMD.

EXPERIMENTAL

General

NMR experiments were performed on Brucker AV-300 and 500 spectrometer. MS data were measured with angilent1100 LC API MSD instrument, IR spectrum were recorded on Jasco FT-4100 spectrophotometer. Thin-layer chromatography (TLC) was performed on plates precoated with silica GF254 (Qingdao Marine Chemistry Factory, China). Column chromatography was carried out with silica gel (200-300 mesh, Marine Chemistry Company, China), ODS (50μm, Beijing H&E Company LTD., China) and Sephadex LH-20 (GE healthcareÿSweden). The chemiluminescence value was recorded by BPCL-1-G-C Ultra-weak Luminescence Analyzer (Beijing Institutes for Biophysics, Chinese Academy of Science). Luminol and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma (St. Louis, MO, USA). Dimethyl sulfoxide and other chemical solvents were analytical grade.

Plant material

The fibrous roots of *O. japonicus* were collected from the Cixi region in Zhejiang Province, P.R. China, in July 2012, and identified by professor Qian Hua, Zhejiang Forestry Academy. A voucher specimen (ZMD120703) was deposited in School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, P.R. China.

Exraction and acid-hydrolysis

The fibrous roots of *O. japonicus* (400 g) were ultrasound-assisted extracted three times with 4 L of 80% ethanol. The ethanol extracts were combined and concentrated under vaccum in a rotary evaporator. The dry ethanol extract (113.74 g) was suspended in 6% sulfuric acid (500 ml) and petroleum ether (boiling range: 60-90□, 500 ml), and then was acid-hydrolyzed at 90□ for 8 hours. The petroleum ether-soluble fraction was concentrated under reduced pressure to obtain a syrup(9.18 g).

Isolation and identification of chemical constituents

The petroleum ether-soluble fraction of acid-hydrolyzed products (9.18 g) was then subjected to silica gel column chromatography with successive elution by a petroleum ether/ethyl acetate gradient and MeOH solvent system. Subfractions with the same thin-layer chromatography (TLC) patterns were combined into one fraction; thus 11 fractions were obtained (No. A-K). Subfractions D and E were further purified on ODS column (with MeOH-H_2O as mobile phase) and Sephadex LH-20 column (eluted with MeOH) to yield compound **1** (8mg), **2** (4mg), **3** (10mg) and **4** (20mg).

prazerigenin A(1)

White amorphous powder. ESIMS *m/z* 429 $[M-H]^-$. IR (KBr) νcm^{-1}:3424, 1641, 980, 918, 893, 864. ^{1}H-NMR (TMS) δppm: 0.78 (d,3H,CH_3-27), 0.94 (s,3H,CH_3-18), 0.98 (d,3H,CH_3-21), 1.05 (s,3H,CH_3-19), 5.38 (brs,1H,H-6). ^{13}C NMR data see at TABLE 1.

1-O-sulfate-ruscogenin (2)

White amorphous powder. ESIMS *m/z* 509$[M-H]^-$. IR (KBr) νcm^{-1}: 3426, 982, 921, 900, 866. ^{1}H NMR (TMS) δppm: 0.67(d,3H,CH_3-27), 0.84(s,3H,CH_3-18), 1.08 (d,3H,CH_3-21), 1.30(s,3H,CH_3-19), 5.39 (brs,1H,H-6). ^{13}C NMR data see at TABLE 1.

ophiogenin(3)

White amorphous powder. ESIMS m/z 445$[M-H]^-$. IR (KBr) νcm^{-1}: 3421, 1639, 1050, 979, 923, 900, 867. ^{1}H NMR (TMS) δppm: 0.67(d,3H,CH_3-27), 1.12(3H, s, CH_3-18), 1.13(3H, s, CH3-19), 1.27(d,3H,CH_3-21), 5.47(brs,1H,H-6).^{13}C NMR data see at TABLE 1.

rugcogenin (4)

White amorphous powder. ESIMS m/z 429$[M-H]^-$. ^{13}C NMR data see at TABLE 1.

Neutrophils respiratory burst inhibitory activity

Neutrophils was obtained using the methods of Wang et al.[10]. A chemiluminescence assay was used to test neutrophil respiratory burst inhibitory activity with a

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method similar to the previous report[11]. Compounds 1,3,4 were dissolved in DMSO for test, and the final concentration of DMSO was <0.1%. In addition, DMSO was used as a blank control, and rutin was chosen as the positive control. We collected the IC_{50} values (the concentration of each substance required for 50% inhibition of neutrophil respiratory burst) of each sample from three replicates, and then mean ± standard deviation values for the results of the IC_{50} assay were obtained.

TABLE 1 : ^{13}C NMR spectral data for compounds 1-4 in pyridine-*d_5*

No.	1	2	3	4
1	37.3	84.3	38.1	78.2
2	31.6	39.7	32.7	44.0
3	71.6	68.0	71.3	68.3
4	42.2	43.6	43.7	43.7
5	140.4	125.2	141.5	140.7
6	121.4	139.0	121.6	124.3
7	26.0	31.8	26.2	33.2
8	34.9	33.0	36.4	32.5
9	43.3	49.8	43.6	51.6
10	36.9	43.1	37.3	43.7
11	19.8	23.6	20.2	24.4
12	31.5	40.6	26.7	40.8
13	44.4	40.2	48.4	40.4
14	87.4	56.7	87.8	57.2
15	39.3	31.9	40.4	32.6
16	80.9	81.1	90.5	81.2
17	58.9	63.1	91.1	63.4
18	19.6	16.6	20.7	16.7
19	19.2	14.7	19.6	15.0
20	41.6	41.9	45.2	42.2
21	15.4	14.9	9.8	15.1
22	109.6	109.2	109.6	109.3
23	30.7	32.3	32.2	32.1
24	29.4	29.2	28.9	29.5
25	30.0	30.6	30.4	30.7
26	66.9	66.8	66.8	67.1
27	17.3	17.2	17.3	17.4

RESULTS AND DISCUSSION

The petroleum ether-soluble fraction of acid-hydrolyzed product of *O. japonicus* was separated using a series of silica-gel, ODS and Sephadex LH-20 to afford four steroidal aglycones, which were identified by comparison of their NMR and MS data with reported values in the literatures[12-15]. Their structures were identified as prazerigenin A(1), 1-O-sulfate-ruscogenin (2), ophiogenin(3) and rugcogenin (4). Compound 1 was firstly isolated from *O. japonicus*.

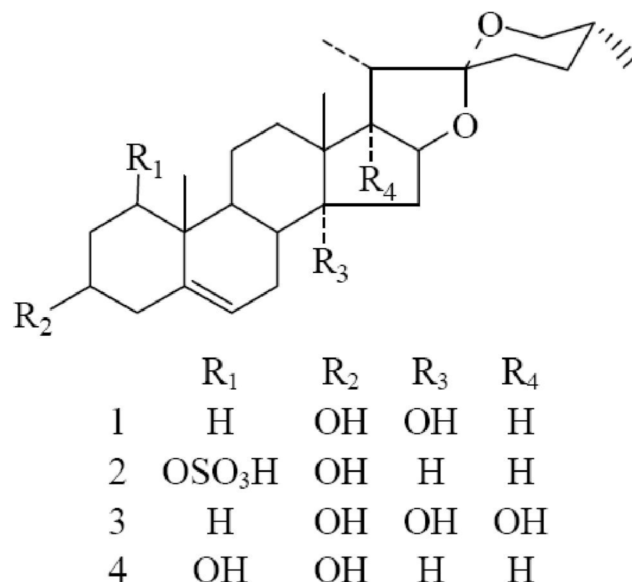

Figure 2 : steroidal aglycones isolated from acid-hydrolyzed products of *Ophiopogon japonicus*

Compound 1 was obtained as a white amorphous powder with positive result to the Liebermann–Burchard reaction. The IR spectrum showed the characteristic absorption of a (25R)-spirosteroid at 980, 918, 893, 864 cm^{-1} (intensity 893>918). The ^{1}H NMR spectrum of 1 showed two singlet methyl signals at δ 0.94 (s, CH_3-18) and 1.05 (s,CH_3-19); two doublet methyl signals at δ 0.78 (d, J = 6.4 Hz, CH_3-27), 0.98 (d, J = 7.0 Hz, CH_3-21) and an olefinic proton signal at δ 5.38 (1H, br s, H-6). The ^{13}CNMR spectrum showed 27 carbon signals, in which the characteristic carbon signals appeared at δ 140.4 (C-5), 121.4 (C-6) and four methyl carbon signals at δ14.6(C-21), 17.1(C-27)ÿ19.2(C-19), 19.6(C-18) were readily assigned. Compared the ^{13}C NMR spectrum with those of diosgnin, rucogenin and ophiogenin in *O. japonicus*, the chemical shift appeared at δ 87.4 suggested that C-14 was substituted by a hydroxyl group. And on the basis of quasi-molecular ion peak at m/z 429 $[M-H]^-$ in its negative ESI-MS, the molecular formula of 1 was

deduced as $C_{44}H_{27}O_4$. Thus, the structure of 1 was determined to be prazerigenin A (1).

Compound 2 was obtained as a white amorphous powder with positive reaction to the Liebermann–Burchard test. ESI-MS spectrum of 2 showed a quasi-molecular peak at *m/z* 509$[M-H]^-$. The IR spectrum showed the characteristic absorption of a (25R)-spirosteroid at 982, 920, 901, 865 cm^{-1} (intensity 901>920). ^1H NMR spectrum of 2 displayed two singlet methyl signals at δ 0.84 (s, CH_3-18) and 1.30 (s, CH_3-19); two doublet methyl signals at δ 0.67 (d, J = 5.2 Hz, CH_3-27), 1.08 (d, J = 7.0 Hz, CH_3-21) and an olefinic proton signal at d 5.39 (1H, br s, H-6). C-27 signal of ^{13}C NMR spectrum could be assigned to the steroidalskeleton with comparsion of ruscogenin. To confirm the sulfate group, this compound was heated with pyridine to afford ruscogenin and sulfate ion. The ^{13}C NMR signal downfield to δ 84.3 indicated a sulfate ion on C-1. On the basis of the above evidence, the structure of 2 was assigned to be 1-O-sulfate-ruscogenin (2).

The steroidal aglycones were evaluated for their anti-inflammation activity in vitro. Compounds 1 and 4 showed inhibitory activity against neutrophil respiratory burst stimulated by PMA with IC50 value of 72.76±2.01 μM and 15.90±0.29 μM, while the IC_{50} value of compound 3 >100μM. In this bioassay, rutin, as an accepted anti-inflammatory agent, was used as a positive control with IC_{50} value of 71.84±3.86 μM. The results showed that compounds 1 and 4 exerted significant inhibitory activity against neutrophil respiratory burst stimulated by PMA and proved their anti-inflammatory potential.

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