Chemistry of Natural Compounds, Vol. 48, No. 4, September, 2012 [Russian original No. 4, July–August, 2012]

A NEW FUROSTANOL GLYCOSIDE WITH FATTY ACID SYNTHASE INHIBITORY ACTIVITY FROM *Ophiopogon japonicus*[a]

Changling Duan,[1,3] Yi Wang,[2] Xiaofeng Ma,[2]* Yong Jiang,[1] Jianxun Liu,[3] and Pengfei Tu[1]*

UDC 547.972

*A new furostanol glycoside, named ophiopogonin J (**1**), was isolated from the fibrous root of* Ophiopogon japonicas. *The structure of the compound was established as (25*R*)-26-[(*O-β-*D-glucopyranosyl-(1→2)-*β-*D-glucopyranosyl)]-20α-hydroxyfurost-5, 22-diene-3-*O-α-*L-rhamnopyranosyl-(1→2)-[*β-*D-xylopyranosyl-(1→4)]-*β-*D-glucopyranoside on the basis of spectroscopic methods, including HR-ESI-MS and 1D and 2D NMR experiments.*

Keywords: *Ophiopogon japonicus*, furostanol glycoside, fatty acid synthase, Liliaceae.

The animal fatty acid synthase (FAS) is a key metabolic enzyme catalyzing the *de novo* synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA in the presence of the reducing substrate NADPH. A number of studies have recently reported that FAS may be a potential target for anti-obesity and anti-cancer drugs [1]. *Ophiopogon japonicus* (Thunb.) Ker-Gawl (known as Maidong in China) is an evergreen perennial herb, widely distributed in Mainland China, especially in Sichuan and Zhejiang Provinces. It has often been used to treat cardiovascular and cerebrovascular diseases in combination with *Panax ginseng* and *Schisandra chinensis* in traditional Chinese medicine. A number of steroidal saponins have been isolated from *O. japonicus* [2–6]. In our previous study, we found that the ethanol extract of *O. japonicus* showed inhibitory activity on FAS. Here, we describe the isolation and structure elucidation of a new furostanol glycoside **1** with FAS inhibitory activity from *O. japonicus*.

Compound **1** was obtained as a white amorphous powder with positive Liebermann–Burchard reaction. The molecular formula was asigned as $C_{56}H_{90}O_{27}$ based on a molecular ion peak at *m/z* 1193.5736 $[M - H]^-$ in its HR-ESI-MS spectrum. The IR spectrum showed absorption bands at 3418 (OH), 2935 (CH), 1548, 1367 (CH_3), and 1032 cm^{-1}.

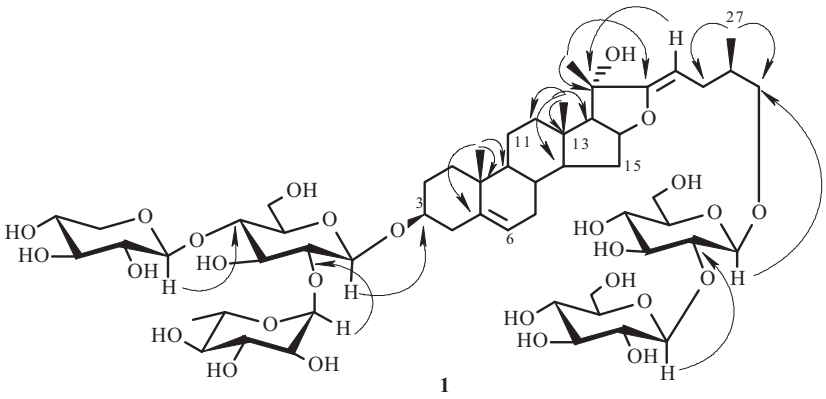
The ^1H NMR spectrum of **1** revealed five signals at δ 0.96 (3H, s), 1.06 (3H, s), 1.76 (3H, s), 1.13 (3H, d, J = 7.0 Hz), and 1.79 (3H, d, J = 6.0 Hz), five anomeric proton signals at δ 4.87 (1H, d, J = 7.5 Hz), 4.98 (1H, d, J = 7.5 Hz), 5.05 (1H, d, J = 7.5 Hz), 5.31 (1H, d, J = 7.5 Hz), and 6.39 (1H, s), and an olefinic proton at δ 5.28 (1H, br.s) (see Table 1). The ^{13}C NMR spectrum consisted of 56 carbon signals, which indicated that compound **1** is a furostanol saponin with five sugar moities and $\Delta^{5(6)}$ [7, 8], in which the characteristic carbon signals at δ 140.8 (C-5), 121.9 (C-6), 19.6 (C-19), 17.5 (C-27), 100.1 (C-1′), 102.1 (C-1″), 105.9 (C-1‴), 103.3 (C-1⁗), 106.7 (C-1⁗′), and 18.8 (C-6″) were readily assigned. The carbon signals at δ 91.9, 163.7 indicated a double bond between C-22 and C-23, and the carbon and proton signals at δ 76.9, 22.0 (δ 1.76, 3H, s) indicated a hydroxy substitution at C-20, which was further verified by gHMBC correlations.

[a]Materials presented at the 9th International Symposium on the Chemistry of Natural Compounds (SCNC, People′s Republic of China, Urumqi, Oct. 16–19, 2011).

1) School of Pharmaceutical Sciences, Peking University, 100191, Beijing, P. R. China, e-mail: pengfeitu@vip.163.com; 2) College of Life Science, Graduate University of Chinese Academy of Sciences, 100049, Beijing, P. R. China, e-mail: maxiaofeng@gucas.ac.cn; 3) Xiyuan Hospital, China Academy of Chinese Medical Sciences, 100091, Beijing, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 551–553. Original article submitted March 5, 2012.

TABLE 1. ^{1}H and ^{13}C NMR Spectral Data of Compound **1** (pyridine-d_5, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	37.6	0.94 (m), 1.73 (o)	3-*O*-Glc 1′	100.1	4.98 (d, J = 7.5)
2	30.3	1.87 (o), 2.11 (o)	2′	77.6	4.24 (o)
3	78.3	3.89 (o)	3′	77.4	4.28 (o)
4	39.1	2.71 (o), 2.76 (o)	4′	81.6	4.20 (o)
5	140.8		5′	76.3	3.86 (o)
6	121.9	5.28 (br.s)	6′	61.8	4.46 (br.d, J = 11.5), 4.55 (o)
7	32.1	1.42 (o), 1.87 (o)	Rha 1″	102.1	6.39 (s)
8	31.2	1.53 (m)	2″	72.5	4.81 (br.d)
9	50.3	0.87 (m)	3″	72.8	4.62 (dd, J = 3.5, 9.0)
10	37.1		4″	74.2	4.37 (o)
11	20.7	1.42 (m)	5″	69.6	5.02 (o)
12	39.5	1.16 (m), 1.90 (o)	6″	18.8	1.79 (d, J = 6.0)
13	40.5		Xyl 1‴	105.9	5.05 (d, J = 7.5)
14	57.0	0.98 (m)	2‴	75.3	3.97 (d, J = 8.0)
15	33.6	1.63 (m), 2.11 (o)	3‴	78.5	4.12 (m)
16	84.2	5.23 (m)	4‴	70.8	4.18 (m)
17	67.9	2.25 (d, J = 6.5)	5‴	67.4	3.65 (m), 4.26 (m)
18	13.7	0.96 (s)	26-*O*-Glc1⁗	103.3	4.87 (d, J = 7.5)
19	19.6	1.06 (s)	2⁗	84.3	4.12 (m)
20	76.9		3⁗	78.0	4.35 (m)
21	22.0	1.76 (s)	4⁗	71.5	4.24 (o)
22	163.7		5⁗	78.4	3.88 (o)
23	91.9	4.56 (t, J = 7.0)	6⁗	62.6	4.38 (o), 4.54 (o)
24	29.7	2.25 (o), 2.53 (m)	Glc 1⁗′	106.7	5.31 (d, J = 7.5)
25	35.1	2.10 (m)	2⁗′	77.1	4.12 (m)
26	75.3	3.70 (t, J = 10.5), 3.99 (m)	3⁗′	78.1	4.29 (o)
27	17.5	1.13 (d, J = 7.0)	4⁗′	71.5	4.33 (o)
			5⁗′	78.6	3.97 (o)
			6⁗′	62.6	4.36 (o), 4.54 (o)



1

Fig. 1. Key HMBC correlations of compound **1.**

By comparison of the ^{13}C NMR signals of the aglycone moiety with the literature values [9] and on the basis of ^{1}H–^{1}H COSY, HSQC, and HMBC experiments, we identify the aglycone of **1** as 3*β*,20,26-trihydroxyfurost-5,22-diene. The *α*-configuration of the C-20 hydroxyl group was deduced from the cross-peak between H-21 (δ 1.76) and H-18 (δ 0.96) in the ROESY spectrum. The 25*R*-configuration of **1** was demonstrated by the chemical shift difference between the two protons of H-26 (Δab = 0.29 < 0.48) [10, 11]. Hence, the aglycone of **1** was identified as (25*R*)-3*β*,20*α*,26-trihydroxyfurost-5,22-diene.

Acid hydrolysis of **1** gave glucose and rhamnose in a ratio of 3:1 as analyzed by GC methods. The sugar sequence of rhamnosyl-(1→2)-[xylosyl-(1→4)]-glucosyl and its linkage at C-3 were identified by gHMBC correlations between H-1‴ (δ_H 5.05) and C-4′ (δ_C 81.6), between H-1″ (δ_H 6.39) and C-2′ (δ_C 77.6), and between H-1′ (δ_H 4.98) and C-3 (δ_C 78.3). The sugar sequence of glucosyl-(1→2)-glucosyl and its linkage at C-26 were ascertained by long-range correlations between

H-1‴″ (δ_H 5.31) and C-2‴″ (δ_C 84.3), between H-1‴″ (δ_H 4.87) and C-26 (δ_C 75.3). Therefore, compound **1** was identified as (25*R*)-26-*O*-[(*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl)]-20*α*-hydroxyfurost-5,22-diene 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside. Comound **1** showed strong inhibitory activity on FAS with an IC_{50} of 76 ± 2 μM.

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded on a Bruker INOVA-500 instrument in pyridine-d_5, δ in ppm and J in Hz. IR spectra were obtained from a Hitachi EPI-2 spectrometer. The HR-ESI-MS spectra were recorded on a Bruker APEX IV FT-MS (7.0 T) mass spectrometer. Optical rotations were studied on a Jasco P-2000 polarimeter. HPLC was carried out on an Agilent 1100 with DAD detector using a Zorbax XDB-C_{18} column (9.4 × 250 mm). GC analysis was performed on an HP 6890 plus instrument equipped with an H_2 flame ionzation detector (Agilent Technologies, USA).

Plant Material. The fibrous roots of *Ophiopogon japonicus* (Thunb.) Ker-Gawl were purchased from Anguo market, Hebei Province, China, in September 2005 and identified by one of the authors (Prof. P. F. Tu). A voucher specimen (MD20050906) is deposited in the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The fibrous roots of *O. japonicus* (45 kg) were extracted with EtOH (70%) under reflux and then filtered by gauze. The extract was concentrated under reduced pressure with a rotary evaporator. The residue was suspended in H_2O and subsequently extracted successively with petroleum ether (PE), EtOAc, and *n*-BuOH. Then the *n*-BuOH-soluble fraction was subjected to Diaion HP-20 resin column chromatography (CC) eluted with 20% EtOH, 55% EtOH, and 80% EtOH in H_2O to afford three fractions (Fr. 1–3). Fraction 2 (100.0 g) was separated by silica gel CC (100–200 mesh) eluted with $CHCl_3$–MeOH (1:0, 100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 2:1, 0:1) to give seven fractions (Fr.2-1–7). Fraction 2-6 was subjected to silica gel, ODS silica gel, and Sephadex LH-20 CC, as well as repeated semi-preparative HPLC (Zorbax XDB-C_{18} column, 9.4 × 250 mm, 5 μm, flow rate 2.0 mL/min, UV 203 nm) to afford **1** (8 mg, MeCN–H_2O 28:72, 7.4 min).

Assay of FAS Inhibition. To examine the fast-binding reversible inhibition effect of **1** on FAS, **1** was added to the assay system before FAS initiated the reaction. The half-inhibitory concentration (IC_{50}) was calculated from the plot of residual activity versus concentration of **1** with Origin 7.5 software.

ACKNOWLEDGMENT

This work was supported by Special Science and Technology Projects for Outstanding Young in Life Sciences (KSCX2-EW-Q-19), the Twelfth Five Year Plan Basic Frontier Project in Life Sciences (Y129015EA2), Program for Changjiang Scholar and Innovative Team in University (No. 985-2-063-112), the President Funding of GUCAS (O95101PY00), as well as the K. C. Wong Education Foundation, Hong Kong.

REFERENCES

1. T. M. Loftus, D. E. Jaworsky, G. L. Frehywot, C. A. Townsend, G. V. Ronnett, M. D. Lane, and F. P. Kuhajda, *Science*, **288**, 2379 (2000).
2. Y. Watanabe, S. Sanada, Y. Ida, and J. Shoji, *Chem. Pharm. Bull.,* **31**, 3486 (1983).
3. Y. Watanabe, Y. Hirai, S. Sanada, and J. Shoji, *Shoyakugaku Zasshi,* **44**, 117 (1990).
4. A. C. Do, K. Y. Jung, and Y. K. Sung, *J. Nat. Prod.,* **58**, 778 (1995).
5. C. L. Duan, Y. J. Li, P. Li, Y. Jiang, J. X. Liu, and P. F. Tu, *Helv. Chim. Acta,* **93**, 227 (2010).
6. C. L. Duan, X. F. Ma, Y. Jiang, J. X. Liu, and P. F. Tu, *J. Asian Nat. Prod. Res.*, **12**, 745 (2010).
7. L. P. Kang, Z. J. Liu, D. W. Tan, Y. Zhao, Y. Zhao, H. B. Chen, and B. P. Ma, *Magn. Reson. Chem.,* **45**, 725 (2007).
8. P. K. Agrawal, D. C. Jain, and A. K. Pathak, *Magn. Reson. Chem.,* **33**, 923 (1995).
9. B. Shao, H. Z. Guo, Y. J. Cui, M. Ye, J. Han, and D. Guo, *Phytochemistry,* **68**, 623 (2006).
10. P. K. Argrawal, *Steroids*, **70**, 715 (2005).
11. T. Zhang, P. Zou, L. P. Kang, H. S. Yu, Y. S. Liu, X. B. Song, and B. P. Ma, *J. Asian Nat. Prod. Res.,* **11**, 824 (2009).