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Two new furostanol glycosides from the fibrous root of *Ophiopogon japonicus* (Thunb.) Ker-Gawl

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ORIGINAL ARTICLE

Two new furostanol glycosides from the fibrous root of *Ophiopogon japonicus* (Thunb.) Ker-Gawl

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Two new furostanol glycosides, ophiopogonins H (**1**) and I (**2**), were isolated from the fibrous root of *Ophiopogon japonicus*. The structures of **1** and **2** were established as (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-22α-hydroxyfurost-5-ene-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside and (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-glucopyranoside on the basis of spectroscopic means including HR-ESI-MS, 1D and 2D NMR experiments.

Keywords: *Ophiopogon japonicus*; furostanol glycoside; Liliaceae

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1. Introduction

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 [1,2]. A number of steroidal saponins were isolated from *O. japonicus* [3–6]. Our phytochemical investigation of the fibrous root of *O. japonicus* led to the isolation of two novel furostanol glycosides, ophiopogonins H (**1**) and I (**2**) (Figure 1). In this paper, we report the isolation and structural elucidation of the new compounds.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with a positive Liebermann–Burchard reaction. The molecular formula was deduced to be $C_{51}H_{84}O_{23}$ on the basis of its molecular ion peaks at *m/z* 1087.5280 $[M + Na]^+$ and 1063.5401 $[M - H]^-$ in its HR-ESI-MS. The IR spectrum showed absorption bands at 3395 (OH), 2903 (CH), 1548, 1367 (CH_3), and 1031 cm^{-1}.

The ^1H NMR spectrum of **1** revealed two singlets at δ 0.88 (3H, s) and 1.01 (3H, s), three methyl doublets at δ 1.03 (3H, d, $J = 6.5$ Hz), 1.29 (3H, d, $J = 7.5$ Hz), 1.74 (3H, d, $J = 6.0$ Hz), four anomeric proton signals at δ 4.81 (1H, d, $J = 7.5$ Hz), 5.00 (1H, d, $J = 7.0$ Hz), 5.25 (1H, d, $J = 7.0$ Hz), 6.34 (1H, br s), and an olefinic proton at δ 5.26 (1H, br s).

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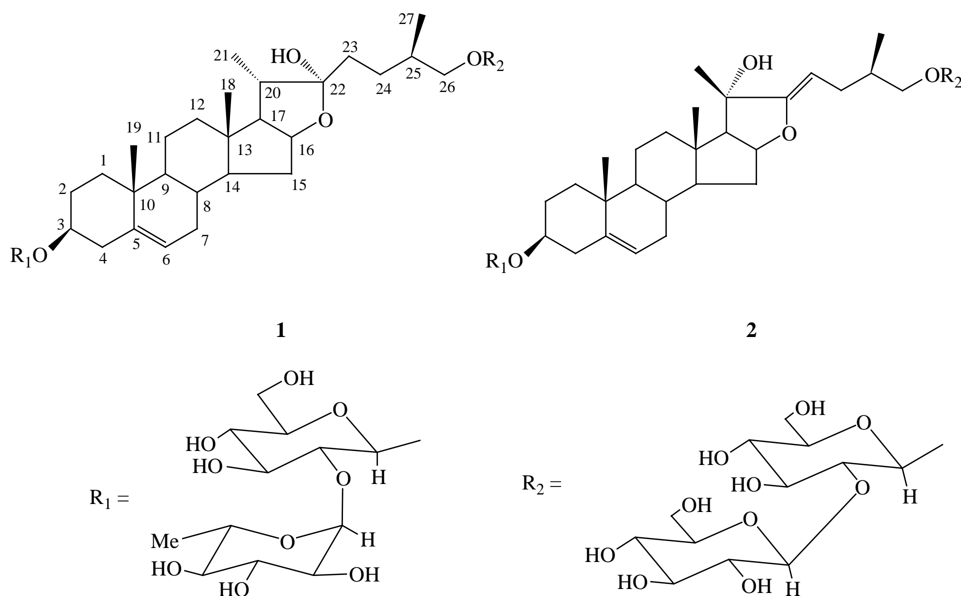

Figure 1. Structures of compounds **1** and **2**.

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The ^{13}C NMR spectrum consisted of 51 carbon signals, which indicated that compound **1** was a furostanol saponin with four sugar moieties and $\Delta^{5(6)}$ [7,8], in which the characteristic carbon signals at δ 140.7 (C-5), 121.7 (C-6), 19.3 (C-19), 17.4 (C-27), 110.6 (C-22), 16.3 (C-21), 100.2 (C-1′), 101.9 (C-1″), 103.0 (C-1‴), 106.3 (C-1⁗), 18.5 (C-6″) were assigned readily. The α-configuration of the C-22 hydroxyl group was deduced from the hemiketal carbon signal at δ 110.6, about 3–4 ppm higher than that of the β-configuration [9,10]. The 25*R* configuration of **1** was demonstrated by the chemical shift difference between the two protons of H-26 ($\Delta ab = 0.28 < 0.48$) [11,12]. The ^1H and ^{13}C NMR spectral data of the aglycone moiety were almost identical with those of protodioscin [12]. Thus, the aglycone of **1** was identified as (25*R*)-3β,22α,26-trihydroxyfurost-5-ene.

Acid hydrolysis of **1** with 1M HCl in dioxane–H_2O (1:1) gave glucose and rhamnose in the ratio of 3:1 by TLC and GC analyses. The four anomeric carbon signals at δ 100.2 (C-1′), 101.9 (C-1″), 103.0 (C-1‴), 106.3 (C-1⁗) showed correlations with their corresponding anomeric proton signals at δ 5.00, 6.34, 4.81, 5.25 in the HSQC spectrum, respectively. The HMBC correlations from H-1′ of Glu at δ 5.00 to C-3 of aglycone at δ 78.1, from H-1″ of Rha at δ 6.34 to C-2′ of Glu at δ 77.7, from H-1‴ of Glu at δ 4.81 to C-26 of aglycone at δ 75.0, from H-1⁗ of Glu at δ 5.25 to C-2⁗ of Glu at δ 84.0 indicated that two sugar chains were attached to C-3; C-26 of the aglycone indicated that two sugar chains were attached to C-3; and C-26 of the aglycone and that of the rhamnose and the glucose were linked to C-2′ and C-2⁗ of the inner glucoses, respectively (Figure 2). The combined use of $^1H-^1H$ COSY, HSQC, and HMBC experiments allowed the sequential assignments of all resonances for each monosaccharide. The proton resonances at δ 4.23 (1H, m), 4.28 (1H, m), 4.14 (1H, m), 4.25 (1H, o), and 4.32 (1H, m) and 4.45 (1H, m) were assigned to H-2′, H-3′, H-4′, H-5′, and H-6′ of the C-3 glucose moiety, respectively. The carbon signals at δ 77.7, 77.9, 71.7, 79.5, and 62.4

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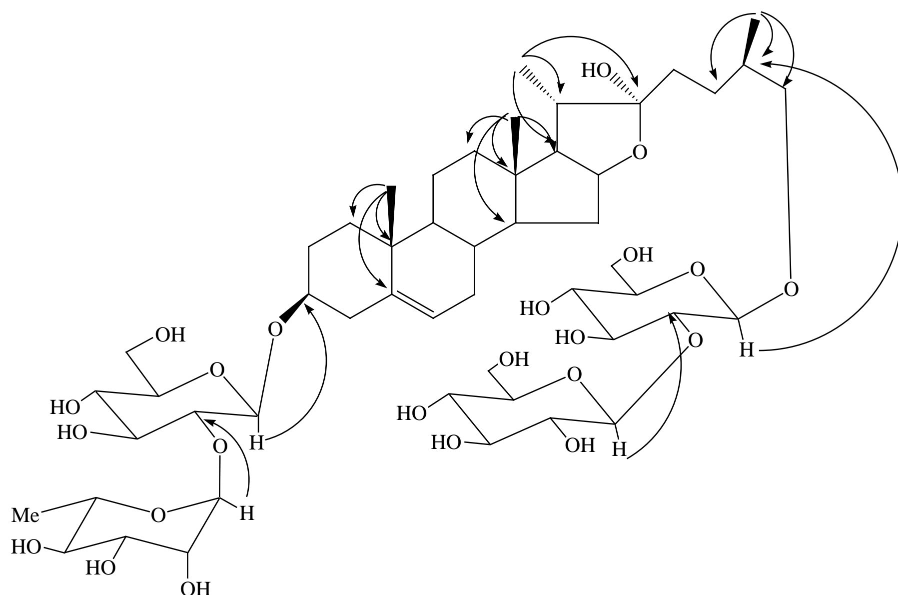

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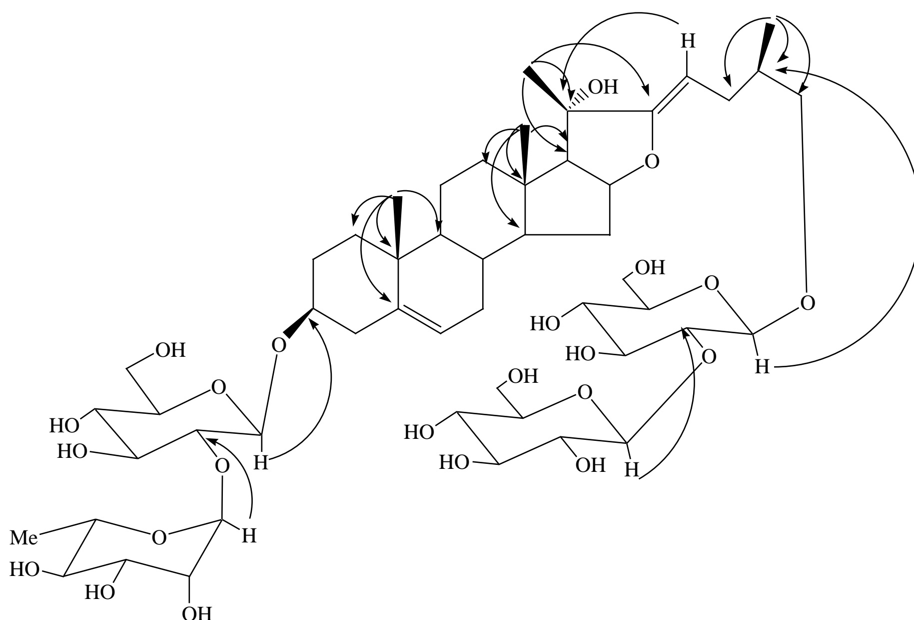

2

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

were assigned to the glucose C-2′, C-3′, C-4′, C-5′, and C-6′ from the HSQC analysis. In the same manner, the proton and carbon signals of other sugar moieties were also assigned. In conclusion, the structure of **1** was elucidated as (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-22α-hydroxyfurost-5-ene-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder with a positive Liebermann–Burchard reaction. The molecular formula was assigned as $C_{51}H_{82}O_{23}$ based on a molecular ion peak at *m/z* 1061.5220 $[M - H]^-$ in its HR-ESI-MS. The IR spectrum showed absorption bands at 3418 (OH), 2935 (CH), 1548, 1367 (CH_3), and 1032 cm^{-1}.

The ^{1}H NMR spectrum of **2** revealed five signals at δ 0.94 (3H, s), 1.06 (3H, s), 1.75 (3H, s), 1.12 (3H, d, $J = 6.8$ Hz), 1.80 (3H, d, $J = 6.0$ Hz), four anomeric proton signals at δ 4.81 (1H, d, $J = 7.5$ Hz), 5.07 (1H, d, $J = 7.0$ Hz), 5.25 (1H, d, $J = 7.0$ Hz), 6.42 (1H, br s), two olefinic proton signals at δ 5.20 (1H, o), 4.52 (1H, m). The ^{13}C NMR spectrum consisted of 51 carbon signals, which indicated that compound **2** is a furostanol saponin with four sugar moieties and $\Delta^{5(6),\ 22(23)}$ [7,8], in which the characteristic carbon signals at δ 140.8 (C-5), 121.7 (C-6), 19.4 (C-19), 17.4 (C-27), 163.6 (C-22), 100.3 (C-1′), 102.0 (C-1″), 103.2 (C-1‴), 106.5 (C-1⁗), 18.7 (C-6″) were assigned readily. The carbon signals at δ 91.8, 163.6 indicated a double bond between C-22 and C-23. The carbon and proton signals at δ_C 76.7, 21.9, and δ_H 1.75, (3H, s) indicated that a hydroxyl group was substituted at C-20. By comparison of the ^{13}C NMR signals of the aglycone moiety with the literature values [13] and on the basis of ^{1}H–^{1}H COSY, HSQC, and HMBC experiments, the aglycone of **2** was identified as 3β,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 1.75) and H-18 (δ 0.94) in the ROESY spectrum. The 25*R* configuration of **2** was demonstrated by the chemical shift difference between the two protons of H-26 ($\Delta ab = 0.34 < 0.48$) [11,12]. Hence, the aglycone of **2** was identified as (25*R*)-3β,20α,26-trihydroxyfurost-5,22-diene.

Acid hydrolysis of **2** gave glucose and rhamnose of 3:1 analyzed by TLC and GC methods. The spectroscopic data of **2** showed that the structure of sugar chains was the same as that of **1**. Therefore, **2** was elucidated 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-glucopyranoside.

3. Experimental

3.1 General experimental procedures

NMR spectra was recorded on a Bruker INOVA-500 spectrometer (Bruker Co., Billerica, MA, USA) or JNM-ECA 400 spectrometer (JEOL Ltd, Tokyo, Japan) in pyridine-d_5, δ in ppm and *J* in Hz. IR spectra were obtained from a Hitachi EPI-2 spectrometer (Hitachi Ltd, Tokyo, Japan). The HR-ESI-MS spectra were recorded on a Bruker APEX IV FT-MS (7.0T) mass spectrometer (Bruker Co.). Optical rotations were performed on a Jasco P-2000 polarimeter (Jasco Co., Tokyo, Japan). HPLC was carried out on Agilent 1100 (Agilent technologies Inc., Santa Clara, CA, USA) with a DAD detector using a Zorbax XDB-C_{18} column (9.4 × 250 mm). GC analysis was performed on an HP 6890 plus instrument (Agilent Technologies Inc.) equipped with an H_2 flame ionization detector. The GC analysis conditions were: HP-5 quartz capillary column (30 m × 0.32 mm × 0.25 μm); column temperature, 140–240°C; programmed oven temperature increase, 10°C/min; carrier gas, N_2 (1.5 ml/min); injector temperature, 240°C; detector temperature, 260°C; injection volume, 1 μl; split ratio, 1:50. Diaion HP-20 polyporous resin (Mitsubishi Chemical Co., Tokyo, Japan), silica gel (100–200, 200–300, and 300–400 mesh; Qingdao Marine Chemical Group, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden) were used for column chromatography (CC). Fractions were monitored by TLC (pre-coated silica gel GF_{254} plates made by Qingdao Marine Chemical Group) and sugars were monitored by TLC (pre-coated cellulose plate; Merck & Co., Darmstadt, Germany).

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** in pyridine-d_5.

Position	δ_C	δ_H, J (Hz)	Position	δ_C	δ_H, J (Hz)
1	37.4	0.94 m, 1.71 o	3-*O*-Glc 1′	100.2	5.00 d (7.0)
2	30.1	1.86 o, 2.11 br d (11.0)	2′	77.7	4.23 o
3	78.1	3.88 o	3′	77.9	4.28 o
4	38.9	2.71 m, 2.78 m	4′	71.7	4.14 o
5	140.7		5′	79.5	4.25 o
6	121.7	5.26 br s	6′	62.4	4.32 o, 4.45 o
7	32.2	1.45 o, 1.86 o	Rha 1″	101.9	6.34 br s
8	31.5	1.52 m	2″	72.4	4.77 br s
9	50.2	0.85 m	3″	72.7	4.60 dd (3.0, 9.5)
10	37.0		4″	74.0	4.32 o
11	21.0	1.41 m	5″	69.4	4.96 m
12	39.8	1.07 m, 1.71 o	6″	18.5	1.74 d (6.0)
13	40.7		26-*O*-Glc1‴	103.0	4.81 d (7.5)
14	56.5	1.03 m	2‴	84.0	4.09 o
15	32.3	1.45 o, 1.97 o	3‴	77.8	4.29 o
16	81.0	4.91 q (7.5)	4‴	71.2	4.14 o
17	64.0	1.91 o	5‴	78.1	3.88 m
18	16.4	0.88 s	6‴	62.5	4.32 o, 4.45 o
19	19.3	1.01 s	Glc 1⁗	106.3	5.25 d (7.0)
20	40.7	2.20 m	2⁗	76.7	4.06 o
21	16.3	1.29 d (7.5)	3⁗	77.8	4.23 o
22	110.6		4⁗	71.3	4.24 o
23	37.1	1.97 o	5⁗	78.6	3.92 o
24	28.1	1.58 m, 1.97 o	6⁗	62.5	4.32 o, 4.45 o
25	34.2	1.95 o			
26	75.0	3.60 o, 3.86 o			
27	17.4	1.03 d (6.5)			

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3.2 Plant material

The fibrous roots of *O. 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 the Peking University Modern Research Center for Traditional Chinese Medicine.

3.3 Extraction and isolation

The fibrous roots of *O. japonicus* (45 kg) were extracted with EtOH (70%) under reflux and then filtered by a 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. The *n*-BuOH soluble fraction was subjected to Diaion HP-20 resin CC eluted with 20% EtOH, 55% EtOH, and 80% EtOH in H_2O to afford three fractions: Fr.1 (100 g), Fr.2 (150 g), Fr.3 (32 g). Fr.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 (10.39 g), Fr.2-2 (11.07 g), Fr.2-3 (15.50 g), Fr.2-4 (12.14 g), Fr.2-5 (11.78 g), Fr.2-6 (11.35 g), Fr.2-7 (10.67 g). Fr.2-6 was subjected to silica gel eluted with EtOAc–EtOH gradiently to afford eight fractions (Fr.2-6-1 ~ 8). Fr.2-6-2 was subjected to ODS silica gel, Sephadex LH-20 CC, as well as 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** (68 mg, MeOH–H_2O 65:35, 8.5 min). Fr.2-6-4 was subjected to ODS silica gel

Table 2. ^{1}H and ^{13}C NMR spectral data of **2** in pyridine-d_5.

Position	δ_C	δ_H, J (Hz)	Position	δ_C	δ_H, J (Hz)
1	37.5	0.94 m, 1.71 o			
2	30.2	1.87 o, 2.11 m	3-*O*-Glc 1′	100.3	5.07 d (7.0)
3	78.2	3.92 m	2′	77.8	4.32 o
4	39.0	2.74 m, 2.80 m	3′	77.9	4.28 o
5	140.8		4′	71.8	4.19 o
6	121.7	5.20 o	5′	79.6	4.31 o
7	32.0	1.41 o, 1.86 o	6′	62.5	4.37 o, 4.54 o
8	31.1	1.52 m	Rha 1″	102.0	6.42 s
9	50.1	0.85 m	2″	72.6	4.83 m
10	37.0		3″	72.8	4.66 dd (3.0, 9.2)
11	20.6	1.44 m	4″	74.1	4.37 o
12	39.3	1.16 m, 1.90 o	5″	69.5	5.02 m
13	40.4		6″	18.7	1.80 d (6.0)
14	57.0	0.96 m			
15	33.5	1.64 m, 2.07 o	26-*O*-Glc1‴	103.2	4.81 d (7.5)
16	84.2	5.16 m	2‴	84.2	4.19 o
17	67.8	2.21 d (6.0)	3‴	77.9	4.36 o
18	13.6	0.94 s	4‴	71.4	4.25 o
19	19.4	1.06 s	5‴	78.5	3.88 o
20	76.7		6‴	62.6	4.37 o, 4.54 o
21	21.9	1.75 s	Glc 1⁗	106.5	5.25 d (7.0)
22	163.6		2⁗	77.0	4.13 o
23	91.8	4.52 m	3⁗	78.2	4.27 o
24	29.5	2.25 m, 2.51 o	4⁗	71.4	4.36 o
25	35.0	2.10 m	5⁗	78.5	3.96 o
26	75.2	3.61 o, 3.95 m	6⁗	62.6	4.37 o, 4.54 o
27	17.4	1.12 d (6.8)			

Note: o: Overlapped

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eluted with MeOH–H_2O, Sephadex LH-20 with MeOH, as well as 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 **2** (10 mg, MeCN–H_2O 28:72, 11.2 min).

3.3.1 *Ophiopogonin H (**1**)*

White powder. $[\alpha]_D^{20}$ 96.8 ($c = 0.17$, DMSO). IR (KBr) ν_{max}: 3395 (OH), 2903 (CH), 1548, 1367 (CH_3) and 1031 cm^{-1}. ^{1}H and ^{13}C NMR (100 MHz) spectral data, see Table 1. HR-ESI-MS *m/z*: 1087.5280 $[M + Na]^+$ (calcd for $C_{51}H_{84}O_{23}Na$, 1087.5301) and 1063.5401 $[M - H]^-$ (calcd for $C_{51}H_{83}O_{23}$, 1063.5325).

3.3.2 *Ophiopogonin I (**2**)*

White powder. $[\alpha]_D^{20}$ 46.6 ($c = 0.22$, DMSO). IR (KBr) ν_{max}: 3418 (OH), 2935 (CH), 1548, 1367 (CH_3), and 1032 cm^{-1}. ^{1}H and ^{13}C NMR (100 MHz) spectral data, see Table 2. HR-ESI-MS *m/z*: 1061.5220 $[M - H]^-$ (calcd for $C_{51}H_{81}O_{23}$, 1061.5169).

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