Spirostanol Saponins from the Fibrous Roots of *Ophiopogon japonicus* (THUNB.) KER-GAWL

by **Chang-Ling Duan***[a][b], **Yu-Juan Li**[b], **Peng Li**[b], **Yong Jiang**[a], **Jian-Xun Liu**[b], and **Peng-Fei Tu***[a]

[a]) School of Pharmaceutical Sciences, Peking University, Beijing 100191, P. R. China (e-mail: pengfeitu@vip.163.com)

[b]) Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, P. R. China (phone/fax: +86-10-62886691; e-mail: changlingd2010@yahoo.com.cn)

Three new steroidal saponins, (25*R*)-ruscogenin-3-yl α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (**1**), diosgenin-3-yl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (**2**), and pennogenin-3-yl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (**3**) were isolated from the fibrous roots of *Ophiopogon japonicus* (THUNB.) KER-GAWL. Their structures were determined by spectroscopic methods including IR, HR-ESI-MS, and 1D- and 2D-NMR. All of these three steroidal saponins exhibited weak cytotoxicities against Hela and Hep2 cell lines.

Introduction. – *Ophiopogon japonicus* (THUNB.) KER-GAWL (known as 'Maidong' in China) is an evergreen perennial, widely distributed in mainland China, especially in Sichuan and Zhejiang provinces. Its tubers were used in traditional Chinese medicine and folk medicine of Vietnam as expectorant, antitussive, and tonic agent, and were often used for the treatment of cardiovascular and cerebrovascular diseases in combination with *Panax ginseng* and *Schisandra chinensis* clinically [1][2]. Previous phytochemical studies of *Ophiopogonis* tubers resulted in the isolation and structure elucidation of steroids [3–7], homoisoflavonoids [8][9], and amides [4], as well as monoterpenoids [10][11]. Our phytochemical investigation on fibrous roots of *O. japonicus* led to the isolation of three new steroidal saponins. Herein, we report the isolation and structural determination of the new constituents. Their cytotoxic activities against Hela and Hep2 cell lines are also described.

Results and Discussion. – Compound **1** was obtained as white amorphous powder with positive reaction to the *Liebermann–Burchard* (*L.–B.*) test (greenish). The specific rotation $[\alpha]_D^{20}$ ($c = 0.32$, pyridine) was −56.3. The molecular formula of **1** was deduced as $C_{44}H_{70}O_{17}$ on the basis of HR-ESI-MS (*m/z* 893.4488 ($[M+Na]^+$; calc. 893.4511)). The IR spectrum showed the characteristic absorption of a (25*R*)-spirosteroid at 980, 930, 910, and 870 cm^{-1} [12]. The structure of **1** was established as (25*R*)-ruscogenin-3-yl α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (see *Fig. 1*) on the basis of ^{1}H- and ^{13}C-NMR (*Table 1*), HSQC, and HMBC spectra.

The ^{1}H-NMR spectrum of **1** revealed the presence of four Me groups (δ(H) 0.91 (*s*, Me(18)), 1.38 (*s*, Me(19)), 1.11 (*d*, $J = 7.0$, Me(21)), 0.70 (*d*, $J = 5.4$, Me(27))), an

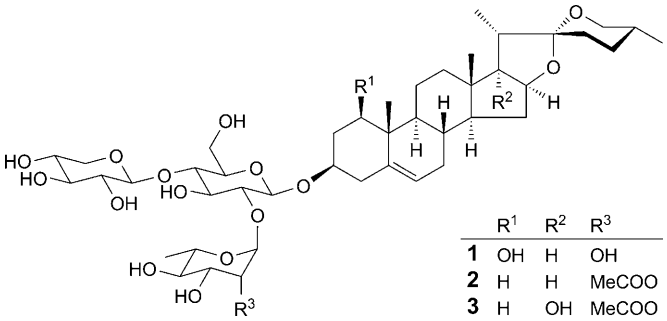

Fig. 1. *Structures of the three spirostanol saponins* **1**–**3**

olefinic H-atom at δ(H) 5.53 (br. *s*), the three anomeric H-atoms (δ(H) 6.27 (br. *s*), 5.04 (*d*, $J = 7.7$), and 5.01 (*d*, $J = 7.2$)) (*Table 1*). The ^{13}C-NMR data were basically consistent with those of (25*R*)-ruscogenin [13], and the glycosidation shifts (− 3.1 ppm at C(2), +6.8 ppm, at C(3), −4.3 ppm at C(4)) indicated a sugar chain linked at HO−C(3), which was further proved by a HMBC between H−C(1′) (δ(H) 5.01 (*d*, $J = 7.2$)) and C(3) (δ(C) 75.1) (*Fig. 2*). Acid hydrolysis of **1** produced D-glucose, L-rhamnose, and D-xylose, identified by TLC and GC analysis. The positions of the L-rhamnosyl and D-xylosyl units were determined as C(2) and C(4) of the D-glucopyranosyl, respectively, by comparison of the ^{13}C-NMR data with the literature [3][14] and by HMBC between H−C(1″) (δ(H) 6.27 (br. *s*)) and C(2′) (δ(C) 77.4), and between H−C(1‴) (δ(H) 5.04 (*d*, $J = 7.7$)) and C(4′) (δ(C) 81.3). The β-configuration of glucose and xylose was determined by large *J* values of the anomeric-H-atom signals at δ(H) 5.01 (*d*, $J = 7.2$, H−C(1′)) and 5.04 (*d*, $J = 7.7$, H−C(1‴)), while the rhamnose had an α-configuration, evidenced by the very small *J* value of the signal for the anomeric H-atom at δ(H) 6.27 (br. *s*, 1 H) in the ^{1}H-NMR spectrum and by the ^{13}C-NMR data. Thus, the structure of compound **1** was established as (25*R*)-ruscogenin-3-yl α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside.

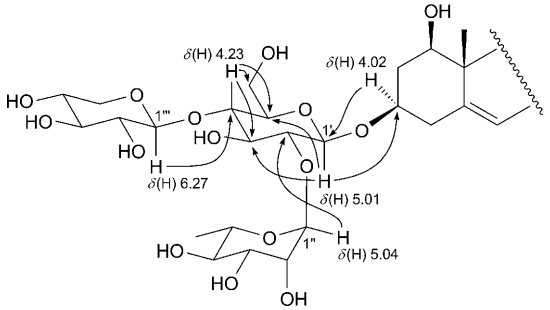

Fig. 2. *Key HMBCs of the sugar moieties of* **1**

Compound **2** was obtained as white amorphous powder with a positive reaction to the *L.–B.* test (brown). The specific rotation $[\alpha]_D^{20}$ ($c = 0.28$, pyridine) was −96.5. The HR-ESI-MS spectrum of **2** (*m/z* 919.4653 ($[M + Na]^+$; calc. 919.4667)) suggested the molecular formula $C_{46}H_{72}O_{17}$. The structure of **2** was established as diosgenin-3-yl 2-*O*-

Table 1. *^{1}H- and ^{13}C-NMR* ((D_5)pyridine) *Data for* **1**–**3**. δ in ppm, J in Hz.

	1		**2**		**3**
	δ(H)	δ(C)	δ(H)	δ(C)	δ(C)
H_α–C(1)	3.69–3.73 (*m*)	77.9	1.72 (br. *d*, *J* = 13.5)	37.4	37.5
H_β–C(1)	–		0.96 (*td*, *J* = 13.5, 3.0)		
H_α–C(2)	2.62 (br. *d*, *J* = 11.5)	40.9	2.08–2.11 (*m*)	30.1	30.1
H_β–C(2)	2.34 (*q*, *J* = 11.5)		1.82–1.85 (*m*)		
H–C(3)	4.00–4.02 (*m*)	75.1	3.84–3.88 (*m*)	78.2	78.2
H–C(4)	2.80–2.82 (*m*)	39.4	2.69–2.74 (*m*)	38.9	39.0
C(5)	–	139.0	–	140.7	140.7
H–C(6)	5.53 (br. *s*)	125.2	5.27 (br. *s*)	121.8	121.8
CH_2(7)	1.88–1.92 (*m*), 1.50–1.54 (*m*)	32.9	1.82–1.85 (*m*), 1.40–1.42 (*m*)	32.3	32.1
H–C(8)	1.60–1.62 (*m*)	32.5	1.50–1.52 (*m*)	31.7	32.3
H–C(9)	1.35–1.36 (*m*)	51.2	0.87 (*td*, *J* = 11.0, 5.5)	50.3	50.2
C(10)	–	43.8	–	37.1	37.1
H_α–C(11)	2.85–2.89 (*m*)	24.1	1.97–1.99 (*m*)	20.1	20.9
H_β–C(11)	1.75–1.77 (*m*)		1.40–1.42 (*m*)		
H_α–C(12)	1.66–1.69 (*m*)	40.5	1.61–1.63 (*m*)	39.8	32.1
H_β–C(12)	1.23–1.25 (*m*)		1.03–1.07 (*m*)		
C(13)	–	40.2	–	40.4	45.1
H–C(14)	1.14–1.16 (*m*)	56.8	1.02–1.04 (*m*)	56.6	53.0
H_α–C(15)	2.05–2.10 (*m*)	32.3	1.99–2.04 (*m*)	32.2	31.8
H_β–C(15)	1.51–1.55 (*m*)		1.40–1.42 (*m*)		
H–C(16)	4.55–4.57 (*m*)	81.1	4.49–4.52 (*m*)	81.1	90.1
H–C(17)	1.81 (*dd*, *J* = 8.0, 6.5)	63.2	1.77–1.79 (*m*)	62.9	90.0
Me(18)	0.91 (*s*)	16.6	0.81 (*s*)	16.3	17.1
Me(19)	1.38 (*s*)	13.7	1.03 (*s*)	19.4	19.4
H–C(20)	2.07–2.09 (*m*)	42.0	1.93 (*dq*, *J* = 7.0, 7.0)	41.9	39.9
Me(21)	1.11 (*d*, *J* = 7.0)	15.1	1.12 (*d*, *J* = 7.0)	15.0	9.8
C(22)	–	109.3	–	109.2	109.8
CH_2(23)	1.66–1.69 (*m*)	31.9	1.62–1.65 (*m*)	31.8	32.4
CH_2(24)	1.60–1.62 (*m*)	29.3	1.57–1.59 (*m*)	29.2	28.8
H–C(25)	1.61–1.63 (*m*)	30.6	1.57–1.59 (*m*)	30.6	30.4
CH_2(26)	3.59 (br. *d*, *J* = 11.5), 3.51 (*t*, *J* = 10.8)	66.9	3.56 (*dd*, *J* = 10.0, 2.5), 3.51 (*t*, *J* = 10.4)	66.8	66.7
Me(27)	0.70 (*d*, *J* = 5.4)	17.4	0.67 (*d*, *J* = 5.2)	17.3	17.3
H–C(1′)	5.01 (*d*, *J* = 7.2)	100.1	4.94 (*d*, *J* = 7.0)	99.9	99.9
H–C(2′)	4.20 (*dd*, *J* = 9.0, 3.5)	77.4	4.19–4.21 (*m*)	77.1	77.1
H–C(3′)	3.82–3.84 (*m*)	76.3	3.78–3.80 (*m*)	76.3	76.2
H–C(4′)	4.23 (*t*, *J* = 7.5)	81.3	4.18–4.20 (*m*)	81.2	81.2
H–C(5′)	4.25–4.27 (*m*)	77.3	4.19–4.22 (*m*)	77.0	77.0
H_α–C(6′)	4.50–4.52 (*m*)	61.5	4.49–4.52 (*m*)	61.5	61.5
H_β–C(6′)	4.36–4.38 (*m*)		4.42 (*dd*, *J* = 9.0, 3.5)		
H–C(1″)	6.27 (br. *s*)	102.0	6.17 (br. *s*)	98.7	98.7
H–C(2″)	4.81 (br. *s*)	72.5	4.71 (*dd*, *J* = 9.0, 4.0)	70.4	70.4
H–C(3″)	4.60–4.64 (*m*)	72.8	6.04 (*d*, *J* = 3.5)	74.0	74.0
H–C(4″)	4.34 (*dd*, *J* = 9.0, 3.5)	74.2	4.23–4.28 (*m*)	74.1	74.1
H–C(5″)	4.93–4.97 (*m*)	69.6	4.90–4.92 (*m*)	69.5	69.5
Me(6″)	1.73 (*d*, *J* = 7.2)	18.7	1.77 (*d*, *J* = 6.0)	18.5	18.6
H–C(1‴)	5.04 (*d*, *J* = 7.7)	105.8	5.01 (*d*, *J* = 7.5)	105.8	105.7
H–C(2‴)	3.98 (*dd*, *J* = 9.0, 4.0)	75.0	3.98 (*t*, *J* = 8.5)	75.0	75.0
HC(3‴)	4.13–4.17 (*m*)	78.3	4.08 (*t*, *J* = 8.5)	78.3	78.3
H–C(4‴)	4.15–4.19 (*m*)	70.8	4.14 (*dd*, *J* = 9.0, 3.0)	70.8	70.8
H_α–C(5‴)	4.26–4.30 (*m*)	67.4	4.18–4.21 (*m*)	67.3	67.4
H_β–C(5‴)	3.68 (*t*, *J* = 10.5)		3.60–3.64 (*m*)		
Me			2.00 (*s*)	21.1	21.1
C=O				170.6	170.6

acetyl-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside by analysis of the ^{1}H- and ^{13}C-NMR (*Table 1*), HSQC, and HMBC spectra.

The ^{1}H-NMR spectrum of **2** showed signals for two tertiary Me groups (δ(H) 0.81 (*s*, Me(18)) and 1.03 (*s*, Me(19))), three secondary Me groups (δ(H) 1.12 (*d*, *J* = 7.0, Me(21)), 0.67 (*d*, *J* = 5.2, Me(27)), and 1.77 (*d*, *J* = 6.0, Me(6″))), an olefinic H-atom at δ(H) 5.27 (br. *s*), and three anomeric H-atoms (δ(H) 6.17 (br. *s*), 5.01 (*d*, *J* = 7.5), 4.94 (*d*, *J* = 7.0)). Acid hydrolysis of **2** gave D-glucose, L-rhamnose, and D-xylose as carbohydrate moieties, identified on the basis of TLC and GC analysis. The presence of an AcO group was indicated by the signals of δ(H) 2.00 (*s*, 3 H), and δ(C) 21.1, 170.6. The acetylation at HO−C(2′) of the α-L-rhamnopyranosyl unit was deduced from a HMBC between H−C(2″) (δ(H) 4.71, *dd*, *J* = 9.0, 4.0) and C=O (δ(C) 170.6). The sugar sequence of **2** was demonstrated by the HMBCs from H−C(1″) (δ(H) 6.17 (br. *s*)) to C(2′) (δ(C) 77.1), from H−C(1‴) (δ(H) 5.01 (*d*, *J* = 7.5)) to C(4′) (δ(C) 81.2), and from H−C(1′) (δ(H) 4.94 (*d*, *J* = 7.0)) to C(3) (δ(C) 78.2). Based on these observations, the structure of **2** was determined as diosgenin-3-yl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside.

Compound **3** was obtained as white amorphous powder with a positive reaction to the *L.–B.* test (red). The specific rotation $[\alpha]_D^{20}$ (*c* = 0.17, MeOH) was −46.9. It was analyzed as $C_{46}H_{72}O_{18}$ on the basis of HR-ESI-MS (*m/z* 935.4598 ($[M+Na]^+$; calc. 935.4616)). The structure was determined as pennogenin-3-yl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside by analysis of HR-ESI-MS, ^{1}H- and ^{13}C-NMR (*Table 1*), HSQC, and HMBC spectra.

The ^{1}H-NMR of **3** revealed the presence of four typical steroid Me groups (δ(H) 0.95 (*s*, Me(18)), 1.09 (*s*, Me(19)), 1.24 (*d*, *J* = 7.0, Me(21)), and 0.68 (*d*, *J* = 5.2, Me(27))), an olefinic H-atom at δ(H) 5.28 (br. *d*, *J* = 4.0, H−C(6)), and three anomeric H-atoms (δ(H) 6.17 (br. *s*), 5.07 (*d*, *J* = 7.2), and 4.94 (*d*, *J* = 7.2)). The ^{13}C-NMR data were basically consistent with those of pennogenin [13], and the glycosylation at HO−C(3) was deduced from the glycosidation shifts at C(2) (−2.9 ppm), C(3) (+7.1 ppm), C(4) (−4.5 ppm). From the ^{1}H- and ^{13}C-NMR data, it was evident that **3** possessed the same sugar chain as **2**. Therefore, the structure of **3** was established as pennogenin-3-yl 2-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside.

These steroidal glycosides were tested for cytotoxicity against Hela and Hep2 cell lines *in vitro*. The IC_{50} values of **1**–**3** are listed in *Table 2*. All of them showed weak cytotoxicity ($IC_{50} > 3$ μg/ml).

Table 2. *Cytotoxic Activities of* **1**–**3** *against HeLa and Hep2 Cell Lines* (IC_{50} [μg/ml])

	Hela	Hep2
1	9.14	11.27
2	10.77	10.08
3	13.46	13.32

This research was financially supported by the *Program for Changjiang Scholar and Innovative Team in University* (No. 985-2-063-112) and the *11th Five Program for Sciences and Technologies Development*

of China (No. 2006BAI08B04-08). Thanks are also extended to Prof. *Shanyi Qiao* for NMR measurements and Dr. *Jifen Guo* for MS measurements.

Experimental Part

General. All solvents and reagents were of anal. grade (*Beijing Chemical Reagent Co., Ltd.*, P. R. China). MeOH and MeCN for HPLC were of HPLC grade (*Fisher Chemicals*, New Jersey, USA). Authentic sugar samples (D-glucose, L-rhamnose, and D-xylose) were purchased from the *National Institute for the Control of Pharmaceutical and Biological Products*, P. R. China. Column chromatography (CC): silica gel (SiO_2; 100–200 and 300–400 mesh, *Qingdao Marine Chemical Co. Ltd.*, P. R. China), *Daion HP-20* polyporous resin (*Mitsubishi Chemical Co.*, Japan), and *Sephadex LH-20* gel (*Amersham Biosciences AB*, Uppsala, Sweden). TLC: pre-coated silica gel GF_{254} plates (*Qingdao Marine Chemical Co. Ltd.*, P. R. China); detection by spraying with 10% H_2SO_4 soln. in EtOH followed by heating; or pre-coated cellulose plates (*Merck*). HPLC: *Waters LC 515* with *2487* detector. GC Analysis: *HP6890 plus* instrument equipped with an H_2 flame ionization detector (*Agilent Technologies*, USA); conditions: *HP-5* quarz capillary column (30 m × 0.32 mm × 0.25 μm), column temp. 140–240°, programmed oven temp. increase 10°/min, carrier gas N_2 (1.5 ml/min), injector temp. 240°, detector temp. 260°, injection volume 1 μl, split ratio 1:50. Optical rotations: *Jasco P-2000* polarimeter. IR Spectra: *Hitachi EPI-2* spectrometer. NMR Spectra: *Varian UNITYINOVA 500* spectrometer and *JNM-ECA 400* spectrometer in (D_5)pyridine, δ in ppm and J in Hz. HR-ESI-MS Spectra: *Nano LC-Q-TOF2* mass spectrometer.

Plant Material. The fibrous roots of *O. japonicus* were purchased from *Anguo traditional Chinese medicine* (TCM) *market*, Hebei province, P. R. China, in September 2005 and identified by one of the authors (Prof. *Peng-Fei Tu*). A voucher specimen (MD20050906) is deposited with 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 through 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), AcOEt, and BuOH. The 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. 1–3*). *Fr. 3* (40.0 g) was separated by SiO_2 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 five fractions (*Fr. 3-1–3-6*). *Fr. 3-3* was subjected to SiO_2, *ODS* SiO_2, and *Sephadex LH-20* CC, as well as repeated semi-prep. HPLC (*Zorbax XDB-C*$_{18}$ column, 9.4 × 250 mm, 5 μm, flow rate 2.0 ml/min, UV 203 nm) to afford **1** (15 mg), **2** (14 mg), and **3** (29 mg).

Acid Hydrolysis of **1** *and* **2**. A soln. of compound **1** or **2** (8 mg, resp.) in MeOH was treated with 1.5M H_2SO_4 (4 ml), and the mixture was refluxed at 100° for 5 h. After cooling, the mixture was neutralized with Na_2CO_3, and then extracted with $CHCl_3$ (3 × 10 ml). The aq. layer was concentrated to appropriate volume (1 ml) for sugar analysis and examined by TLC (cellulose) with the solvent system AcOEt/pyridine/AcOH/H_2O 5:5:1:3. The remaining aq. layer was concentrated to dryness. Then, 1 ml of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the dry residue, and the mixture was heated to 100° for 1 h. After cooling, Ac_2O (1.5 ml) was added, followed by heating to 100° for another 1 h, and then, the mixture was evaporated to dryness under reduced pressure. The resulting residue was solved in $CHCl_3$ and analyzed by GC, by comparing with aldononitrile peracetates of authentic samples, which indicated that both the sugar moieties of **1** and **2** consisted of D-glucose, L-rhamnose, and D-xylose in a ratio of 1:1:1.

*(25*R*)-Ruscogenin-3-yl α-*L*-Rhamnopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→4)]-β-*D*-glucopyranoside* (=*(1β,3β,25*R*)-1-Hydroxyspirost-5-en-3-yl 6-Deoxy-α-*L*-mannopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→4)]-β-*D*-glucopyranoside*; **1**). White amorphous powder with a positive reaction to the *L.–B.* test (greenish). $[\alpha]_D^{20} = -56.3$ ($c = 0.32$, pyridine). IR (KBr): 980, 930, 910, 870. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Table 1*. HR-ESI-MS: 893.4488 ($[M+Na]^+$, $C_{44}H_{70}NaO_{17}^+$; calc. 893.4511).

Diosgenin-3-yl 2-O-*Acetyl-α-*L*-rhamnopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→4)]-β-*D*-glucopyranoside* (=*(3β,25*R*)-Spirost-5-en-3-yl 2*-O-*Acetyl-6-deoxy-α-*L*-mannopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→4)]-β-*D*-glucopyranoside*; **2**). White amorphous powder with a positive reaction to the

L.–B. test (brown). $[\alpha]_D^{20} = -96.5$ ($c = 0.28$, pyridine). ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): *Table 1*. HR-ESI-MS: 919.4653 ($[M+Na]^+$, $C_{46}H_{72}NaO_{17}^+$; calc. 919.4667).

*Pennogenin-3-yl 2-*O*-Acetyl-α-*L*-rhamnopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 4)]-β-*D*-glucopyranoside* (*=(3β,25*R*)-17-Hydroxyspirost-5-en-3-yl 2-*O*-Acetyl-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 4)]-β-*D*-glucopyranoside*; **3**). White amorphous powder with a positive reaction to the *L.–B.* test (red). $[\alpha]_D^{20} = -46.9$ ($c = 0.17$, pyridine). ^{1}H-NMR (400 MHz, (D$_5$)pyridine): 6.17 (*s*, H–C(1″)); 5.28 (br. *d*, *J* = 4.0, H–C(6)); 5.07 (*d*, *J* = 7.2, H–C(1‴)); 4.94 (*d*, *J* = 7.2, H–C(1′)); 4.75 (*d*, *J* = 8.5, H–C(16)); 3.82–3.85 (*m*, H–C(3)); 3.50 (br. *d*, *J* = 10.5, H–C(26)); 2.79–2.83 (*m*, H–C(4)); 2.22–2.26 (*m*, H–C(20)); 2.21–2.24 (*m*, H_α–C(15)); 2.03–2.07 (*m*, H_α–C(2)); 1.93 (*s*, MeCOO)); 1.81–1.84 (*m*, H_β–C(2)); 1.80 (*d*, *J* = 7.0, Me(6″)); 1.72–1.75 (*m*, H_α–C(1)); 1.67–1.69 (*m*, H_α–C(12)); 1.56–1.60 (*m*, H–C(11)); 1.56–1.59 (*m*, H–C(23)); 1.55–1.58 (*m*, H–C(25)); 1.51–1.54 (*m*, H–C(24)); 1.51–1.54 (*m*, H_β–C(15)); 1.44–1.48 (*m*, H_β–C(12)); 1.24 (*d*, *J* = 7.0, Me(21)); 1.09 (*s*, Me(19)); 0.95–0.97 (*m*, H_β–C(1)); 0.94–0.97 (*m*, H_β–C(9)); 0.95 (*s*, Me(18)); 0.68 (*d*, *J* = 5.2, Me(27)). ^{13}C-NMR: *Table 1*. HR-ESI-MS: 935.4598 ($[M+Na]^+$, $C_{46}H_{72}NaO_{18}^+$; calc. 935.4616).

Cytotoxicities against Hela and Hep2 Cell Lines. The cytotoxicities against HeLa and Hep2 cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay on 96-well microplates as described in a previous article [15], and expressed as IC_{50} values (50% inhibition of cell proliferation, μg/ml), which was calculated by GraphPad Prism. The *OD* value was read on a SYNERY™ 4 multi-mode microplate reader (*BioTek Instrument Inc.*, USA) at 490 nm.

REFERENCES

[1] Jiangsu College of New Medicine, 'Dictionary of Chinese Traditional Medicine' 1986, p. 2082.
[2] N. T. H. Anh, T. V. Sung, A. Porzel, K. Franke, L. A. Wessjohann, *Phytochemistry* **2003**, *62*, 1153.
[3] Y. Watanabe, S. Sanada, Y. Ida, J. Shoji, *Chem. Pharm. Bull.* **1983**, *31*, 3486.
[4] Y. Watanabe, Y. Hirai, S. Sanada, J. Shoji, *Shoyakugaku Zasshi* **1990**, *44*, 117.
[5] J. T. Branke, E. Haslinger, *Liebigs Ann.* **1995**, 587.
[6] A. C. Do, K. Y. Jung, Y. K. Sung, J. H. Jung, K. H. Son, *J. Nat. Prod.* **1995**, *58*, 778.
[7] Z.-H. Chen, T. Wu, B.-Y. Yu, *J. Asian Nat. Prod. Res.* **2006**, *8*, 555.
[8] Y. Zhu, K. Yan, G. Tu, *Phytochemistry* **1987**, *26*, 2873.
[9] J.-M. Chang, C.-C. Shen, Y.-L. Huang, M.-Y. Chien, J.-C. Ou, B.-J. Shieh, C.-C. Chen, *J. Nat. Prod.* **2002**, *65*, 1731.
[10] Z.-H. Cheng, T. Wu, S. W. A. Bligh, A. Bashall, B.-Y. Yu, *J. Nat. Prod.* **2004**, *67*, 1761.
[11] M. Adinolfi, M. Parrilli, Y. Zhu, *Phytochemistry* **1990**, *29*, 1696.
[12] R. N. Jones, K. Katzenellenbogen, K. Dobriner, *J. Am. Chem. Soc.* **1953**, *75*, 158.
[13] P. K. Agrawal, D. C. Jain, R. K. Gupta, R. S. Thakur, *Phytochemistry* **1985**, *24*, 2499.
[14] J. Z. Wang, L. M. Ye, X. B. Chen, *Chin. Chem. Lett.* **2008**, *19*, 82.
[15] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

Received May 5, 2009