Diosgenin Glucuronides from *Solanum lyratum* and their Cytotoxicity against Tumor Cell Lines

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Bioassay-directed fractionation of the cytotoxicity active fraction of the whole plant from *Solanum lyratum* led to the isolation of a new steroidal saponin, diosgenin 3-*O*-*β*-D-glucopyranosiduronic acid methyl ester (**2**), as well as four known compounds, diosgenin (**1**), diosgenin 3-*O*-*β*-D-glucopyranosiduronic acid (**3**), diosgenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosiduronic acid (**4**), diosgenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucuronidu-ronic acid methyl ester (**5**). The structures of the isolated compounds were elucidated on the basis of their spectral data and chemical evidences. Compound **1** was isolated for the first time from this plant, and compound **3** was isolated as a new natural product. Cytotoxic activities of the isolated compounds were evaluated and the cytotoxicities of compounds **2**–**5** reported for the first time.

Key words: Solanum lyratum, Cytotoxicity, Diosgenin Glucuronides

Introduction

The whole plants of *Solanum lyratum* Thunb. (Solanaceae) are called "Baimaoteng" in Chinese medicine. The aerial part of *Solanum lyratum* had traditionally been used in therapies against cancer, malaria, jaundice, edema, hepatitis *etc.* (Shim *et al.*, 1995). Previously, it was reported that the aqueous extract of *Solanum lyratum* has cytotoxic activity against HL60 cell lines (Shi and Yan, 2002) and the EtOAC extract has apoptosis-inducing activity in Bel-7404 cell lines (Shan *et al.*, 2001). Many steroidal glucuronides and steroidal alkaloid glycosides had been isolated from *Solanum lyratum.* Some of them have inhibitory activity for cancer cell growth (Murakami *et al.*, 1985; Yahara *et al.*, 1985; Yung *et al.*, 1997). However, bioactivity components responsible for its anticancer activity have not been well understood.

To systematically evaluate its potential anticancer activity, the bioactivity ingredients were isolated from the whole plant of *Solanum lyratum* by activity-guided fractionation. The structures of the isolated compounds were elucidated on the basis of their spectral data and chemical evidences, and their cytotoxic activities were determined in this study.

Abbreviations: HeLa, human uterine carcinoma; A375-S2, human malignant melanoma; L929, murine fibrosarcoma; MCF7, human breast cancer; Bel-7402, human hepatocellular carcinoma; SGC-7901, human gastric cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC_{50}, the 50% growth inhibition; 5-Fu, 5-fluorouracil.

Materials and Methods

General

The NMR spectra were recorded with a Bruker-ARX 300 or 600 spectrometer operating at 300 or 600 MHz for ^1H and 75 or 150 MHz for ^{13}C NMR, respectively. Chemical shifts were reported in ppm on the δ scale with TMS as the internal standard. ESI-MS were recorded on a Finnigan LCQ LC-MS analyzer. The IR absorption spectra were recorded in KBr discs using a Bruker IFS-55 infrared spectrometer. Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, Qingdao, People's Republic of China) and Sephadex LH-20 (25–100 μm, Pharmacia). TLC was performed on precoated Si gel 60 F254 plates (0.2 mm thick, Merck) and spots were detected by spraying with 10% ethanolic H_2SO_4 reagent.

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Plant

The whole plants of *Solanum lyratum* were purchased from ShangHai XuHui Chinese Herbal Medicine Factory (Shanghai, China) in January 2003. This plant was identified by Professor Qi-Shi Sun, College of Chinese Traditional Medicine, Shenyang Pharmaceutical University.

Extraction and isolation

The air-dried aerial parts of *Solanum lyratum* (15 kg) were extracted with 95% EtOH. The EtOH solutions were combined, filtered and evaporated under vacuum. The dry EtOH extract (705 g, 4.7%) was further processed by successive solvent partitioning to give petroleum ether (180 g), EtOAc (135 g), *n*-BuOH (150 g) and aqueous (210 g) fractions, respectively. Each fraction was evaluated for the antiproliferative activity on the tumor cell lines. Consequently, it was shown that the activity resided predominantly in the EtOAc fraction (Table I). Thus, the EtOAC fraction was subjected to column chromatography on silica gel. Using petroleum ether/acetone (100:0 ~ 0:100) as the eluting solvent, it gave 15 fractions (E_1–E_{15}), as follows: E_1 (0.5 g), E_2 (0.1 g), E_3 (0.4 g), E_4 (0.2 g), E_5 (1.2 g), E_6 (0.7 g), E_7 (0.5 g), E_8 (5.1 g), E_9 (10.9 g), E_{10} (9.1 g), E_{11} (2.7 g), E_{12} (3.6 g), E_{13} (10.2 g), E_{14} (5.4 g), E_{15} (25.2 g). Fractions E_1–E_{15} were evaluated for the cytotoxic activity against HeLa and A375-S2 (Table II).

Fraction E_5 was separated by silica gel using $CHCl_3$/MeOH (100:0 ~ 0:100) and HPLC (ODS, 95% MeOH) to give compound **1** (36 mg). Fraction E_{14} was chromatographed on a silica gel column using $CHCl_3$/MeOH (80:0 ~ 0:1) and 15 fractions (E_{14}-01–E_{14}-15) were collected and evaporated under reduced pressure. Fraction E_{14}-07 was purified by a Sephadex LH20 column using $CHCl_3$/MeOH (1:1 ~ 0:1) and recrystallization to give compound **2** (40 mg). Fraction E_{14}-09 was chromatographed on a silica gel column using $CHCl_3$/MeOH (20:1 ~ 0:1) and a Sephadex LH20 column using $CHCl_3$/MeOH (1:1 ~ 0:1) to offer compound **3** (10.4 mg). Separation of fraction E_{15} by a silica gel column using $CHCl_3$/MeOH (60:1 ~ 0:1) and RPHPLC (ODS, 60% MeOH) afforded compound **4** (11.5 mg) and compound **5** (13 mg).

*Diosgenin (**1**):* Light green solid. – IR: ν = 3364.6 (-OH), 2916.0, 2849.4, 1452.9 (>C=C<), 1372.6, 1240.9, 1054.0, 977.8, 921.7, 863.3 cm^{-1}. – EI-MS: *m/z* (rel. int.) = 415 $[M]^+$ (2), 414 $[M-1]^+$ (3), 397 $[M-H_2O]^+$ (2), 342 (5), 300 (7), 282 (23), 271 (17), 253 (9), 139 (100). – ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) = 0.69 (3H, d, J = 6.0 Hz, 27-CH_3), 0.79 (3H, s, 18-CH_3), 0.97 (3H, d, J = 4.6 Hz, 21-CH_3), 1.03 (3H, s, 19-CH_3). – ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) = 37.2 (C-1), 31.6 (C-2), 71.7 (C-3), 42.3 (C-4), 140.8 (C-5), 121.4 (C-6), 32.0 (C-7), 31.4 (C-8), 50.0 (C-9), 36.6 (C-10), 20.8 (C-11), 39.8 (C-12), 40.2 (C-13), 56.5 (C-14), 31.8 (C-15), 80.8 (C-16), 62.1 (C-17), 16.3 (C-18), 19.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.3 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 17.1 (C-27).

*Diosgenin 3-O-β-D-glucopyranosiduronic acid methyl ester (**2**):* White amorphous powder. – IR: ν = 3329.7 (-OH), 2949.1, 1743.8 (-**CO**OMe), 1451.5 (>C=C<), 1381.8, 1170.0, 1051.7, 981.9, 921.7, 899.3 cm^{-1}. – Positive ESI-MS: *m/z* = 605.3 $[M+H]^+$, 627.4 $[M+Na]^+$; negative ESI-MS: *m/z* = 621.1 $[M+H_2O-H]^-$, 639.1 $[M+Cl]^-$. – ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.73 × 2 (6H, br s, H-18, H-27), 0.90 (3H, d, J = 6.88 Hz, H-21), 0.96 (3H, s, H-19), 3.65 (3H, s, O**C**$\mathbf{H}_3$), 4.38 (1H, d, J = 7.79 Hz, H-1 of Glc), 5.34 (H, s, H-6). – ^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) = 36.8 (t, C-1), 31.1 (t, C-2), 77.5 (d, C-3), 38.3 (t, C-4), 140.4 (s, C-5), 121.2 (d, C-6), 31.6 (t, C-7), 29.3 (d, C-8), 49.6 (d, C-9), 36.4 (s, C-10), 20.5 (t, C-11), 39.3 (t, C-12), 39.6 (s, C-13), 55.8 (d, C-14), 31.6 (t, C-15), 80.3 (d, C-16), 61.9 (d, C-17), 16.1 (q, C-18), 19.2 (q, C-19), 41.2 (d, C-20), 14.8 (q, C-21), 108.5 (s, C-22), 31.0 (t, C-23), 28.6 (t, C-24), 29.9 (d, C-25), 66.0 (t, C-26), 17.2 (q, C-27), Glu 101.2 (d, C-1), 73.2 (d, C-2), 75.8 (d, C-3), 71.7 (d, C-4), 75.4 (d, C-5), 169.6 (s, C-6), 51.9 (3H, q, O**C**H_3).

*Diosgenin 3-O-β-D-glucopyranosiduronic acid (**3**):* White amorphous powder. – Negative ESI-MS: *m/z* = 589.2 $[M-H]^-$. – ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.74 (3H, d, J = 4.3 Hz, H-27), 0.76 (3H, s, H-18), 0.91 (3H, d, J = 6.94 Hz, H-21), 0.98 (3H, s, H-19), 4.19 (1H, d, J = 7.79 Hz, H-1 of Glu), 5.33 (H, s, H-6). – ^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) = 36.9 (C-1), 31.1 (C-2), 77.5 (C-3), 38.3 (C-4), 140.5 (C-5), 121.0 (C-6), 31.6 (C-7), 29.3 (C-8), 49.6 (C-9), 36.4 (C-10), 20.2 (C-11), 39.3 (C-12), 39.6 (C-13), 55.8 (C-14), 31.5 (C-15), 80.3 (C-16), 61.7 (C-17), 16.1 (C-18), 19.1 (C-19), 41.1 (C-20), 14.6 (C-21), 108.5 (C-22), 31.0 (C-23), 28.5 (C-24), 29.8 (C-25), 66.0 (C-26), 17.1 (C-27), Glu 101.7 (C-1), 73.4 (C-2), 76.8 (C-3), 72.2 (C-4), 76.6 (C-5), 172.5 (C-6).

*Diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid (**4**):* White amorphous powder. – Positive ESI-MS: *m/z* = 782.2 $[M+2Na]^+$, 588.4 [M-rha], 413.2 [M-glu-rha]. – ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.72 (3H, d, *J* = 3.7 Hz, H-27), 0.73 (3H, s, H-18), 0.90 (3H, d, *J* = 6.84 Hz, H-21), 0.96 (3H, s, H-19), 1.08 (3H, d, *J* = 6.12 Hz, H-6 of Rha), 4.35 (1H, d, *J* = 7.80 Hz, H-1 of Glu), 5.03 (1H, s, H-1 of Rha), 5.33 (H, s, H-6). – ^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) = 36.8 (C-1), 29.0 (C-2), 76.4 (C-3), 37.6 (C-4), 140.5 (C-5), 121.3 (C-6), 31.6 (C-7), 31.1 (C-8), 49.7 (C-9), 36.5 (C-10), 20.5 (C-11), 39.7 (C-12), 39.7 (C-13), 55.8 (C-14), 31.6 (C-15), 80.3 (C-16), 61.9 (C-17), 16.2 (C-18), 19.1 (C-19), 41.2 (C-20), 14.8 (C-21), 108.5 (C-22), 31.1 (C-23), 28.6 (C-24), 29.9 (C-25), 66.0 (C-26), 17.2 (C-27), Glu 97.9 (C-1), 78.0 (C-2), 78.0 (C-3), 70.6 (C-4), 73.3 (C-5), 172.9 (C-6), Rha 100.2 (C-1), 72.5 (C-2), 72.7 (C-3), 73.9 (C-4), 68.0 (C-5), 17.9 (C-6).

*Diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuroniduronic acid methyl ester (**5**):* White amorphous powder. – IR: ν = 3319.3 (-OH), 2931.0, 1741.0 (-**CO**OMe), 1452.1 (>C=C<), 1382.3, 1047.2, 981.9, 921.7, 899.9 cm^{-1}. – Positive ESI-MS: *m/z* = 773.3 $[M+Na]^+$, 585.2 $[M-rha-H_2O]^+$, 413.2 [M-glu-rha]; negative ESI-MS: *m/z* = 785.2 $[M+Cl]^-$. – ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.73 (3H, d, *J* = 5.8 Hz, H-27), 0.74 (3H, s, H-18), 0.90 (3H, d, *J* = 6.84 Hz, H-21), 0.95 (3H, s, H-19), 1.08 (3H, d, *J* = 6.12 Hz, H-6 of Rha), 3.66 (3H, s, OC**H**$_3$), 4.55 (1H, d, *J* = 7.80 Hz, H-1 of Glu), 5.04 (1H, s, H-1 of Rha), 5.33 (H, s, H-6). – ^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) = 36.8 (C-1), 29.9 (C-2), 76.6 (C-3), 37.6 (C-4), 140.3 (C-5), 121.5 (C-6), 31.6 (C-7), 31.1 (C-8), 49.6 (C-9), 36.5 (C-10), 20.5 (C-11), 39.7 (C-12), 39.7 (C-13), 55.8 (C-14), 31.6 (C-15), 80.3 (C-16), 61.9 (C-17), 16.1 (C-18), 19.0 (C-19), 41.2 (C-20), 14.8 (C-21), 108.5 (C-22), 31.1 (C-23), 28.6 (C-24), 31.6 (C-25), 66.0 (C-26), 17.2 (C-27), Glu 98.5 (C-1), 76.0 (C-2), 76.9 (C-3), 71.9 (C-4), 75.1 (C-5), 169.6 (C-6), Rha 100.3 (C-1), 70.6 (C-2), 70.7 (C-3), 72.0 (C-4), 68.1 (C-5), 17.9 (C-6), 52.0 (3H, O**C**H$_3$).

Cell culture

HeLa (human uterine carcinoma) cell lines, A375-S2 (human malignant melanoma) cell lines, MCF7 (human breast cancer) cell lines, L929 (murine fibrosarcoma) cell lines, SGC-7901 (human gastric cancer) cell lines and Bel-7402 (human hepatocellular carcinoma) cell lines were obtained from American Type Culture Collection (ATCC, #CRL, 1872, MD, USA) and cultured in RPMI 1640 medium (GIBCO, USA) including L-glutamine with 10% FBS. All cells were maintained at 37 °C, 5% CO_2 in a humidified atmosphere incubator. The confluent cells were used for the cytotoxicity assay.

Cytotoxicity assay

The inhibition of the cellular growth was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Mosmann (1983).

Results and Discussion

The ethanol extract of *Solanum lyratum* had a high cytotoxic activity against A375-S2, SGC-7901 and Bel-7402 cells, a medium cytotoxic activity against HeLa and L929 cells and a low cytotoxic activity against MCF7 cells (Table I). Compared with the cytotoxic activity of the *n*-BuOH fraction,

Table I. The cytotoxic activity (IC_{50}, μg/ml) of extract/fraction against HeLa, A375-S2, SGC-7901, MCF7, L929, and Bel-7402 cell lines.

	IC_{50} [μg/ml][a]					
Extract/fraction	HeLa	A375-S2	SGC-7901	Bel-7402	L929	MCF7
Ethanol extract	320.1 ± 1.0	210.0 ± 1.4	176.1 ± 1.8	287.4 ± 0.9	460.5 ± 1.0	> 500
EtOAC fraction	20.9 ± 1.4	9.7 ± 1.6	65.4 ± 0.9	106.2 ± 1.1	76.5 ± 0.9	129.2 ± 0.8
n-BuOH fraction	64.4 ± 1.6	31.7 ± 1.2	114.8 ± 0.7	180.2 ± 1.0	357.7 ± 1.4	266.3 ± 0.5
Petroleum ether fraction	> 500	> 500	–	–	> 500	> 500
Aqueous fraction	> 500	> 500	–	–	> 500	> 500

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

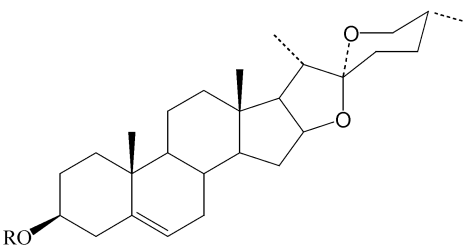
Table II. The cytotoxic activity (IC_{50}, μg/ml) of fractions E_1–E_{15} against HeLa and A375-S2 cell lines.

Fraction	IC_{50} [μg/ml][a]	
	HeLa	A375-S2
E_1	> 100	> 100
E_2	> 100	> 100
E_3	> 100	> 100
E_4	49.7 ± 1.4	38.9 ± 1.2
E_5	44.5 ± 0.9	29.8 ± 1.9
E_6	29.7 ± 1.0	15.0 ± 1.3
E_7	45.7 ± 0.9	27.3 ± 0.8
E_8	41.7 ± 0.7	32.3 ± 1.1
E_9	39.2 ± 1.1	16.5 ± 1.5
E_{10}	36.5 ± 1.2	43.7 ± 1.7
E_{11}	55.4 ± 0.6	52.8 ± 0.9
E_{12}	55.3 ± 0.9	43.7 ± 1.0
E_{13}	42.0 ± 0.8	28.1 ± 1.2
E_{14}	60.0 ± 1.0	42.3 ± 0.9
E_{15}	42.1 ± 1.8	30.3 ± 0.9

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

the EtOAC fraction showed stronger cytotoxic activity. As for 15 fractions of the EtOAC fraction, fractions E_1–E_3, which had low polarity, showed no cytotoxic activity, and fractions E_4–E_{15} exhibited significant dose-dependent cytotoxic activity for HeLa and A375-S2 cells (Table II).

Bioassay-directed fractionation of the EtOAC fraction from *Solanum lyratum* led to the isolation of five compounds. The structures (Fig. 1) were identified as diosgenin (**1**), diosgenin 3-*O*-β-D-glucopyranosiduronic acid methyl ester (**2**), diosgenin 3-*O*-β-D-glucopyranosiduronic acid (**3**), diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid (**4**) and diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuroniduronic acid methyl ester (**5**). Compound **2** was a new compound, and compounds **1** and **3** were isolated for the first time from this plant. Compound **3** had been prepared by a standardized, simplified Koenigs-Knorr synthesis (Schneider, 1971), and was isolated as a new natural product in the present study. Compounds **2** and **5** were the methyl esters of compounds **3** and **4**, respectively. It was also confirmed by TLC that compounds **2** ~ **5** were the intact constituents in the plant.



	R
1	H
2	-^{1}GlcUA methyl ester
3	-^{1}GlcUA
4	-^{1}GlcUA^2-^{1}Rha
5	-^{1}GlcUA methyl ester^2-^{1}Rha

Fig. 1. The structure of the isolated compounds: diosgenin (**1**), diosgenin 3-*O*-β-D-glucopyranosiduronic acid methyl ester (**2**), diosgenin 3-*O*-β-D-glucopyranosiduronic acid (**3**), diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid (**4**), and diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuroniduronic acid methyl ester (**5**).

Compound **2** was obtained as white amorphous powder. The molecular formula $C_{34}H_{52}O_9$ was determined by positive ESI-MS (at *m/z* 605.3 $[M+H]^+$, 627.4 $[M+Na]^+$) and negative ESI-MS (at *m/z* 639.1 $[M+Cl]^-$), and from ^{13}C and ^{13}C distortionless enhancement by polarization transfer (DEPT) NMR spectral data. The ^1H NMR spectrum of compound **2** exhibited signals characteristic for two methyl singlets at δ 0.73 (s, CH_3-18), 0.96 (s, CH_3-19), two methyl doublets at δ 0.73 (CH_3-27), 0.90 (d, J = 6.88 Hz, CH_3-21), one trisubstituted olefinic proton at δ 5.34 and one anomeric proton at δ 4.38. The ^{13}C NMR spectrum of **2** showed signals of a pair of olefinic carbon atoms at δ 121.2 (C-6) and 140.4 (C-5), one anomeric carbon atom of sugar at δ 101.2, and a carboxyl carbon atom at δ 169.6 (Glu C-6). It was also possible to demonstrate a spiroketal ring system through the presence of carbon quaternary signals at δ_c 108.5 (C-22), -OCH_2 group at δ_c 66.0 (C-26), and OCH group at δ_c 80.3 (C-16). Careful comparison of ^1H NMR and ^{13}C NMR spectral data of **2** with **1** allowed to establish compound **2** as diosgenin [(25*R*)-spirost-5-en-3β-ol] glycoside with a sugar attached at C-3 position. An IR spectrum also confirmed the presence of a C=C (1451.5 cm^{-1}), and the stereochemistry of the spiroketal [intensity at 899.3 > 921.7 cm^{-1} indicated 25(*R*)-stereochemistry] (Asami *et al.*, 1991). The sugar was identified as glucuronic acid by co-TLC with authentic samples after acid hydrolysis. The chemical shifts of the sugar moiety in the ^{13}C NMR spectrum also confirmed the presence of one glucuronic acid. The anomeric configuration of the sugar was determined to be β for glucuronic acid on the basis of the $J_{H\text{-}H}$ values (7.79 Hz). The

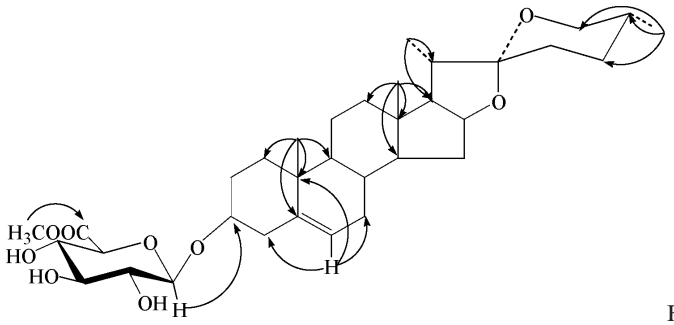


Fig. 2. The key HMBC correlations of compound **2**.

HMBC correlation (Fig. 2) was observed between a proton signal at δ 3.65 (-OC**H**$_3$) and a carbon signal at δ 169.6. All this confirmed the fact that compound **2** was diosgenin with a glucuronic methyl ester at C-3. From the above evidences, the structure of **2** was concluded to be diosgenin 3-*O*-β-D-glucopyranosiduronic acid methyl ester. It has not been reported yet, and it is a new compound.

Compound **3** was obtained as white amorphous powder. ESI-MS (negative mode) of compound **3** exhibited ions at *m/z* 589.2 $[M-H]^-$. Careful comparison of NMR spectral data of **3** with that of **2** showed that **3** has the same structure as **2**, and that **3** differs structurally from **2** only by the elimination of -O**CH**$_3$ group signal in the sugar moiety. This assumption was confirmed by HMBC, HMQC and ^{1}H,^{1}H-COSY experiments. From the above evidences, the structure of **3** was concluded to be diosgenin 3-*O*-β-D-glucopyranosiduronic acid. Compound **3** has been prepared by a standardized, simplified Koenigs-Knorr synthesis (Schneider, 1971), and was isolated as a new natural product in the present study.

Compounds **1**, **4** and **5** were identified by comparison of their spectral data with the literature data (Agrawal *et al.*, 1985; Yahara *et al.*, 1986).

The cytotoxic activity of the isolated compounds was determined against HeLa, A375-S2, SGC-7901 and Bel-7402 cells. The results are summarized in Table III. Compound **1** showed significant cytotoxic activities. It was reported that diosgenin induced apoptosis in HeLa cells via activation of the caspase pathway (Hou *et al.*, 2004). Compounds **2** and **3** exhibited no cytotoxic activities. Compounds **4** and **5** exhibited moderated cytotoxic activities against HeLa, A375-S2, SGC-7901 and Bel-7402 cells in a concentration-dependent manner. Their cytotoxicity is reported for the first time.

Acknowledgement

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Table III. The cytotoxic activity (IC_{50}, μg/ml) of the isolated compounds against HeLa, A375-S2, SGC-7901, and Bel-7402 cell lines.

	IC_{50} [μg/ml][a]			
Compound	HeLa	A375-S2	SGC-7901	Bel-7402
1	16.4 ± 1.3	8.2 ± 0.6	21.3 ± 0.9	20.1 ± 1.5
2	> 50	> 50	> 50	> 50
3	> 50	> 50	> 50	> 50
4	18.2 ± 1.6	12.2 ± 0.8	30.5 ± 0.8	35.6 ± 1.2
5	19.8 ± 1.3	16.2 ± 0.6	37.2 ± 1.1	39.2 ± 0.9
5-Fu[b]	17.5 ± 1.4	15.0 ± 0.7	3.9 ± 1.0	14.1 ± 0.4

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.
[b] 5-Fu (5-fluorouracil) was used as a positive control.

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