A Cytotoxic Triterpene Saponin from the Root Bark of *Aralia dasyphylla*

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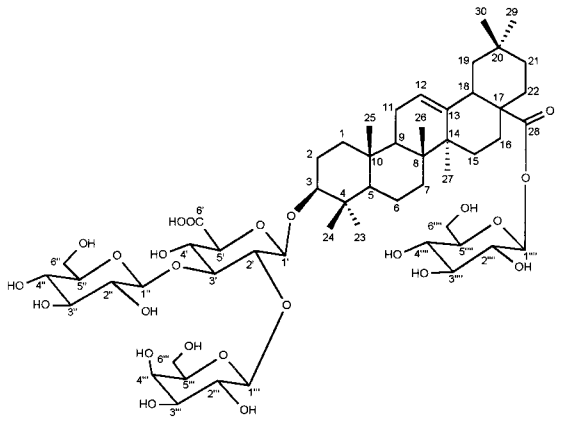
A novel triterpene saponin (**1**) was isolated from an ethanol extract of the root bark of *Aralia dasyphylla*. Its structure was elucidated as 3-*O*-[*β*-D-glucopyranosyl (1→3)-*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside, according to spectral and chemical evidence. Compound **1** showed significant cytotoxic activity against KB and Hela-S_3 cells.

Aralia dasyphylla Miq. (Araliaceae) has been used as folk medicine for hepatitis and diabetes and as a tonic. Its tender buds are edible.[1] No phytochemical work, however, has been performed on this plant to date. This paper deals with the isolation and structure elucidation of a novel oleanolic acid-based saponin (**1**) and its cytotoxic activity against two human cancer cell lines.



Compound **1** was obtained as white amorphous powder. Its FABMS showed an $[M + Na + H]^+$ ion at *m*/*z* 1142, indicating a molecular weight of 1118, compatible with a molecular formula of $C_{54}H_{86}O_{24}$, as determined from elemental analysis. Compound **1** showed positive results in the Liebermann−Burchard and Molisch reactions, which suggested that **1** was a triterpene saponin. On acid hydrolysis, **1** liberated oleanolic acid as the genin, which was identified with an authentic sample by co-TLC, IR, and NMR. In addition, **1** also afforded D-glucose and D-galactose on acid hydrolysis (in a molar ratio of 2:1 as estimated by GC analysis after conversion into their thiazolidine derivatives) and D-glucuronic acid (identified by co-TLC and paper chromatography with an authentic sample). The configuration of the glucuronic acid moiety was assumed to be that most commonly encountered among the plant glycosides. On alkaline hydrolysis, only glucose was detected by co-TLC with an authentic sample, indicating that the glucose was bound to the genin by a glycosidic ester linkage at C-28.[2]

The ^1H NMR and ^{13}C NMR spectra of **1**, which are presented in Table 1, showed that most of the signals of the aglycon were in good agreement with literature data for oleanolic acid.[3] Glycosylation shifts were observed at C-3 and C-28 of the aglycon, indicating that the saccharide units were attached at these two positions (i.e., signals at *δ* 89.8 and 176.2 represented a downfield shift by 10 ppm and an upfield shift by 3 ppm, respectively, when compared with the analogous data for oleanolic acid). Compound **1** was shown to contain four sugar residues in a HMQC NMR experiment, which revealed the correlations between anomeric carbons in the *δ* 105−93 range and anomeric proton signals resonating between *δ* 4.0 and 6.1. Thus, the anomeric ^{13}C NMR signals at *δ* 103.2, 102.5, 101.2, and 94.8 gave cross-peaks with anomeric protons at *δ* 4.79 (d, $J = 8.0$ Hz), 5.28 (d, $J = 7.6$ Hz), 5.54 (d, $J = 8.0$ Hz), and 6.01 (d, $J = 7.9$ Hz), respectively. The sugar moieties of **1** were assigned mainly from the $^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC NMR spectra obtained. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one galactopyranose (gal) unit with the anomeric proton at *δ* 5.28, and the H-4 signal was a singlet in the HOHAHA NMR spectrum, which is characteristic of galactose.[4,5] Two glucopyranose (glc) units were observed with anomeric protons resonating at *δ* 6.01 and 5.54, respectively, with the former linked to the carboxylic group of the aglycon through an ester linkage and the latter being linked to C-3 of a glucuronic acid (glcA) substituent. The sequence of the sugar moieties in **1** was determined from the HMBC and NOESY NMR spectra. In the HMBC spectrum (Table 1), long-range $^{13}C-^1H$ correlations were observed between the signals at δ_C 176.2 and δ_H 6.01, δ_C 89.8 and δ_H 4.79, δ_C 83.9 and δ_H 5.54, and δ_C 76.7 and δ_H 5.28. Accordingly, in turn, the glucuronic acid unit was linked to C-3 of the aglycon, while the galactose and one of the glucose (*δ* 5.54) units were linked to C-2 and C-3 positions of the glucuronic acid unit, respectively. The other glucose unit (*δ* 6.01) was linked to C-28 of the aglycon. These linkages were confirmed from the NOESY spectrum of **1** (Table 1): correlations between glcA H-1/aglycon H-3 (*δ* 3.11), glc H-1 (*δ* 5.54)/ glcA H-3 (*δ* 4.44), and gal H-1/ glcA H-1 were observed. All the sugar units were *β* according to their anomeric proton coupling constants.[6] The accumulated evidence described above indicated that the structure of **1** was 3-*O*-[*β*-D-glucopyranosyl (1→3)-*β*-D-galactopyranosyl (1→2)]-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside.

Compound **1** was evaluated for cytotoxic activity against two cultured human cancer cell lines (KB and Hela-S_3 cells) using established protocols.[7] The IC_{50} values were 1.2 *μ*g/mL and 0.02 *μ*g/mL, respectively. Positive controls were 5-fluorouracil (5FU) and cytosine arabinoside, which exhibited IC_{50} values of 0.93 (KB), 0.44 *μ*g/mL (Hela-S_3), 0.81 (KB), and 0.23 *μ*g/mL(Hela-S_3), respectively.

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Table 1. ^{1}H and ^{13}C NMR Data and NOESY and HMBC Correlation of Compound **1** in $C_5D_5N + D_2O$

aglycon moiety					sugar moiety				
position	proton	carbon	NOESY	HMBC	position	proton	carbon	NOESY	HMBC
1	*a*	37.3			3-glcA				
2	*a*	26.4		H-3	1′	4.79 d (8.0)	103.2	H-3/H-1‴	H-3/H-2′/H-3′
3	3.11 m	89.8	H-1′/H-23	H-1′/H-23/H-24	2′	4.39[b]	76.7	H-1‴/H-4′	H-1‴/H-3′
4		38.4			3′	4.44[b]	83.9	H-1″/H-1′/H-5′	H-2′/H-1″
5	*a*	54.5			4′	4.19 dd (6.7, 9.0)	68.6	H-2′	H-2′/H-3′
6	*a*	17.2			5′	4.31 d (9.0)	76.2	H-1′/H-3′	H-6′/H-3′
7	*a*	31.1			6′		174.4		H-4′/H-5′
8		38.4		H-27	glc at C-3 of glcA				
9	*a*	46.5		H-12	1″	5.54 d (8.0)	101.2	H-3′/H-3″/H-5″	H-3′/H-2″/H-3″
10		35.5			2″	3.85 dd (8.0, 8.5)	74.3	H-4″	H-1″/H-3″
11	*a*	22.3			3″	4.18 dd (8.5, 8.7)	76.3	H-1″/H-5″	H-2″
12	5.33 m	121.6	H-18/H-25/H-26	H-18	4″	3.75 dd (8.7, 9.0)	70.5	H-2″	H-2″/H-5″
13		142.8		H-18	5″	4.01[b]	75.4	H-1″/H-3″	H-6″
14		40.4		H-18/H-12/H-27	6″	4.0, 4.4[a]	61.5		H-4″/H-5″
15	*a*	26.9		H-27	gal at C-2 of glcA				
16	*a*	22.3			1‴	5.28 d (7.6)	102.5	H-1′/H-2′/H-3‴/H-5‴	H-2′/H-3‴
17		45.9		H-18	2‴	4.31 dd (7.6, 9.0)	71.1		H-3‴
18	3.03 m	40.8	H-12/H-26/H-30		3‴	4.07 dd (9.0, 1.8)	73.0	H-1⁗	H-1‴/H-2‴/H-4‴
19	*a*	45.0		H-29	4‴	4.22[b]	70.6		H-2‴
20		29.3		H-29	5‴	3.85[b]	74.3		H-3‴
21	*a*	32.6		H-29	6‴	*a*	60.8		
22	*a*	31.8			glc at C-28				
23	1.05 s	26.9	H-3/H-27	H-3	1⁗	6.01 d (7.9)	94.8	H-3⁗/H-5⁗	H-2⁗/H-3⁗
24	0.93 s	15.2		H-3	2⁗	4.10 dd (7.9, 8.4)	72.0	H-4⁗	H-1⁗/H-3⁗
25	0.93 s	14.1			3⁗	4.20 dd (8.4, 8.0)	77.1	H-1⁗/H-5⁗	H-1⁗/H-2⁗
26	0.82 s	16.1			4⁗	4.14 dd (8.0, 8.8)	69.3	H-2⁗	H-2⁗/H-5⁗
27	1.16 s	24.8			5⁗	3.93[b]	76.7	H-1⁗/H-3⁗	H-6⁗
28		176.2		H-1⁗	6⁗	4.1, 4.3[a]	60.4		H-5⁗
29	0.70 s	31.8							
30	0.87 s	21.9	H-12/H-18						

[a] Obscured by other signals: chemical shifts could not be accurately determined. [b] Obscured by other signals: coupling constants could not be accurately determined.

Experimental Section

General Experimental Procedures. The melting point was determined on a micromelting point apparatus and is uncorrected. The optical rotation was measured on a Perkin−Elmer 241 automatic digital polarimeter. The IR spectrum was obtained on a Hitachi 275−50 IR spectrometer. NMR spectra were obtained on a Bruker AMX-400 spectrometer (^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz) in $C_5D_5N + D_2O$ with TMS as an internal standard. FABMS was obtained with a Varian-MAT 212 instrument. Semipreparative HPLC was performed on a Waters PAD 996 instrument. Gas chromatography (GC) was run on a Hitachi G-3000 gas chromatograph.

Plant Material. *Aralia dasyphylla* Miq. was collected from Xupu in Hunan Province, People's Republic of China, in July 1995. The plant was identified by Dr. Zhong-Zhuang Wang, School of Pharmacy, Second Military Medical University, Shanghai. A voucher specimen (No.C005) is deposited at the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The root bark of the plant (1.8 kg) was extracted with 70% EtOH under reflux. The extract was evaporated to generate an aqueous suspension. The suspension was partitioned with petroleum ether, $CHCl_3$, and *n*-BuOH (saturated with H_2O), successively. The *n*-BuOH layer was evaporated to dryness, yielding a brown mass (80 g), and it was chromatographed on a dry column of silica gel and eluted with $CHCl_3$−MeOH−H_2O (80:20:10), increasing the proportion of MeOH to $CHCl_3$−MeOH−H_2O (65:35:10), to give three fractions. The saponin fraction (fraction 3) was rechromatographed on silica gel using the solvent system $CHCl_3$-MeOH−H_2O (65:35:10) and purified by HPLC on RP-18 reversed-phase material [Bondpak C_{18}, Waters, 10−20 μm, 7.5 × 380 mm, eluted with MeOH−H_2O (1:1)] to give **1** (50 mg, 0.0028%).

Compound **1** was obtained as white amorphous powder; Liebermann−Burchard reaction, reddish purple; Molisch reaction, purple; mp188−190 °C (dec); $[\alpha]^{20}_D$ +15.8° (*c* 9.2 H_2O); IR (KBr) ν_{max} 3450 (OH), 2960 (CH_3), 1760 (COOR), 1630 (C=C), 1080 (CHOH), 1030 (CH_2OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; FABMS *m/z* $[M + Na + H]^+$ 1142; *anal.* C 56.98%, H 7.79%, calcd for $C_{54}H_{86}O_{24}\cdot H_2O$, C 57.04%, H 7.75%.

Acidic Hydrolysis of 1. A solution of **1** (5 mg) and 7% HCl−EtOH (3:7) was refluxed for 4 h. The mixture was diluted with H_2O and extracted with Et_2O. The Et_2O layer was evaporated to dryness. The residue was recrystallized in EtOH to afford oleanolic acid, which was compared with an authentic sample (co-TLC, IR, NMR). The NMR data were in good agreement with literature values.[3] A sample of the aqueous layer was neutralized with 1 N NaOH and was then subjected to TLC analysis on Kieselgel 60 F_{254} (Merck) [using $CHCl_3$−MeOH−H_2O (30:12:4), 9 mL and HOAc, 1 mL] and paper chromatography [using *n*-BuOH−HOAc−H_2O (4:1:5)] with standard sugars, in which the presence of glucose, glucuronic acid, and galactose was established. The aqueous layer was then passed through an Amberlite IRA-60E column. The aqueous eluate was concentrated and derivatized with thiazolidine as described previously.[8] Monosaccharides were detected by GC: D-glc and D-gal in the ratio 2:1. GC conditions: column, Supelco SPB-1, 0.25 mm × 27 m, column temperature 230 °C; carrier gas, N_2; t_R, D-glc 17.8 min, L-glc 17.2 min; D-gal 19.5 min, L-gal 18.3 min.

Alkaline Hydrolysis of 1. Compound **1** (6 mg) in 70% EtOH and 2% KOH was refluxed for 6 h. After slow neutralization with 0.1 N HCl, the reaction mixture was concentrated under vacuum, and the residue was subjected to co-TLC and paper chromatographic analysis by the same method as described in acid hydrolysis. Glucose was detected.

Cytotoxic Assay. Compound **1** was evaluated for cytotoxic activity using a previously described protocol.[7]

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