Biologically Active Triterpenoid Saponins from *Ardisia japonica*

Xiaolong Chang,[†] Wei Li,[†] Zhonghua Jia,[†] Tadaaki Satou,[†] Shinji Fushiya,[‡,§] and Kazuo Koike*[,†]

Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan, and Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-8578, Japan

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Eleven new triterpenoid saponins, ardisianosides A (**1**), B (**2**), C (**4**), D (**5**), E (**6**), F (**7**), G (**15**), H (**16**), I (**17**), J (**18**), and K (**19**), together with 10 known saponins, were isolated from the whole plants of *Ardisia japonica*. The structures of the new saponins were established on the basis of extensive 1D and 2D NMR and MS studies coupled with chemical degradations. The cytotoxic activities of saponins **1**−**21** are reported against three human cancer cell lines, namely, HL-60 myeloid leukemia, KATO-III stomach adenocarcinoma, and A_{549} lung adenocarcinoma cells.

Ardisia japonica (Thunb.) Bl. (Myrsinaceae) is a widely occurring shrub in Southeast Asia and North America. Its roots have been used traditionally in the treatment of coughs and uterine bleeding and showed significant anti-HIV effects.[1] Previous chemical investigation on the whole plants of *A. japonica* has demonstrated the occurrence of benzoquinones, phenols, flavonoids, chromones, triterpenes, and triterpene saponins.[1] To date, four triterpenoid saponins have been reported from this plant.[1c,d] As a part of an ongoing investigation on medicinal plants in the genus *Ardisia*[2] and an interest in the chemistry of bioactive triterpenoid saponins,[3] we have carried out a chemical investigation on the whole plants of *A. japonica*, resulting in the isolation of 21 triterpenoid saponins, including 11 new triterpenoid saponins, ardisianosides A, B (**1**, **2**), C−F (**4**−**7**), and G−K (**15**−**19**). In this paper, we report the isolation and structure elucidation of these new triterpenoid saponins, along with the cytotoxic activities of these 21 saponins (**1**−**21**) against three human cancer cell lines: HL-60 myeloid leukemia, KATO-III stomach adenocarcinoma, and A_{549} lung adenocarcinoma.

Results and Discussion

A methanolic extract of the whole plants of *A. japonica* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction was subjected to passage over a Diaion HP-20 column, followed by washing with MeOH and H_2O mixtures in different ratios. Further purification of the 70% and 100% MeOH fractions using combinations of silica gel column chromatography, ODS column chromatography, and preparative HPLC afforded 21 triterpenoid saponins, namely, 11 new compounds, ardisianosides A (**1**), B (**2**), C (**4**), D (**5**), E (**6**), F (**7**), G (**15**), H (**16**), I (**17**), J (**18**), and K (**19**), and 10 known saponins (**3**, **8**−**14**, **20**, and **21**).

Ardisianoside A (**1**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −15.5 (*c* 1.0, MeOH). Its molecular formula, $C_{70}H_{116}O_{36}$, was determined from the positive-ion HRFABMS (*m*/*z* 1555.7100, $[M + Na]^+$). The positive-ion ESIMS showed a $[M + Na]^+$ ion peak at *m*/*z* 1555 and fragment peaks at *m*/*z* 1401 $[M + H - C_5H_8O_4]^+$ (due to the loss of a pentose unit), *m*/*z* 1371 $[M + H - C_6H_{10}O_5]^+$ (due to the loss of a hexose unit), and *m*/*z* 457 $[M + H - C_{40}H_{66}O_{33}]^+$ (due to the aglycon moiety). Compound **1** displayed 70 carbon signals in its ^{13}C NMR spectrum, of which 30 could be assigned to the signals of the aglycon. The ^{13}C NMR spectrum exhibited seven sp^3 carbon signals at δ 16.5, 16.7, 18.6, 19.6, 24.8, 28.1, and 33.8 and signals for an oxygenated methylene at δ 77.8 and a quaternary carbon at δ 86.5. These data, when coupled with information from the ^1H NMR spectrum [seven methyl singlets at δ 0.87, 0.99, 1.06, 1.09, 1.19, 1.35, and 1.54 and a pair of oxygenated methylene protons at δ 3.32 and 3.61 (each, d, $J = 7.4$ Hz)], indicated that the aglycon of **1** is based on an 13,28-epoxyoleanane skeleton.[4] Further, in the ^1H NMR spectrum, two carbinylic proton signals assignable to H-3 and H-16 of the aglycon were observed at δ 3.13 (dd, $J = 11.6$, 4.5 Hz) and 4.21 (brs), suggesting the carbinylic protons could be placed at 3α and 16β, respectively.[4] Thus, the aglycon was identified as 13,28-epoxy-3β,16α-dihydroxyoleanane (protoprimulagenin A).[5] On acid hydrolysis, **1** afforded the component sugars as L-arabinose, D-xylose, and D-glucose in a ratio of 1:1:5, which were identified by gas−liquid chromatographic (GLC) analysis of their trimethylsilyl D-cysteine derivatives.[6] The ^1H NMR spectrum showed seven anomeric proton signals at δ 5.45 (d, $J = 7.8$ Hz, GlcI-H-1), 5.36 (d, $J = 7.2$ Hz, GlcIV-H-1), 5.34 (d, $J = 7.3$ Hz, GlcV-H-1), 5.20 (d, $J = 7.8$ Hz, GlcIII-H-1), 5.14 (d, $J = 6.9$ Hz, Xyl-H-1), 4.94 (d, $J = 7.6$ Hz, GlcII-H-1), and 4.83 (d, $J = 5.2$ Hz, Ara-H-1). All proton signals due to sugars were assigned by careful analysis of the DQF-COSY, TOCSY, and NOESY spectra, and the carbon signals were assigned by HMQC and HMQC-TOCSY spectra. The β-anomeric configurations for the glucopyranose and xylopyranose units were determined from their $^3J_{H1,H2}$ coupling constants (6.9−7.8 Hz). The arabinopyranose unit was determined to have an α-configuration on the basis of the $^3J_{H1,H2}$ value (5.2 Hz) and the correlation between H-1 and H-3 and between H-1 and H-5 in the NOESY experiment observed for the $^4C_1$ form.[8] When the ^{13}C NMR data of **1** were compared with those of protoprimulagenin A,[5] a glycosylation shift was observed at C-3 (+10.1 ppm), suggesting that **1** is a monodesmosidic glycoside. The arabinose was connected to C-3 of the aglycon, which was deduced from the HMBC correlation between δ_H 4.83 (Ara-H-1) and δ_C 89.1 (C-3). The sequence of the sugar chain at C-3 was further determined by analysis of the HMBC and NOESY NMR spectra. Thus, HMBC correlations were observed between δ_H 5.45 (GlcI-H-1) and δ_C 79.3 (Ara-C-2), δ_H 4.92 (GlcII-H-1) and δ_C 77.7 (Ara-C-4), δ_H 5.12 (Xyl-H-1) and δ_C 81.3 (GlcII-C-2), δ_H 5.19 (GlcIII-H-1) and δ_C 86.9 (GlcII-C-3), δ_H 5.35 (GlcIV-H-1) and δ_C 81.5 (GlcIII-C-3), and δ_H 5.33 (GlcV-H-1) and δ_C 74.0 (GlcIII-C-4). The NOESY correlations were observed between δ_H 5.45 (GlcI-H-1) and δ_H 4.58 (Ara-H-2), δ_H 4.92 (GlcII-H-1) and δ_H 4.29 (Ara-H-4), δ_H 5.12 (Xyl-H-1) and δ_H 4.10 (GlcII-H-2), δ_H 5.19 (GlcIII-H-1) and δ_H 4.00 (GlcII-H-3), δ_H 5.35 (GlcIV-H-1) and δ_H 4.51 (GlcIII-H-3), and δ_H 5.33 (GlcV-H-1) and δ_H 4.49 (GlcIII-H-4). On the basis of the above results, the structure of ardisianoside A (**1**) was concluded to be 3β-*O*-β-D-glucopyranosyl-(1→2)-{β-D-xylopyranosyl-(1→2)-{β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-glu-

* Corresponding author. Tel: +81-47-4721396. Fax: +81-47-4721404. E-mail: koike@phar.toho-u.ac.jp.
[†] Toho University.
[‡] Tohoku University.
[§] Current address: Nihon Pharmaceutical University, Komuro 10281, Ina-machi, Kita-Adachi-gun, Saitama 362-0806, Japan.

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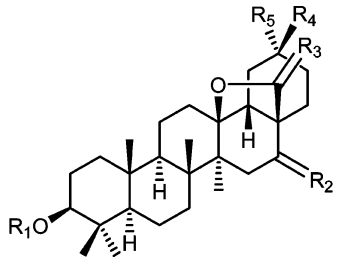
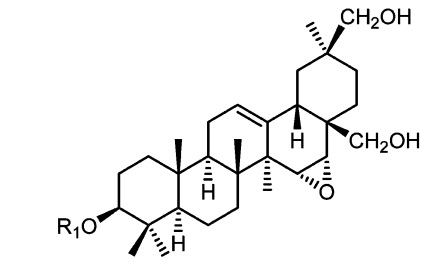
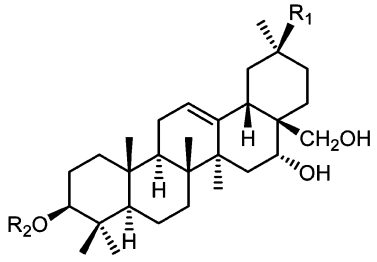
Chart 1



	R_1	R_2	R_3	R_4	R_5
1	S_1	α-OH, β-H	$=H_2$	CH_3	CH_3
2	S_2	α-OH, β-H	$=H_2$	CH_3	CH_3
3	S_4	α-OH, β-H	$=H_2$	CH_3	CH_3
4	S_5	α-OH, β-H	$=H_2$	CH_3	CH_3
5	S_6	α-OH, β-H	$=H_2$	CH_3	CH_3
6	S_7	α-OH, β-H	$=H_2$	CH_2OH	CH_3
7	S_3	α-OH, β-H	$=H_2$	CH_2OH	CH_3
8	S_4	α-OH, β-H	$=H_2$	CH_2OH	CH_3
9	S_3	α-OH, β-H	$=H_2$	CHO	CH_3
10	S_4	α-OH, β-H	$=H_2$	CHO	CH_3
11	S_5	α-OH, β-H	$=H_2$	CHO	CH_3
12	S_6	α-OH, β-H	$=H_2$	CHO	CH_3
13	S_5	=O	$=H_2$	CHO	CH_3
14	S_4	α-OH, β-H	$=H_2$	COOH	CH_3
15	S_4	α-OH, β-H	$=H_2$	OH	CH_3
16	S_1	α-OH, β-H	=O	CH_3	CH_3
17	S_4	α-OH, β-H	=O	CH_2OH	CH_3
18	S_4	α-OH, β-H	=O	CHO	CH_3
22	S_4	α-OH, β-H	$=H_2$	$(OCH_3)_2$	CH_3
23	S_4	α-OH, β-H	$=H_2$	CH_3	COOH
24	S_8	α-OH, β-H	$=H_2$	CHO	CH_3

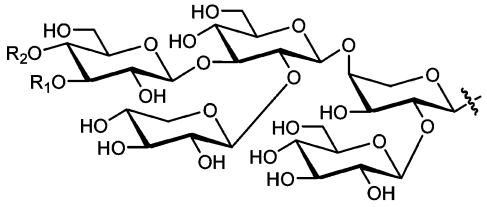


19 $R_1 = S_4$

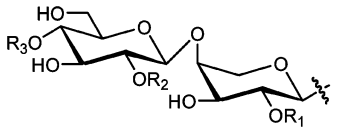


20 $R_1 = CH_2OH$ $R_2 = S_4$
21 $R_1 = COOH$ $R_2 = S_4$

sugar chain



	R_1	R_2
S_1	β-D-glucopyranosyl	β-D-glucopyranosyl
S_2	β-D-glucopyranosyl	H
S_3	H	H



	R_1	R_2	R_3
S_4	β-D-glucopyranosyl	α-L-rhamnopyranosyl	H
S_5	H	α-L-rhamnopyranosyl	H
S_6	H	β-D-xylopyranosyl	H
S_7	β-D-glucopyranosyl	H	H
S_8	β-D-glucopyranosyl	α-L-rhamnopyranosyl	β-D-xylopyranosyl

copyranosyl-(1→3)}-*β*-D-glucopyranosyl-(1→4)}-α-L-arabinopyranosyl-13*β*,28-epoxy-16α-hydroxyoleanane.

Ardisianoside B (**2**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −15.7 (*c* 1.0, MeOH). Its molecular formula, $C_{64}H_{106}O_{31}$, was determined from the positive-ion HRFABMS (*m*/*z* 1393.6564, $[M + Na]^+$). Acid hydrolysis afforded L-arabinose, D-xylose, and D-glucose in a ratio of 1:1:4. The ^1H and ^{13}C NMR data assignable to the aglycon moiety of **2** were identical to those of **1** (Tables 1 and 3), suggesting the aglycon also to be protoprimulagenin A. Further comparison of the ^1H and ^{13}C NMR data assignable to the sugar chain between **1** and **2** (Tables 2 and 4) suggested the absence of one set of terminal *β*-D-glucopyranose moiety signals (Glc-V) at GlcIII-C-4 in **2** that was present in **1**. The sequence of the sugar chain was confirmed using HMBC and NOESY correlations. Thus, HMBC correlations were observed between δ_H 5.44 (GlcI-H-1) and δ_C 79.4 (Ara-C-2), δ_H 4.92 (GlcII-H-1) and δ_C 78.0 (Ara-C-4), δ_H 5.14 (Xyl-H-1) and δ_C 81.2 (GlcII-C-2), δ_H 5.22 (GlcIII-H-1) and δ_C 87.7 (GlcII-C-3), and δ_H 5.20 (GlcIV-H-1) and δ_C 87.7 (GlcIII-C-3). NOESY correlations were observed between δ_H 5.44 (GlcI-H-1) and δ_H 4.56 (Ara-H-2), δ_H 4.92 (GlcII-H-1) and δ_H 4.26 (Ara-H-4), δ_H 5.14 (Xyl-H-1) and δ_H 4.06 (GlcII-H-2), δ_H 5.22 (GlcIII-H-1) and δ_H 4.09 (GlcII-H-3), and δ_H 5.20 (GlcIV-H-1) and δ_H 4.23 (GlcIII-H-3). On the basis of the above results, the structure of ardisianoside B (**2**) was elucidated as 3*β*-*O*-*β*-D-glucopyranosyl-(1→2)-{*β*-D-xylopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)}-α-L-arabinopyranosyl-13*β*,28-epoxy-16α-hydroxyoleanane.

Ardisianoside C (**4**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −21.3 (*c* 1.0, MeOH). Its molecular formula, $C_{47}H_{78}O_{16}$, was determined from the positive-ion HRFABMS (*m*/*z* 921.5166, $[M + Na]^+$). The ^1H and ^{13}C NMR data revealed that **4** is a monodesmosidic glycoside with the same aglycon as that in **1** and **2**. On acid hydrolysis, **4** afforded L-arabinose, L-rhamnose, and D-glucose in the ratio 1:1:1. For the rhamnopyranose moiety, the small $^3J_{H1,H2}$ coupling constant (1.2 Hz) of the anomeric proton and the three-bond HMBC correlations from the anomeric proton to C-3 and C-5 of the rhamnose indicated that the anomeric proton is equatorial, thus possessing an α-configuration in the $^1C_4$ form.[8] Compound **4** showed identical ^1H and ^{13}C NMR data for the sugar moieties as 3-*O*-(α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-α-L-arabinopynanosyl) cyclamiretin A (**11**),[9] suggesting the same sugar sequences occur in both **4** and **11**. This conclusion was confirmed by HMBC correlations between δ_H 4.75 (Ara-H-1) and δ_C 89.1 (C-3), δ_H 5.14 (GlcI-H-1) and δ_C 74.6 (Ara-C-4), and δ_H 6.23 (Rha-H-1) and δ_C 78.2 (GlcI-H-2). Thus, the structure of ardisianoside C (**4**) was elucidated as 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl-13*β*,28-epoxy-16α-hydroxyoleanane.

Ardisianoside D (**5**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −46.5 (*c* 0.9, MeOH). Its molecular formula, $C_{46}H_{76}O_{16}$, was determined from the positive-ion HRFABMS (*m*/*z* 907.5027, $[M + Na]^+$). On acid hydrolysis, **5** afforded L-arabinose, D-xylose, and D-glucose in a ratio of 1:1:1. Its ^1H and ^{13}C NMR data were similar with those of **4** except that the α-L-rhamnose moiety in **4** was replaced by a *β*-D-xylose unit in **5**. This conclusion was also confirmed by the HMBC correlation between δ_H 4.91 (Xyl-H-1) and δ_C 86.3 (GlcI-H-2). Thus, the structure of ardisianoside D (**5**) was elucidated as 3*β*-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl-13*β*,28-epoxy-16α-hydroxyoleanane.

Table 1. ^{13}C NMR Spectroscopic Data (δ) of the Aglycon Moieties of Compounds **1**, **2**, **4**−**7**, and **15**−**19** (125 MHz in pyridine-d_5)[a]

position	1	2	4	5	6	7	15	16	17	18	19
1	39.2	39.2	39.3	39.4	39.3	39.2	39.3	39.1	39.1	39.1	38.6
2	26.5	26.5	26.7	26.9	26.6	26.5	26.6	26.5	26.5	26.5	26.4
3	89.1	89.1	89.1	88.9	89.0	89.1	89.3	88.9	89.0	89.0	89.1
4	39.7	39.7	39.7	39.8	39.7	39.7	39.7	39.7	39.6	39.6	39.5
5	55.7	55.8	55.8	55.8	55.7	55.7	55.7	55.6	55.5	55.5	55.6
6	18.0	18.0	18.0	18.0	18.0	18.0	18.0	17.9	17.9	17.9	18.5
7	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.1	34.0	34.0	32.6
8	42.5	42.5	42.5	42.5	42.5	42.6	42.5	42.2	42.2	42.3	39.5
9	50.6	50.6	50.6	50.6	50.6	50.6	50.6	49.9	49.8	49.8	47.8
10	37.0	37.0	37.0	37.0	37.0	37.0	36.9	36.8	36.8	36.8	37.1
11	19.3	19.3	19.3	19.3	19.3	19.3	19.4	18.7	18.7	18.6	23.6
12	32.9	32.9	32.9	33.0	32.9	33.1	32.8	31.4	31.5	31.3	123.3
13	86.5	86.5	86.4	86.5	86.5	86.6	86.5	92.4	92.6	92.1	140.5
14	44.7	44.7	44.7	44.7	44.7	44.7	44.7	42.3	42.4	42.4	42.0
15	37.0	37.0	37.0	37.1	37.1	37.1	36.9	37.2	37.2	37.1	56.8
16	77.2	77.2	77.2	77.2	77.2	77.3	77.4	72.6	72.6	72.4	62.9
17	44.7	44.6	44.4	44.6	44.7	44.7	44.4	47.9	47.9	48.0	38.0
18	51.6	51.6	51.6	51.6	51.6	51.1	50.1	51.8	51.3	53.0	43.0
19	39.1	39.1	39.0	39.1	39.1	33.6	39.7	38.5	32.3	32.5	40.4
20	31.9	31.9	31.9	31.9	31.9	37.0	69.8	31.6	36.6	47.3	35.8
21	36.9	36.9	36.9	36.9	36.9	32.7	37.5	36.1	32.1	30.0	30.7
22	31.9	31.9	31.9	31.9	31.9	31.7	31.5	28.7	28.6	29.4	28.7
23	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.0	28.0	28.0
24	16.7	16.7	16.7	16.8	16.6	16.7	16.5	16.6	16.5	16.5	16.6
25	16.5	16.5	16.5	16.5	16.5	16.5	16.4	16.3	16.2	16.2	15.5
26	18.6	18.6	18.6	18.6	18.6	18.6	18.6	18.0	18.0	18.0	18.6
27	19.6	19.6	19.6	19.6	19.6	19.8	19.7	19.4	19.5	19.6	23.2
28	77.8	78.0	78.0	78.0	78.0	78.0	78.0	179.2	179.4	178.5	66.6
29	33.8	33.8	33.8	33.8	33.8	29.1	32.8	33.4	28.7	23.8	28.0
30	24.8	24.8	24.8	24.8	24.8	66.1		24.6	65.8	206.8	66.8

[a] Assignments based on DEPT, HMQC, HMQC-TOCSY, and HMBC experiments.

Ardisianoside E (**6**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −7.8 (*c* 1.0, MeOH). Its molecular formula, $C_{47}H_{78}O_{17}$, was determined from the positive-ion HRFABMS (*m*/*z* 937.5157, $[M + Na]^+$). On acid hydrolysis, **6** afforded L-arabinose and D-glucose in a ratio of 1:2. On comparing the ^1H and ^{13}C NMR data of **6** and **4**, it was apparent that **6** possesses the same aglycon as **4**, but differs in its saccharide units. Thus, a glycosylation shift was observed at Ara-C-2 (+7.0 ppm) in **6**, instead of that observed at Glc-C-2 in **4**, suggesting the sugar chain in **6** to be a β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl moiety. This conclusion was confirmed by the HMBC correlations between δ_H 5.12 (GlcI-H-1) and δ_C 80.8 (Ara-H-2), δ_H 5.17 (GlcII-H-1), and δ_C 77.0 (Ara-H-4). Accordingly, the structure of ardisianoside E (**6**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyoleanane.

Ardisianoside F (**7**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −13.2 (*c* 0.3, MeOH). Its molecular formula, $C_{58}H_{96}O_{27}$, was determined from the positive-ion HRFABMS (*m*/*z* 1247.6060, $[M + Na]^+$). The ^{13}C NMR data of the aglycon of **7** were similar to that of **1**, but the methyl group signal for C-30 was replaced by a hydroxymethyl at δ_C 66.1. This assignment was confirmed by the HMBC correlation between δ_H 1.35 (H-29) and δ_C 66.1 (C-30) and the NOESY correlations between δ_H 1.87 (H-18) and δ_H 3.76 and 4.01 (H_2-30). Therefore, the aglycon of **7** was established as 13,28-epoxy-3β,16α,30-trihydroxyoleanane.[4] On acid hydrolysis, **7** afforded L-arabinose, D-xylose, and D-glucose in a ratio of 1:1:3. The ^1H and ^{13}C NMR data assignable to the sugars in **7** were similar to those in **2** except for the absence of the signals of the terminal β-D-glucopyranol moiety (Glc-IV) at GlcIII-C-3 in **2**. The sequence of the sugar chains was further confirmed from HMBC and NOESY correlations. Hence, HMBC correlations were observed between δ_H 5.45 (GlcI-H-1) and δ_C 79.3 (Ara-C-2), δ_H 4.95 (GlcII-H-1) and δ_C 78.0 (Ara-C-4), δ_H 5.19 (Xyl-H-1) and δ_C 81.4 (GlcII-C-2), and δ_H 5.23 (GlcIII-H-1) and δ_C 88.0 (GlcII-C-3). In turn, NOESY correlations were observed between δ_H 5.45 (GlcI-H-1) and δ_H 4.56 (Ara-H-2), δ_H 4.95 (GlcII-H-1) and δ_H 4.29 (Ara-H-4), δ_H 5.19 (Xyl-H-1) and δ_H 4.11 (GlcII-H-2), and δ_H 5.23 (GlcIII-H-1) and δ_H 4.09 (GlcII-H-3). Thus, the structure of ardisianoside F (**7**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-{β-D-xylopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)}-α-L-arabinopyranosyl-13β,28-epoxy-16α,30-dihydroxyoleanane.

Ardisianoside G (**15**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −19.7 (*c* 0.9, MeOH). Its molecular formula, $C_{52}H_{86}O_{22}$, was determined from the positive-ion HRFABMS (*m*/*z* 1085.5469, $[M + Na]^+$). On acid hydrolysis, **15** gave L-arabinose, L-rhamnose, and D-glucose in a ratio of 1:1:2. The ^{13}C NMR spectrum displayed 52 carbons, of which 29 were assigned to the aglycon part and 23 to the sugar moiety. Comparison of the ^1H and ^{13}C NMR data of the aglycon units of **15** and **1** revealed similar signals for the A−D rings, but differences were observed for the respective E rings. The replacement of the methyl group at C-20 in **1** by a hydroxyl group in **15** was deduced from the downfield shift of C-20 (+37.9 ppm). This assignment was confirmed by the HMBC correlation between δ_H 0.99 (H-29) and δ_C 69.8 (C-20). Therefore, the aglycon was established as 13,28-epoxy-3β,16α,20-trihydroxy-30-noroleanane. The sequence of the sugar chain of **15** was assigned with the same structure as that of ardisicrenoside A (**8**),[4] by comparing their ^1H and ^{13}C NMR data. Thus, the structure of ardisianoside G (**15**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α,20-dihydroxy-30-noroleanane.

Ardisianoside H (**16**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −19.4 (*c* 1.0, MeOH). Its molecular formula, $C_{70}H_{114}O_{37}$, was determined from the positive-ion HRFABMS (*m*/*z* 1569.7031, $[M + Na]^+$). On acid hydrolysis, **16** afforded L-arabinose, D-xylose, and D-glucose in a ratio of 1:1:5. The structure of the sugar chain was determined to be the same as that of **1** by careful comparison of their ^1H and ^{13}C NMR data. The ^{13}C NMR spectroscopic data of the aglycon in **16** were similar to those of **1** except that the carbon signal of the oxygenated methylene at δ_C 77.8 (C-28) in **1** was replaced by a saturated carbonyl signal at δ_C 179.2 in **16**, which was supported by the HMBC correlation between δ_H 4.48 (H-16) and δ_C 179.2 (C-28), the molecular formula calculated from HRFABMS, and the IR spectrum (1756 cm^{-1}). Thus, the structure

Table 2. ^{13}C NMR Spectroscopic Data (δ) of the Sugar Moieties of Compounds **1**, **2**, **4**−**7**, and **15**−**19** (125 MHz in pyridine-d_5)[a]

3-*O*-sugar	**1**	**2**	**4**	**5**	**6**	**7**	**15**	**16**	**17**	**18**	**19**
Ara-1	104.5	104.5	107.0	107.6	104.3	104.5	104.4	104.5	104.4	104.5	104.6
2	79.3	79.4	72.8	73.8	80.8	79.3	80.7	79.4	80.8	80.9	81.0
3	72.8	72.7	73.7	74.6	72.3	72.7	72.3	72.8	72.4	72.4	72.7
4	77.7	78.0	78.6	81.3	77.0	78.0	74.6	77.7	74.7	74.7	74.6
5	63.5	63.5	65.2	66.5	63.5	63.5	63.5	63.6	63.5	63.5	63.6
Glc-I-1	104.8	104.9	105.0	105.4	105.8	104.9	105.4	104.8	105.4	105.5	105.5
2	76.1	76.1	78.2	86.3	75.7	76.2	76.3	76.1	76.4	76.4	76.4
3	78.3	78.2	78.9	77.7	78.2	78.3	78.1	78.3	78.1	78.2	78.1
4	71.9	71.9	71.4	71.1	71.7	71.9	71.9	71.9	71.8	71.9	71.9
5	77.8	78.0	78.2	78.4	78.1	78.0	78.1	77.8	78.1	78.1	78.1
6	63.1	63.0	62.6	62.5	62.7	62.5	62.9	63.1	62.9	63.0	63.0
Glc-II-1	104.1	104.0			105.6	104.1	103.1	104.1	103.1	103.1	103.1
2	81.3	81.2			76.2	81.4	77.4	81.3	77.4	77.4	77.3
3	86.9	87.7			78.4	88.0	79.6	86.9	79.5	79.6	79.6
4	69.7	69.7			71.4	69.8	71.9	69.7	71.9	71.9	71.9
5	77.9	77.9			78.7	78.0	78.4	77.9	78.4	78.4	78.4
6	62.2	62.2			62.7	62.3	62.7	62.2	62.7	62.7	62.7
Xyl-1 (Rha-1)	105.9	105.9	101.9	108.1		106.2	101.6	105.9	101.6	101.6	101.6
2	75.7	75.7	72.3	76.4		75.8	72.4	75.7	72.4	72.4	72.4
3	78.0	78.3	72.0	78.0		78.4	72.3	78.0	72.7	72.8	72.8
4	70.9	70.9	74.5	70.5		71.0	74.9	71.0	74.8	74.9	74.9
5	67.3	67.1	70.0	67.3		67.4	69.4	67.3	69.4	69.5	69.4
6			18.6				18.9		18.9	19.0	19.0
Glc-III-1	103.9	104.3				105.0		103.9			
2	75.0	73.9				75.3		75.0			
3	81.5	87.7				78.7		81.5			
4	74.0	69.9				71.7		73.9			
5	77.4	78.6				78.7		77.4			
6	61.2	62.4				63.1		61.2			
Glc-IV-1	103.8	105.2						103.8			
2	74.8	75.1						74.7			
3	78.0	78.0						78.0			
4	71.0	71.5						71.0			
5	78.6	78.0						78.6			
6	62.4	62.1						62.4			
Glc-V-1	102.7							102.7			
2	75.0							74.9			
3	78.0							78.0			
4	71.2							71.5			
5	78.2							78.2			
6	62.1							62.1			

[a] Assignments based on DEPT, HMQC, HMQC-TOCSY, and HMBC experiments.

of ardisianoside H (**16**) was concluded to be 3β-*O*-β-D-glucopyranosyl-(1→2)-{β-D-xylopyranosyl-(1→2)-{β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→3)}-β-D-glucopyranosyl-(1→4)}-α-L-arabinopyranosyl-16α-hydroxyolean-28,13β-olide.

Ardisianoside I (**17**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −29.4 (*c* 1.0, MeOH). Its molecular formula, $C_{53}H_{86}O_{23}$, was determined from the positive-ion HRFABMS (*m*/*z* 1113.5466, $[M + Na]^+$). On acid hydrolysis, **17** gave L-arabinose, L-rhamnose, and D-glucose in the ratio of 1:1:2. The ^{13}C NMR data of the aglycon in **17** was similar to that in **16**, except that the signal due to the methyl group at C-30 in **16** was replaced by a signal for a hydroxymethyl group [δ_H 3.65 and 3.98 (each, d, J = 10.7 Hz) and δ_C 65.8] in **17**, which was confirmed by the HMBC correlation between δ_H 1.29 (H-29) and δ_C 65.8 (C-30) and the NOESY correlation between δ_H 2.30 (H-18) and δ_H 3.65, 3.98 (H_2-30). Comparison of the ^1H and ^{13}C NMR data of **17** with those of **15** revealed that the signals of the sugar moieties were superimposable, suggesting the sugar structure at C-3 was the same as that in **15**. Thus, the structure of ardisianoside I (**17**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-16α,30-dihydroxyolean-28,13β-olide.

Ardisianoside J (**18**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −28.2 (*c* 0.3, MeOH). Its molecular formula, $C_{53}H_{84}O_{23}$, was determined from the positive-ion HRFABMS (*m*/*z* 1111.5347, $[M + Na]^+$). On acid hydrolysis, **18** gave L-arabinose, L-rhamnose, and D-glucose in a ratio of 1:1:2. The ^1H and ^{13}C NMR data **18** were similar to those of **17** except that the hydroxymethyl group at C-30 was replaced by an aldehyde group (δ_H 9.48, δ_C 206.8), which was confirmed by the HMBC correlation between δ_H 1.17 (H-29) and δ_C 206.8 (C-30). Thus, the structure of ardisianoside J (**18**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-16α-hydroxyolean-30-al-28,13β-olide.

Ardisianoside K (**19**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −18.6 (*c* 1.0, MeOH). Its molecular formula, $C_{53}H_{86}O_{22}$, was determined from the positive-ion HRFABMS (*m*/*z* 1097.5465, $[M + Na]^+$). On acid hydrolysis, **19** gave L-arabinose, L-rhamnose, and D-glucose in a ratio of 1:1:2. The ^{13}C NMR spectroscopic data of **19** showed 53 carbon signals, of which 30 were assigned to the aglycon part, while 23 were assigned to the sugar moieties. The six sp^3 quaternary carbon signals at δ 15.5, 16.6, 18.6, 23.2, 28.0, and 28.0 and two sp^2-hybridized carbons at 123.3 and 140.5 indicated that the aglycon of **19** is an olean-12-en skeleton. Comparison of the ^{13}C NMR spectral data of **19** with that of ardisimamilloside C (**20**),[10] a known saponin from *A. mamillata* with a structure differing from **19** only by the absence of the signals for a hydroxymethylene at δ_C 73.8 (C-16) and a methylene at δ_C 34.7 (C-15) and the appearance of the signals for two hydroxymethylenes at δ_C 56.8 and 62.9, suggested the presence of a 15,16-epoxy moiety in **19**. This assignment was confirmed by the HMBC correlation between δ_H 1.51 (H-27) and δ_C 56.8 (C-15). The configuration of the 15,16-epoxide was determined to be *cis* by the $^3J_{H15,H16}$ coupling constants (3.5 Hz), since in the case of a *trans* epoxide, the coupling constants are reported as being less than 2

Table 3. ^{1}H NMR Spectroscopic Data (δ) of the Aglycon Moieties of Compounds **1**, **2**, **4**−**7**, and **15**−**19** (500 MHz in pyridine-d_5)

position	1	2	4	5	6	7	15	16	17	18	19
1	0.84[a]	0.84[a]	0.90 (td, 12.5, 2.8)	0.96[a]	0.90[a]	0.85[a]	0.81 (t, 13.1)	0.84 (dd, 13.7, 3.2)	0.84[a]	0.83 (td, 13.5, 3.5)	0.84 (td, 13.5, 3.7)
	1.65 (dd, 13.4, 3.7)	1.65 (dd, 13.5, 3.7)	1.69[a]	1.74 (d, 13.3)	1.70 (dd, 14.0, 4.1)	1.63[a]	1.64 (dd, 12.4, 3.7)	1.63[a]	1.65 (dd, 9.4, 3.7)	1.63[a]	1.41 (d, 13.1)
2	1.81 (d, 12.3)	1.82[a]	1.85[a]	1.85[a]	1.81 (t, 14.0)	1.82[a]	1.83[a]	1.81 (d, 12.6)	1.87 (m)	1.83[a]	1.82[a]
	1.97 (dd, 13.5, 3.6)	1.97 (dd, 13.5, 3.4)	2.08 (dd, 11.9, 5.2)	2.16 (dd, 13.5, 3.9)	2.09 (dd, 14.2, 5.3)	1.94 (dd, 11.2, 4.7)	1.99 (dd, 13.5, 3.4)	2.01[a]	2.01 (dd, 13.3, 4.8)	2.02 (dd, 13.3, 3.5)	2.00 (dd, 13.8, 4.1)
3	3.13 (dd, 11.7, 4.3)	3.13 (dd, 11.6, 4.5)	3.27 (dd, 11.9, 4.4)	3.34 (dd, 10.8, 4.3)	3.17 (dd, 12.2, 4.1)	3.12 (dd, 11.8, 4.4)	3.17 (dd, 11.7, 4.5)	3.13 (dd, 11.7, 4.4)	3.15 (dd, 11.7, 4.4)	3.16 (dd, 11.2, 4.1)	3.18 (dd, 11.9, 4.4)
5	0.67 (dd, 10.6, 1.9)	0.68 (d, 11.2)	0.75 (d, 11.0)	0.79 (d, 11.0)	0.79 (d, 10.6)	0.67 (d, 11.3)	0.67 (d, 11.3)	0.67 (d, 11.4)	0.64 (d, 11.5)	0.65 (d, 11.7)	0.75 (d, 11.9)
6	1.44[a]	1.45[a]	1.45[a]	1.46[a]	1.43[a]	1.41 (d, 14.3)	1.45 (dd, 14.0, 4.3)	1.41 (d, 8.1)	1.41 (m)	1.42 (m)	1.37 (m)
7	1.23[a]	1.23[a]	1.24 (dd, 9.2, 2.7)	1.25 (m)	1.22 (t, 13.3)	1.24[a]	1.25[a]	1.15 (d, 12.2)	1.15[a]	1.11 (dd, 12.8, 4.6)	1.23[a]
	1.55[a]	1.55[a]	1.55[a]	1.58[a]	1.57[a]	1.57[a]	1.52[a]	1.47 (d, 12.9)	1.49[a]	1.48 (d, 11.9)	1.58[a]
9	1.30 (d, 12.1)	1.29 (d, 8.7)	1.29 (d, 13.6)	1.35[a]	1.26 (d, 11.9)	1.27 (d, 13.0)	1.25 (d, 13.6)	1.29 (d, 10.8)	1.28[a]	1.27 (d, 11.7)	1.57[a]
11	1.47 (t, 13.7)	1.47 (t, 13.7)	1.48 (t, 13.7)	1.51 (d, 13.1)	1.49 (d, 13.7)	1.47[a]	1.47[a]	1.48 (m)	1.39[a]	1.32 (d, 12.4)	1.71[a]
	1.79 (d, 13.3)	1.79 (d, 13.1)	1.78 (d, 13.1)	1.82[a]	1.80[a]	1.74[a]	1.79[a]	1.79 (d, 13.1)	1.63[a]	1.79 (d, 13.1)	1.81[a]
12	1.47 (d, 13.7)	1.47 (t, 13.7)	1.46 (t, 13.7)	1.48 (dd, 12.2, 1.9)	1.49[a]	1.47[a]	1.47 (t, 13.0)	1.55 (dd, 14.3, 3.3)	1.58 (dd, 13.5, 4.1)	1.53 (dd, 8.1, 4.4)	5.33 (t, 3.3)
	2.05 (td, 14.4, 5.2)	2.07 (dd, 14.4, 5.7)	2.06 (m)	2.08 (td, 13.5, 5.5)	2.04 (dd, 11.4, 3.2)	2.12 (td, 13.7, 5.1)	2.14 (dd, 13.9, 5.2)	2.03 (dd, 13.5, 4.6)	2.09 (dd, 13.6, 5.5)	2.13[a]	
15	1.48 (d, 13.3)	1.49 (d, 13.7)	1.49 (d, 13.7)	1.49 (d, 13.7)	1.29 (d, 12.1)	1.51 (d, 14.2)	1.48 (d, 13.9)	1.67 (d, 14.9)	1.68 (d, 14.9)	1.65 (d, 14.1)	3.14 (d, 3.5)
	2.25 (dd, 14.4, 5.2)	2.25 (dd, 14.7, 5.3)	2.24 (dd, 14.7, 5.3)	2.26 (dd, 14.3, 5.2)	2.25 (dd, 14.4, 5.0)	2.27 (dd, 14.5, 5.3)	2.29 (dd, 14.7, 5.3)	2.46 (dd, 15.1, 2.2)	2.28 (dd, 14.9, 4.4)	2.21 (dd, 10.1, 6.4)	
16	4.21 (br s)	4.21 (br s)	4.19 (br s)	4.20 (br s)	4.20 (br s)	4.21 (br s)	4.27 (br s)	4.48 (br s)	4.52 (br s)	4.50 (br s)	3.41 (d, 3.5)
18	1.68 (dd, 11.5, 2.3)	1.69 (dd, 11.7, 2.3)	1.69 (dd, 11.7, 2.3)	1.72 (dd, 12.4, 2.7)	1.71 (dd, 14.1, 3.7)	1.87 (dd, 14.7, 2.8)	2.35 (dd, 14.2, 2.5)	2.08 (dd, 12.2, 2.5)	2.30 (dd, 14.6, 2.3)	1.87 (d, 12.4)	2.54 (dd, 13.8, 7.7)
19	1.36[a]	1.36[a]	1.36[a]	1.35[a]	1.36[a]	2.05 (dd, 12.1, 2.5)	1.94 (d, 10.5)	1.37 (d, 10.5)	2.13 (dd, 13.5, 3.2)	2.21 (d, 13.8)	1.73[a]
	2.79 (dd, 14.2, 12.6)	2.78 (dd, 14.2, 12.1)	2.77 (dd, 14.0, 12.1)	2.79 (dd, 14.2, 12.1)	2.79 (dd, 14.0, 12.1)	2.79 (dd, 14.1, 12.3)	3.02 (dd, 14.0, 12.1)	2.82 (t, 12.3)	2.81 (dd, 13.8, 12.4)	2.88 (dd, 14.2, 12.1)	2.45 (t, 13.5)
21	1.26 (dd, 13.0, 8.7)	1.25 (dd, 13.6, 8.7)	1.25 (dd, 13.6, 8.7)	1.26 (dd, 12.6, 2.8)	1.27[a]	1.71 (dd, 13.6, 8.7)	1.86 (dd, 13.6, 8.1)	1.27 (dd, 13.0, 4.5)	1.62 (m)	2.09 (dd, 13.5, 6.0)	1.80[a]
	2.57 (td, 12.8, 4.8)	2.56 (td, 13.6, 5.1)	2.55 (td, 13.7, 4.8)	2.56 (td, 13.5, 4.8)	2.56 (td, 13.3, 4.6)	2.60 (td, 14.6, 9.8)	2.82 (td, 13.3, 5.0)	2.49 (td, 13.3, 5.1)	2.52 (td, 13.5, 5.5)	2.51 (td, 13.7, 5.2)	1.93 (d, 12.9)
22	1.59 (dd, 13.3, 5.0)	1.59 (dd, 13.7, 5.0)	1.59 (dd, 13.7, 5.0)	1.61 (td, 13.6, 4.6)	1.62 (dd, 13.7, 5.0)	1.69 (dd, 13.7, 5.0)	2.00 (dd, 13.4, 5.2)	1.96 (dd, 14.4, 5.2)	2.06 (dd, 13.6, 4.5)	1.91 (td, 13.7, 5.0)	2.09 (dt, 13.8, 4.8)
	1.91 (dd, 12.6, 3.3)	1.91 (dd, 13.5, 3.0)	1.90 (dd, 14.0, 3.0)	1.91 (dd, 14.2, 3.0)	1.91 (dd, 13.3, 3.7)	1.96 (dd, 11.2, 4.7)	2.16 (dd, 13.5, 3.5)	2.19 (dd, 14.4, 3.0)	2.23 (dd, 13.5, 4.4)	2.26 (dd, 13.8, 4.8)	2.47 (td, 14.0, 3.7)
23	1.19 (s)	1.19 (s)	1.21 (s)	1.29 (s)	1.18 (s)	1.19 (s)	1.17 (s)	1.19 (s)	1.16 (s)	1.04 (s)	1.21 (s)
24	1.06 (s)	1.06 (s)	0.96 (s)	0.99 (s)	0.99 (s)	1.05 (s)	1.03 (s)	1.04 (s)	1.01 (s)	1.00 (s)	1.06 (s)
25	0.86 (s)	0.87 (s)	0.88 (s)	0.92 (s)	0.91 (s)	0.85 (s)	0.85 (s)	0.80 (s)	0.78 (s)	0.77 (s)	0.78 (s)
26	1.35 (s)	1.35 (s)	1.35 (s)	1.37 (s)	1.36 (s)	1.35 (s)	1.36 (s)	1.22 (s)	1.20 (s)	1.17 (s)	1.01 (s)
27	1.55 (s)	1.54 (s)	1.55 (s)	1.57 (s)	1.55 (s)	1.61 (s)	1.59 (s)	1.61 (s)	1.66 (s)	1.62 (s)	1.51 (s)
28	3.32 (d, 7.4)	3.32 (d, 7.4)	3.32 (d, 7.4)	3.33 (d, 7.0)	3.33 (d, 7.3)	3.32 (d, 7.5)	3.44 (d, 7.3)				3.89 (d, 10.6)
	3.62 (d, 7.4)	3.61 (d, 7.4)	3.61 (d, 7.4)	3.62 (d, 7.0)	3.61 (d, 7.3)	3.62 (d, 7.5)	3.66 (d, 7.3)				3.95 (d, 10.6)
29	1.09 (s)	1.09 (s)	1.08 (s)	1.09 (s)	1.09 (s)	1.35 (s)	1.55 (s)	1.04 (s)	1.29 (s)	1.17 (s)	1.27 (s)
30	0.99 (s)	0.99 (s)	0.99 (s)	1.02 (s)	1.04 (s)	3.76 (d, 11.3)		0.94 (s)	3.65 (d, 10.7)	9.84 (s)	3.65 (d, 10.7)
						4.01 (d, 11.3)			3.98 (d, 10.7)		3.98 (d, 10.7)

[a] Overlapped signals.

Table 4. ^{1}H NMR Spectroscopic Data (δ) of the Sugar Moieties of Compounds **1**, **2**, **4**−**7**, and **15**−**19** (500 MHz in pyridine-d_5)

position	**1**	**2**	**4**	**5**	**6**	**7**	**15**	**16**	**17**	**18**	**19**
Ara-1	4.82 (d, 5.2)	4.83 (d, 5.1)	4.75 (d, 6.2)	4.67 (d, 6.3)	4.96 (d, 5.0)	4.83 (d, 5.5)	4.95 (d, 4.6)	4.83 (d, 5.1)	4.94 (d, 4.6)	4.95 (d, 4.9)	4.95 (d, 5.4)
2	4.58[a]	4.56 (d, 5.1)	4.42 (dd, 8.7, 6.2)	4.31 (dd, 8.5, 6.3)	4.56 (dd, 8.5, 6.2)	4.56 (d, 7.1)	4.58 (dd, 7.3, 5.1)	4.58 (d, 5.1)	4.57 (dd, 7.1, 5.5)	4.58[a]	4.59 (d, 5.8)
3	4.35 (m)	4.33[a]	4.24 (dd, 8.7, 3.5)	4.01 (dd, 9.6, 4.3)	4.45 (dd, 7.8, 2.8)	4.34[a]	4.50[a]	4.35 (m)	4.48[a]	4.49[a]	4.33 (dd, 5.1, 2.0)
4	4.29[a]	4.26[a]	4.38 (dd, 5.3, 3.0)	4.14 (m)	4.51 (dd, 6.0, 2.8)	4.29[a]	4.58[a]	4.29[a]	4.59 (m)	4.60[a]	4.60 (m)
5	3.67 (d, 10.7)	3.69 (d, 10.0)	3.82 (d, 10.6)	3.74 (d, 12.3)	3.83 (d, 11.7)	3.68 (d, 8.9)	3.80[a]	3.68 (d, 11.6, 1.8)	3.80 (m)	3.80 (m)	3.80 (m)
	4.52[a]	4.53 (dd, 10.0, 4.6)	4.64 (dd, 11.6, 3.9)	4.65 (dd, 12.3, 2.2)	4.41 (dd, 11.7, 3.9)	4.55[a]	4.40 (dd, 11.4, 5.5	4.52[a]	4.41 (dd, 11.7, 5.3)	4.41 (dd, 11.3, 5.0)	4.40 (dd, 11.6, 5.2)
Glc-I-1	5.45 (d, 7.5)	5.44 (d, 7.6)	5.14 (d, 7.1)	5.03 (d, 7.8)	5.12 (d, 7.8)	5.45 (d, 7.6)	5.37 (d, 7.8)	5.45 (d, 7.9)	5.37 (d, 7.8)	5.39 (d, 7.6)	5.40 (d, 7.8)
2	4.07 (t, 8.1)	4.05 (t, 8.7)	4.17 (t, 7.1)	3.96 (t, 8.3)	4.04 (d, 8.1)	4.05 (d, 8.6)	4.06 (t, 8.2)	4.07 (t, 7.9)	4.07 (t, 8.9)	4.08 (t, 8.4)	4.09 (d, 9.0)
3	4.21 (t, 7.8)	3.99 (t, 7.4)	3.85 (dd, 8.6, 7.1)	4.25 (t, 7.4)	4.16 (d, 9.0)	4.24 (d, 8.6)	4.29 (t, 9.0)	4.24 (t, 7.9)	4.29 (d, 8.1)	4.30 (t, 8.7)	4.29 (t, 8.2)
4	4.22 (t, 9.2)	4.23 (t, 6.6)	4.14 (t, 8.6)	4.26 (t, 7.4)	4.28 (t, 9.0)	4.24 (t, 8.6)	4.13 (t, 9.0)	4.22 (t, 8.9)	4.22 (t, 9.7)	4.22 (t, 9.2)	4.22 (t, 9.4)
5	4.07[a]	4.04[a]	4.04 (dd, 8.6, 3.0)	3.86 (m)	3.77 (m)	4.05[a]	4.07 (m)	4.09[a]	4.07 (m)	4.08[a]	4.05 (m)
6	4.38 (dd, 11.5, 5.0)	4.40 (dd, 11.4, 5.1)	4.30 (dd, 11.8, 5.3)	4.34 (dd, 11.8, 5.3)	4.40 (dd, 11.9, 5.3)	4.26 (dd, 12.1, 5.5)	4.37 (dd, 11.7, 5.1)	4.39 (dd, 11.4, 5.1)	4.38 (dd, 11.4, 4.6)	4.38 (dd, 11.6, 5.1)	4.38 (dd, 11.5, 4.7)
	4.55 (dd, 11.5, 2.7)	4.54 (dd, 11.4, 2.9)	4.45 (dd, 11.8, 2.1)	4.67 (dd, 11.8, 2.1)	4.40[a]	4.56 (dd, 12.1, 2.7)	4.50 (dd, 11.7, 2.5)	4.55 (dd, 11.4, 2.7)	4.51 (dd, 11.4, 2.5)	4.51 (dd, 11.6, 2.5)	4.53 (dd, 11.5, 2.3)
Glc-II-1	4.92 (d, 7.4)	4.92 (d, 6.9)			5.17 (d, 7.8)	4.95 (d, 7.3)	5.25 (d, 7.7)	4.94 (d, 7.6)	5.25 (d, 7.6)	5.27 (d, 7.5)	5.30 (d, 7.8)
2	4.10 (t, 8.2)	4.06 (t, 8.7)			4.06 (t, 8.3)	4.11 (t, 8.3)	4.28 (t, 8.9)	4.10 (t, 8.8)	4.27 (t, 8.1)	4.29 (t, 8.0)	4.28 (t, 8.3)
3	4.00 (t, 8.9)	4.09 (t, 8.9)			4.20 (t, 8.7)	4.09 (t, 8.9)	4.20 (t, 8.9)	4.03 (t, 8.8)	4.20 (t, 9.3)	4.21 (t, 8.7)	4.21 (t, 8.9)
4	3.98[a]	4.01[a]			4.24 (t, 9.1)	4.06 (t, 8.9)	4.22 (t, 8.9)	3.99[a]	4.05 (t, 9.3)	4.14 (t, 9.3)	4.13 (t, 9.1)
5	3.65 (m)	3.68 (m)			3.88 (m)	3.70[a]	3.80[a]	3.67 (m)	3.81 (m)	3.82 (m)	3.81 (m)
6	4.14 (dd, 11.7, 5.6)	4.14 (dd, 11.8, 5.9)			4.36 (dd, 12.1, 4.9)	4.18 (dd, 12.1, 5.3)	4.30 (dd, 11.7, 5.2)	4.15 (dd, 11.6, 5.9)	4.28 (dd, 12.2, 4.9)	4.21 (dd, 11.3, 4.8)	4.30 (dd, 11.7, 5.3)
	4.33 (d, 11.7)	4.34 (dd, 11.8, 2.5)			4.50 (dd, 12.1, 2.1)	4.35 (dd, 12.1, 2.3)	4.46 (dd, 11.7, 2.0)	4.32[a]	4.47 (dd, 12.2, 2.3)	4.57 (dd, 12.2, 2.0)	4.47 (dd, 11.7, 2.0)
Xyl-1 (Rha-1)	5.12 (d, 6.8)	5.14 (d, 6.9)	6.23 (d, 1.2)	4.91 (d, 6.9)		5.19 (d, 7.1)	6.40 (d, 1.2)	5.14 (d, 6.9)	6.39 (d, 1.3)	6.1 (br s)	6.41 (d, 1.1)
2	3.96 (t, 6.7)	4.00 (t, 6.7)	4.71 (d, 3.8)	3.98 (d, 8.4)		4.02 (d, 6.9)	4.72 (dd, 3.2, 1.2)	3.96 (t, 6.9)	4.72 (dd, 3.0, 1.3)	4.73 (dd, 3.1, 1.1)	4.73 (dd, 3.4, 1.6)
3	3.97 (t, 8.9)	4.24 (t, 6.2)	4.72 (dd, 6.1, 3.7)	3.98 (d, 8.4)		4.01 (t, 6.4)	4.67 (dd, 9.3, 3.2)	3.97 (t, 6.9)	4.67 (dd, 9.7, 3.0)	4.68 (dd, 9.3, 3.1)	4.67 (dd, 9.4, 3.4)
4	4.21[a]	4.20[a]	4.20 (t, 6.1)	3.99[a]		4.21[a]	4.26 (t, 9.3)	4.20[a]	4.29 (t, 9.0)	4.29[a]	4.31[a]
5	3.74 (t, 11.0)	3.63[a]	4.93 (dd, 9.4, 6.2)	3.48 (t, 10.1)		3.69 (d, 11.3)	5.03 (dd, 9.3, 6.2)	3.74 (t, 10.6)	5.03 (dt, 12.1, 6.1)	5.05 (dd, 9.3, 6.1)	5.04 (dd, 9.4, 6.1)
(6)	4.52 (dd, 10.2, 3.3)	4.50 (dd, 11.7, 2.5)	1.66 (d, 6.2)	4.28 (dd, 11.0, 3.7)		4.50 (dd, 11.3, 5.5)	1.80 (d, 6.2)	4.56 (dd, 10.6, 3.3)	1.81 (d, 6.1)	1.80 (d, 6.1)	1.81 (d, 6.1)
Glc-III-1	5.19 (d, 7.8)	5.22 (d, 7.6)				5.23 (d, 7.8)		5.20 (d, 7.8)			
2	3.98 (t, 8.9)	4.01 (t, 8.6)				4.02 (t, 8.3)		4.01 (t, 8.7)			
3	4.51 (t, 8.1)	4.23 (t, 8.6)				4.21 (t, 9.0)		4.53 (t, 8.7)			
4	4.49 (t, 9.6)	4.02[a]				4.13 (t, 9.6)		4.51 (t, 8.7)			
5	4.02[a]	3.94 (m)				4.04[a]		4.02[a]			
6	4.27 (dd, 11.7, 5.8)	4.27 (dd, 11.7, 5.8)				4.41 (dd, 11.2, 5.0)		4.27 (dd, 11.7, 5.8)			
	4.48 (dd, 11.7, 2.6)	4.48 (dd, 11.7, 2.6)				4.55 (dd, 11.2, 2.0)		4.48 (dd, 11.7, 2.6)			
Glc-V-1	5.35 (d, 7.1)	5.20 (d, 8.0)						5.36 (d, 7.2)			
2	4.10 (t, 8.7)	4.05 (t, 8.7)						4.14 (t, 8.7)			
3	4.04 (t, 8.7)	4.27 (t, 8.7)						4.04 (t, 8.7)			
4	4.11 (t, 8.0)	4.15 (t, 8.4)						4.11 (t, 8.7)			
5	3.87 (m)	4.00[a]						3.87 (m)			
6	4.29 (dd, 11.5, 4.1)	4.13 (dd, 11.5, 5.8)						4.29 (dd, 11.5, 5.8)			
	4.42 (dd, 11.5, 1.8)	4.44 (dd, 11.5, 1.6)						4.42 (dd, 11.5, 1.6)			
Glc-V-1	5.33 (d, 7.3)							5.34 (d, 7.3)			
2	4.13 (t, 8.1)							4.10 (t, 8.7)			
3	4.18 (t, 8.7)							4.18 (t, 8.7)			
4	4.17 (t, 8.4)							4.18 (t, 8.7)			
5	3.94 (m)							3.94 (m)			
6	4.26 (dd, 11.5, 4.7)							4.27 (dd, 11.5, 5.8)			
	4.42 (dd, 11.5, 1.9)							4.41 (dd, 11.5, 1.6)			

[a] Overlapped signals.

Hz.[11] Further, H-16 was determined with the β-configuration from the NOESY correlation between δ_H 3.41 (H-16) and δ_H 3.80 (H-28). Thus, the structure of ardisianoside K (**19**) was elucidated as 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-15α,16α-epoxy-28,30-dihydroxyoleanan-12-ene.

The known saponins were identified as 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyoleanane (**3**),[12] ardisicrenoside A (**8**),[4] cyclamin (**9**),[13] ardisiacrispin B (**10**),[4] 3β-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopynanosyl)cyclamiretin A (**11**),[9] primulanin (**12**),[9] ardisiamamilloside H (**13**),[10] ardisiamamilloside F (**14**),[14] ardisiamamillosede C (**20**),[10] and ardisicrenoside G (**21**),[2d] by detailed NMR analysis and comparison with literature data.

It is worth noting that four triterpene saponins, namely, 3β-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}cyclamiretin A, 3β-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}-13β,28-epoxy-30,30-dimethoxyolean-16α-ol, 3β-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}-16α-hydroxy-13β,28-epoxyolean-29-oic acid, and 3β-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}cyclamiretin A, have been previously reported from *A. japonica*.[1c,d] However, 3β-*O*-{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}cyclamiretin A showed identical ^{1}H and ^{13}C NMR data to those of ardisiacrispin B (3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyoleanane, **10**) in the same NMR solvent (CD_3OD). Since these saponins bear the same sugar chain, we consider their structures should be corrected to ardisiacrispin B (**10**), 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxy-30,30-dimethoxyoleanane (**22**), 3β-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyolean-29-oic acid (**23**), and 3β-*O*-β-D-glucopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)]}-α-L-arabinopyranosyl-13β,28-epoxy-16α-hydroxyoleanane (**24**).

The isolated saponins **1**−**21** were evaluated for their cytotoxic activities against HL-60 human myeloid leukemia, KATO-III human stomach adenocarcinoma, and A_{549} human lung adenocarcinoma cells. All cells were treated continuously with each sample for 72 h, and cell growth was measured by a MTT reduction assay procedure (Table 5).[15] Saponins with the aglycons protoprimulagenin A (**1**−**6**) and cyclamiretin A (**9**−**13**) showed moderate cytotoxic activity against HL-60 cells and A_{549} adenocarcinoma cells, with IC_{50} values of 1.9−30.8 and 3.1−37.2 μM, respectively, while the other saponins (**7**−**8**, **14**−**21**) were inactive (>100 μM) compared with etoposide used as a positive control (IC_{50} 0.3 and 20.9 μM), respectively. Among the cytotoxic saponins, comparison between **4** (2.7 μM) and **11** (18.1 μM) suggested that the glucopyranosyl moiety at Ara-C-2 has an effect on cytotoxic activity. Saponins **1**−**6** and **8**−**13** showed moderate cytotoxic activity against KATO-III adenocarcinoma cells, with IC_{50} values of 0.3−21.5 μM, while the other saponins (**7**, **14**−**21**) were inactive (>100 μM), as compared with etoposide used as a positive control (IC_{50} 0.1 μM). Among the cytotoxic saponins, comparison between **11** (1.7 μM) and **13** (21.5 μM) suggested that the hydroxyl moiety at C-16 has an effect on the activity. It is worth noting that ardisicrenoside A (**8**) showed selective cytotoxic activity against KATO-III adenocarcinoma cells, with an IC_{50} value of 14.5 μM, compared to the other cells examined.

Table 5. Cytotoxic Activity of Compounds **1**−**21** against Three Human Cancer Cell Lines[a,b]

	IC_{50} (μM)		
sample	HL-60	KATO-III	A549
1	2.7 ± 0.4	0.4 ± 0.2	6.0 ± 3.7
2	2.4 ± 0.9	0.3 ± 0.1	3.1 ± 0.3
3	1.9 ± 0.5	0.4 ± 0.3	3.7 ± 0.3
4	2.7 ± 0.5	1.3 ± 0.3	3.2 ± 2.1
5	3.5 ± 0.4	1.5 ± 0.7	24.2 ± 3.5
6	3.4 ± 0.8	0.4 ± 0.2	3.1 ± 0.3
7	>100	38.3 ± 6.2	36.2 ± 7.6
8	>100	14.5 ± 4.4	>100
9	3.0 ± 0.2	0.3 ± 0.3	3.3 ± 0.4
10	3.2 ± 0.1	1.2 ± 0.5	3.6 ± 1.0
11	18.1 ± 7.0	1.7 ± 0.8	29.2 ± 4.1
12	24.7 ± 5.8	3.3 ± 0.9	27.7 ± 4.7
13	30.8 ± 7.9	21.5 ± 5.2	37.2 ± 6.5
16	28.4 ± 6.3	8.3 ± 1.2	33.4 ± 9.1
18	45.6 ± 8.4	33.5 ± 6.8	32.9 ± 5.6
etoposide[c]	0.3 ± 0.05	0.1 ± 0.02	20.9 ± 15.2

[a] The data shown represent the mean ± SEM of two independent experiments with three determinations in each. HL-60, human myeloid leukemia cells; KATO-III, human stomach KATO-III adenocarcinoma cells; A549,human lung A549 adenocarcinoma cells. [b] Compounds **14**, **15**, **17**, and **19**−**21** were inactive for all cell lines (IC_{50} > 100 μM). [c] Positive control substance.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The IR spectra were run on a JASCO FT/IR-300E spectrometer. The ^{1}H and ^{13}C NMR were measured with a JEOL ECP-500 or JEOL AL-400 spectrometer in δ (ppm) referring to TMS. The ESIMS and HRFABMS data were taken on LCQ and JEOL JMS-700 MStation mass spectrometers, respectively. Preparative HPLC was performed on a JASCO model PU-2080 HPLC system, equipped with a Shodex RI-101 refractive index detector and YMC-Pack RP-C_{18} column (150 × 20 mm i.d.). Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica gel 60N, Kanto Chemical Co., Inc., Tokyo, Japan), and ODS (100−200 mesh, Chromatorex DM1020T ODS, Fuji Silysia Chemical Co., Ltd., Aichi, Japan) were used for column chromatography. TLC was conducted on Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS instrument.

Plant Material. *Ardisia japonica* was collected at Sendai, Miyagi Prefecture, Japan, in September 2000, and was identified by one of the authors (K.K.). A specimen of the plant (TH2000004) is kept in the herbarium of the Faculty of Pharmaceutical Sciences, Toho University.

Extraction and Isolation. The fresh whole plants (15.1 kg) of *A. japonica* were extracted with MeOH (100 L) at room temperature. Evaporation of the solvent under reduced pressure gave an extract (800 g), which was then partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was evaporated under reduced pressure at below 40 °C to give a residue (450 g), which was subjected to passage over a Diaion HP-20 column and eluted with H_2O and 30%, 70%, and 100% MeOH, successively. The 70% MeOH and 100% MeOH eluates were concentrated to give two fractions, A (66 g) and B (112 g). Fraction A was chromatographed over a silica gel column with a gradient of $CHCl_3$−MeOH−H_2O (60:20:3, 60:29:6, 6:4:1) to give three fractions, A1−A3. The saponin-containing fraction A2 (7.08 g) was further separated by repeated HPLC purification with aqueous MeOH or CH_3CN at a flow rate of 5 mL/min to afford **1** (54 mg, t_R = 40.0 min, 75% MeOH), **2** (17 mg, t_R = 45.0 min, 75% MeOH), **7** (3 mg, t_R = 17.8 min, 35% CH_3CN), **8** (65 mg, t_R = 15.6 min, 70% MeOH), **9** (62 mg, t_R = 16.4 min, 75% MeOH), **10** (87 mg, t_R = 21.0 min, 70% MeOH), **14** (3 mg, t_R = 17.8 min, 35% CH_3CN), **15** (12 mg, t_R = 22.8 min, 60% MeOH), **16** (17 mg, t_R = 21.2 min, 73% MeOH), **17** (32 mg, t_R = 18.0 min, 57% MeOH), **19** (15 mg, t_R = 19.0 min, 60% MeOH), **20** (20 mg, t_R = 18.0 min, 57% MeOH), and **21** (41 mg, t_R = 18.6 min, 57% MeOH). Fraction B was chromatographed over a silica gel column with a gradient of $CHCl_3$−MeOH−H_2O (60:20:3, 60:29:6, 6:4:1) to give three fractions, B1−B3. The saponin-containing fraction B2 (20.50 g) was further separated by repeated HPLC purification with aqueous MeOH

or CH_3CN at a flow rate of 5 mL/min to afford **3** (22 mg, t_R = 16.8 min, 80% MeOH), **4** (39 mg, t_R = 25.2 min, 85% MeOH), **5** (14 mg, t_R = 22.0 min, 850% MeOH), **6** (17 mg, t_R = 23.2 min, 82% MeOH), **11** (98 mg, t_R = 71.2 min, 75% MeOH), **12** (5 mg, t_R = 24.4 min, 73% MeOH), **13** (21 mg, t_R = 19.2 min, 43% CH_3CN), and **18** (4 mg, t_R = 19.2 min, 70% MeOH).

Ardisianoside A (1): amorphous powder, $[\alpha]^{22}_D$ −15.5 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3396, 2924, 1636, 1371, 1259, 1073 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1555 $[M + Na]^+$, 1401 $[M + H - C_5H_8O_4]^+$, 1371 $[M + H - C_6H_{10}O_5]^+$, 457 $[M + H - C_{40}H_{66}O_{33}]^+$; positive-ion HRFABMS *m/z* 1555.7100 (calcd for $C_{70}H_{116}O_{36}Na$, 1555.7144).

Ardisianoside B (2): amorphous powder, $[\alpha]^{22}_D$ −15.7 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3405, 1733, 1632, 1591, 1383, 1256, 1071 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1393 $[M + Na]^+$, 589 $[M + H - C_{29}H_{49}O_{24}]^+$, 457 $[M + H - C_{34}H_{56}O_{28}]^+$; positive-ion HRFABMS *m/z* 1393.6564 (calcd for $C_{64}H_{106}O_{31}Na$, 1393.6616).

Ardisianoside C (4): amorphous powder, $[\alpha]^{22}_D$ −21.3 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3427, 2922, 2859, 1632, 1376, 1074 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 921 $[M + Na]^+$, 559 $[M + H - C_{12}H_{21}O_9]^+$, 457 $[M + H - C_{17}H_{28}O_{13}]^+$; positive-ion HRFABMS *m/z* 921.5166 (calcd for $C_{41}H_{78}O_{16}Na$, 921.5188).

Ardisianoside D (5): amorphous powder, $[\alpha]^{22}_D$ −46.5 (*c* 0.9, MeOH); IR (KBr) ν_{max} 3424, 2925, 1630, 1377, 1086 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 907 $[M + Na]^+$, 457 $[M + H - C_{16}H_{26}O_{13}]^+$; positive-ion HRFABMS *m/z* 907.5027 (calcd for $C_{46}H_{76}O_{16}Na$, 907.5031).

Ardisianoside E (6): amorphous powder, $[\alpha]^{22}_D$ −7.8 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3415, 2926, 1632, 1368, 1074 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 937 $[M + Na]^+$, 589 $[M + H - C_{12}H_{21}O_{10}]^+$, 457 $[M + H - C_{17}H_{28}O_{14}]^+$; positive-ion HRFABMS *m/z* 937.5157 (calcd for $C_{47}H_{78}O_{11}Na$, 937.5137).

Ardisianoside F (7): amorphous powder, $[\alpha]^{22}_D$ −13.2 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3406, 2923, 1632, 1376, 1075 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1247 $[M + Na]^+$, 607 $[M + H - C_{23}H_{39}O_{19}]^+$, 473 $[M + H - C_{28}H_{46}O_{23}]^+$; positive-ion HRFABMS *m/z* 1247.6060 (calcd for $C_{58}H_{96}O_{27}Na$, 1247.6037).

Ardisianoside G (15): amorphous powder, $[\alpha]^{22}_D$ −19.7 (*c* 0.9, MeOH); IR (KBr) ν_{max} 3403, 2925, 1386, 1073 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1085 $[M + Na]^+$, 883 $[M + H - C_6H_{10}O_5]^+$; positive-ion HRFABMS *m/z* 1085.5469 (calcd for $C_{52}H_{86}O_{22}Na$, 1085.5508).

Ardisianoside H (16): amorphous powder, $[\alpha]^{22}_D$ −19.4 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3416, 2924, 1756, 1632, 1376, 1074 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1569 $[M + Na]^+$, 1385 $[M + H - C_6H_{10}O_5]^+$, 1113 $[M + Na - C_{17}H_{30}O_{14}]^+$; positive-ion HRFABMS *m/z* 1569.7031 (calcd for $C_{70}H_{114}O_{37}Na$, 1545.6961).

Ardisianoside I (17): amorphous powder, $[\alpha]^{22}_D$ −29.4 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3417, 2929, 1743, 1632, 1369, 1249, 1074 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1113 $[M + Na]^+$, 937 $[M + H - C_7H_{13}O_6]^+$; positive-ion HRFABMS *m/z* 1113.5466 (calcd for $C_{53}H_{86}O_{23}Na$, 1113.5458).

Ardisianoside J (18): amorphous powder, $[\alpha]^{22}_D$ −28.2 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3418, 2928, 743, 1632, 1386, 1260, 1073 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1111 $[M + Na]^+$, 927 $[M + H - C_6H_{10}O_5]^+$, 619 $[M + H - C_{18}H_{31}O_{14}]^+$, 485 $[M + H - C_{23}H_{38}O_{18}]^+$; positive-ion HRFABMS *m/z* 1111.5347 (calcd for $C_{53}H_{84}O_{23}Na$, 1111.5301).

Ardisianoside K (19): amorphous powder, $[\alpha]^{22}_D$ −18.6 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3408, 2927, 1638, 1376, 1268, 1075 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1−4; positive-ion ESIMS *m/z* 1097 $[M + Na]^+$, 913 $[M + H - C_6H_{10}O_5]^+$, 603 $[M + H - C_{18}H_{31}O_{14}]^+$, 471 $[M + H - C_{23}H_{38}O_{18}]^+$; positive-ion HRFABMS *m/z* 1097.5465 (calcd for $C_{53}H_{86}O_{22}Na$, 1097.5508).

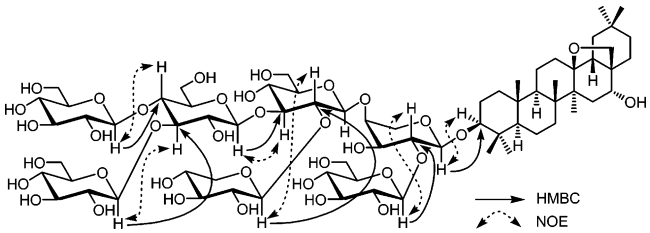


Figure 1. Key HMBC and NOE correlations for the saccharide units of ardisianoside A (**1**).

Acid Hydrolysis of the Saponins and Determination of the Absolute Configuration of Monosaccharides. A solution of **1** (10 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 100 °C for 2 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (2 mL × 3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column and concentrated under reduced pressure to dryness to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M D-cysteine methyl ester hydrochloride in pyridine (0.15 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 mL each), and the hexane extract was analyzed by GC-MS under the following conditions: capillary column, Equity-1 (30 m × 0.25 mm × 0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier, N_2 gas. In the acid hydrolysate of **1**, D-glucose, L-arabinose, and D-xylose were confirmed by comparison of the retention times of their derivatives with those of the derivatives of D-glucose, L-glucose, L-arabinose, and D-xylose prepared in a similar way, which showed retention times of 20.00, 19.43, 13.20, and 13.05 min, respectively. The constituent sugars of compounds **2**, **4**−**7**, and **15**−**19** were also identified by the same method.

Cytotoxicity Assay. Cells of human HL-60 myeloid leukemia, human stomach KATO-III adenocarcinoma, and human lung A549 adenocarcinoma were obtained from RIKEN Cell Bank (Tsukuba, Japan) and maintained in RPMI 1640 medium (Gibco RBL Co., Grand Island, NY) containing heat-inactivated 10% fetal bovine serum (Bio-Whittaker, Walkersville, MD) supplemented with L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (MeijiSeika, Tokyo, Japan). The leukemia cells were washed and resuspended in the above medium to 3 × 10^4 cells/mL, and 180 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% CO_2−air for 24 h at 37 °C. After incubation, 20 μL of a EtOH−H_2O (1:9) solution containing the sample was added to give the final concentrations of 0.1−100 μM/mL; 20 μL of EtOH−H_2O (1:9) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure. At the end of incubation, 10 μL of 5 mg/mL MTT (Sigma, St. Louis, MO) in phosphate-buffered saline was added to each well, and the plate was further incubated in 5% CO_2−air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* for 5 min to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from each well, and 175 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. The *T*/*C* (%) score was calculated by the formula given below, and graphs of the concentration of samples and *T*/*C* (%) were constructed. The concentration values of samples that crossed the *T*/*C* (%) were measured as the IC_{50} values. Data are mean values of two experiments performed in triplicate. *T*/*C* (%) = (*T* − *S*)/(*C* − *S*) × 100. *T*: OD_{550} values of the cell with samples after 3 days incubation. *C*: OD_{550} values of the cell without samples. *S*: OD_{550} values of the cell before samples were added. The IC_{50} value was defined as the concentration of sample necessary to inhibit the growth to 50% of the control.

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