Labdane Diterpenes from *Aster spathulifolius* and Their Cytotoxic Effects on Human Cancer Cell Lines

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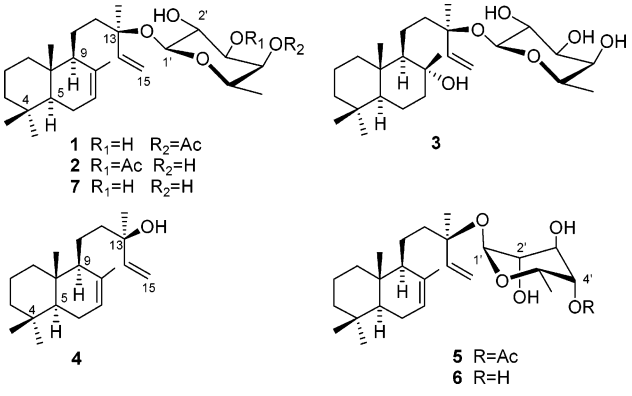
Three new labdane diterpenes (**1**−**3**), together with eight known diterpenoids, were isolated from a methanol extract of the aerial parts of *Aster spathulifolius*. The structures of **1**−**3** were determined as (13*R*)-labda-7,14-diene 13-*O*-*β*-D-(4′-*O*-acetyl)fucopyranoside (**1**), (13*R*)-labda-7,14-diene 13-*O*-*β*-D-(3′-*O*-acetyl)fucopyranoside (**2**), and (13*R*)-labda-14(15)-en-8,13-diol 13-*O*-*β*-D-fucopyranoside (**3**), on the basis of spectroscopic and chemical methods. Compounds **1**, **2**, and four of the known compounds exhibited generally nonspecific cytotoxicity against human A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 tumor cells.

Aster spathulifolius Maxim. (Asteraceae) is a perennial herb distributed along the eastern and southern coasts of South Korea, and its aerial parts have been used to treat asthma and diuresis in Korean traditional medicine.[1,2] We have investigated the secondary metabolites produced by plants belonging to the genus *Aster* and have reported cytotoxic diterpenoids and sesquiterpene peroxides from *Aster oharai* and *A. scaber*, respectively.[3,4] In the present study, three new diterpene glycosides (**1**−**3**) and eight known substances were isolated from methanol extracts of aerial parts of *A. spathulifolius* and their cytotoxicities against five human tumor cell lines were evaluated. The eight known compounds **7**α-hydroxymanool,[5] labda-7,14-dien-13-ol (**4**),[6] (13*R*)-labda-7,14-diene 13-*O*-α-L-(4′-*O*-acetyl)-6′-deoxyidopyranoside (**5**),[7,9] (13*R*)-labda-7,14-diene 13-*O*-α-L-6′-deoxyidopyranoside (**6**),[3,7,8] 13-*epi*-sclareol,[10,11] 13-*epi*-*ent*-manoyl oxide 18-oic acid,[12] (13*R*)-labda-7,14-diene 13-*O*-*β*-D-fucopyranoside (**7**),[7−9] and (13*R*)-labda-14(15)-ene-8,13-diol 13-*O*-α-L-6′-deoxyidopyranoside[9] were identified by comparing their physical and spectroscopic data with those reported in the literature.



Results and Discussion

Compound **1** was obtained as a colorless oil, and its molecular formula was assigned as $C_{28}H_{46}O_6$ on the basis of the sodiated molecular ion peak $[M + Na]^+$ at *m/z* 501.3187 in the HRFABMS. The IR spectrum showed the presence of hydroxyl and ester groups at 3446 and 1743 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectra of **1** were similar to those of a known diterpene glycoside, (13*R*)-labda-7,14-diene 13-*O*-*β*-D-fucopyranoside (**7**), except for the presence of signals for an acetyl group at δ_H 2.16 (3H, s) and δ_C 20.8 and 171.4 in the ^1H and ^{13}C NMR spectra of **1**. The H-4′ (δ 5.15) and C-4′ (δ 74.3) signals of **1** appeared more downfield than those of **7** (H-4′, δ 3.71; C-4′, δ 72.4). This supported the presence of an acetyl group at C-4′ in **1**. The glycosyl and acetyl linkages were also confirmed by the HMBC data, which showed correlations of H-1′ (δ 4.31) to C-13 (δ 81.1) and H-4′ (δ 5.15) to an acetyl carbon (δ 171.4), respectively (Figure 1). Alkaline hydrolysis[13] of **1** afforded **7**, which was identified by its ^1H NMR spectrum as well as by direct comparison by co-TLC (R_f 0.26, *n*-hexane−EtOAc, 1:1) using an authentic sample. Acid hydrolysis[14] of **7** yielded the aglycon, labda-7,14-dien-13-ol (**4**),[6] and a sugar. The sugar was identified by a GC analysis as an acetylated derivative as well as by co-TLC using an authentic fucose (R_f 0.61, $CHCl_3$−MeOH−H_2O, 9:5:0.5). Treatment of a sugar from the acid hydrolysis of **7** with L-cysteine methyl ester and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) afforded the trimethylsilyl ether of the methyl 2-(fucotetrahydroxybutyl)-thiazolidine-4(*R*)-carboxylate, which enabled the L- and D-monosaccharide derivatives to be readily separated from each other in a GC capillary column.[15] This experiment

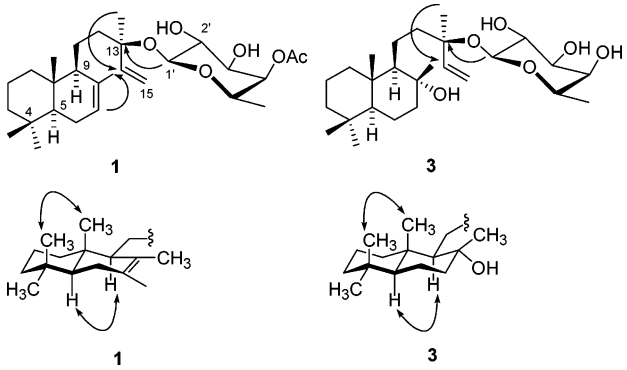


Figure 1. Key HMBC (the upper part) and NOESY (the lower part) correlations for compounds **1** and **3**.

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1**−**3** in $CDCl_3$[a]

	1		**2**		**3**	
position	δ_H (mult., Hz)	δ_C	δ_H (mult., Hz)	δ_C	δ_H (mult., Hz)	δ_C
1α	0.95 (td, 3.5, 12.5)	39.3	0.93 (td, 3.5, 12.5)	39.3	0.94 (m)	39.4
1*β*	1.78 (br d, 12.5)		1.81 (br d, 12.5)		1.81 (br d, 12.5)	
2	1.54 (m)	18.8	1.58 (m)	18.8	1.59 (m)	19.1
3α	1.16 (m)	42.3	1.17 (m)	42.3	1.13 (m)	42.6
3*β*	1.41 (m)		1.42 (m)		1.42 (m)	
4		32.9		33.0		33.3
5α	1.15 (m)	50.2	1.17 (m)	50.2	1.13 (m)	56.2
6α	1.95 (m)	23.8	1.96 (br d, 17.5)	23.8	1.98 (br d, 17.5)	22.3
6*β*	1.91 (m)		1.84 (m)		1.84 (m)	
7	5.39 (s)	122.3	5.38 (s)	122.3	1.74 (m)	43.6
8		135.3		135.3		74.6
9α	1.53 (m)	55.5	1.53 (m)	55.4	1.59 (m)	62.6
10		37.1		37.1		38.7
11	1.45 (m)	22.3	1.46 (m)	21.8	1.46 (m)	22.1
12	1.54 (m) 1.85 (m)	43.3	1.55 (m) 1.84 (m)	43.5	1.55 (m) 1.84 (m)	44.8
13		81.1		81.2		82.4
14	6.00 (dd, 10.5, 17.5)	142.2	6.02 (dd, 11.0, 17.5)	142.2	5.92 (dd, 10.5, 17.5)	146.8
15	5.19 (dd, 1.0, 17.5) 5.21 (dd, 1.0, 11.0)	115.1	5.18 (dd, 1.0, 17.5) 5.21 (dd, 1.0, 11.0)	115.1	5.20 (dd, 1.5, 17.5) 5.00 (dd, 1.5, 10.5)	111.8
16	1.34 (s)	21.8	1.34 (s)	21.9	1.27 (s)	27.4
17	1.69 (s)	22.3	1.68 (s)	22.3	1.37 (s)	23.9
18	0.86 (s)	33.1	0.86 (s)	33.2	1.02 (s)	34.0
19	0.88 (s)	21.5	0.88 (s)	21.5	0.89 (s)	22.2
20	0.75 (s)	13.6	0.75 (s)	13.6	0.79 (s)	15.9
fucose						
1′	4.31 (d, 8.0)	97.4	4.36 (d, 8.0)	97.9	4.32 (d, 8.0)	97.6
2′	3.60 (dd, 8.0, 10.0)	71.7	3.73 (dd, 8.0, 10.0)	69.3	3.59 (dd, 8.0, 10.0)	71.8
3′	3.76 (dd, 4.0, 10.0)	72.4	4.84 (dd, 3.0, 10.0)	75.7	3.54 (br dd, 3.0, 10.0)	71.4
4′	5.15 (br d, 3.0)	74.3	3.81 (br d, 3.0)	70.3	3.63 (br d, 3.0)	72.6
5′	3.65 (dq, 1.0, 6.0)	69.2	3.61 (dq, 1.0, 6.0)	70.2	3.58 (br dq, 1.0, 6.0)	70.9
6′	1.18 (d, 6.0)	16.5	1.18 (d, 6.0)	16.3	1.21 (d, 6.0)	16.6
$COCH_3$	2.16 (s)	20.8 171.4	2.17 (s)	21.1 170.6		

[a] Assignments were based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

allowed us to determine the absolute stereochemistry of the sugar unit in **1** as D-fucose. The *β*-configuration of the D-fucose was determined by the coupling constant (8.0 Hz) of the anomeric proton signal in the ^{1}H NMR spectrum.[16] The absolute configuration of C-13 of **1** was assigned the *R*-form by comparing the optical rotation data of (13*R*)-labda-7,14-dien-13-ol ($[\alpha]_D$ −1.8°) and (13*S*)-labda-7,14-dien-13-ol ($[\alpha]_D$ +20.0°).[6] The optical rotation of the aglycon ($[\alpha]_D$ −2.3°) obtained from acid hydrolysis with **1** was almost the same as that of (13*R*)-labda-7,14-dien-13-ol. Furthermore, the optical rotation ($[\alpha]_D$ −63.3°) of **1** was in accordance with that of **7** ($[\alpha]_D$ −58.4°). The NOESY spectrum of **1** showed corresponding NOE correlations of the stereochemistry of **7** (Figure 1). Therefore, the structure of compound **1** was determined as (13*R*)-labda-7,14-diene 13-*O*-*β*-D-(4′-*O*-acetyl)fucopyranoside.

Compound **2** was obtained as a colorless oil, and its molecular formula was determined as $C_{28}H_{46}O_6$ on the basis of the quasimolecular ion peak $[M + Na]^+$ at *m/z* 501.3190 in the HRFABMS. The IR and NMR spectra of **2** were almost the same as those of **1**. The only difference was the position of the acetyl group, whose location was determined to be at C-3′ by a comparison with the NMR data of the *β*-D-fucopyranose unit in **7**. The H-3′ (*δ* 4.84) and C-3′ (*δ* 75.7) signals in **2** appeared more downfield than those of **7** (H-3′, *δ* 3.58; C-3′, *δ* 71.3). The HMBC spectrum of **2** showed a correlation between H-3′ (*δ* 4.84) and an acetyl carbon (*δ* 170.6). Alkaline hydrosis[13] of **2** afforded (13*R*)-labda-7,14-diene 13-*O*-*β*-D-fucopyranoside, which was identified from its ^{1}H NMR spectrum as well as by direct comparison by co-TLC (R_f 0.26, *n*-hexane−EtOAc, 1:1) with **7**. Analysis of its ^{1}H−^{1}H COSY, HMQC, and HMBC spectra allowed the assignment of all ^{1}H and ^{13}C NMR signals for **2** (Table 1). Therefore, the structure of **2** was determined as (13*R*)-labda-7,14-diene 13-*O*-*β*-D-(3′-*O*-acetyl)fucopyranoside.

Compound **3** was obtained as a colorless oil with a molecular formula of $C_{26}H_{46}O_6$ on the basis of the quasimolecular ion peak $[M + Na]^+$ at *m/z* 477.2958 in the HRFABMS. The IR spectrum showed the presence of a hydroxyl group at 3452 cm^{-1}. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **3** with those of (13*R*)-labda-14(15)-ene-8,13-diol 13-*O*-α-L-6′-deoxyidopyranoside showed that these two compounds were identical except for the nature of the sugar moiety. The signals in the sugar unit in the ^{1}H and ^{13}C NMR spectra of **3** appeared at δ_H 1.21 (3H, d, J = 6.0 Hz), 3.54 (1H, br dd, J = 3.0, 10.0 Hz), 3.58 (1H, br dq, J = 1.0, 6.0 Hz), 3.59 (1H, dd, J = 8.0, 10.0 Hz), 3.63 (1H, br d, J = 3.0 Hz), and 4.32 (1H, d, J = 8.0 Hz) and δ_C 16.6, 70.9, 71.4, 71.8, 72.6, and 97.6, respectively. The ^{1}H and ^{13}C NMR signals in the sugar moiety of **3** were almost the same as those of (13*R*)-labda-7,14-diene 13-*O*-*β*-D-fucopyranoside,[8,9] which was also isolated from *A. spathulifolius*, indicating that the sugar in **3** is *β*-D-

Table 2. Cytotoxicity of Compounds Isolated from *Aster spathulifolius*[a]

	cell line				
compound[b]	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	3.9	5.8	3.4	8.9	4.1
2	3.6	3.8	3.5	5.1	3.8
4	2.4	8.7	4.4	9.5	9.1
5	3.2	3.4	3.5	3.8	3.1
6	3.9	5.7	4.2	8.9	3.2
7	3.5	3.6	3.5	4.2	3.9
etoposide	1.5	2.7	0.08	2.6	2.2
doxorubicin	0.012	0.12	0.003	0.01	0.35

[a] ED_{50} is defined as the concentration (μg/mL) causing a 50% inhibition of cell growth in vitro. [b] All other known compounds obtained in this investigation exhibited ED_{50} values of >5 μg/mL for all cell lines.

fucopyranose. Acidic hydrolysis[14] of **3** yielded the aglycon, labda-14(15)-ene-8,13-diol, and a sugar. The aglycon was confirmed by comparison of the optical rotation, ^{1}H NMR spectroscopic, and EIMS data with literature values,[10,11,17] and the sugar was identified by co-TLC and by GC.[15] The analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra of **3** allowed the assignment of all ^{1}H and ^{13}C NMR signals for this substance (Table 1). Therefore, the structure of compound **3** was determined as (13*R*)-labda-14(15)-ene-8,13-diol 13-*O*-*β*-D-fucopyranoside.

Compounds **1**−**3** and eight of the known compounds isolated from *A. spathulifolius* were evaluated for their cytotoxicity against five human tumor cell lines (Table 2). Compounds **1**, **2**, and **4**−**7** showed generally nonspecific cytotoxicity against the cell lines tested. The other compounds were inactive (ED_{50} >5 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO P-1020 polarimeter. The UV spectra were recorded on a Shimazu UV 1601 spectrophotometer, and the IR spectra were recorded on a Nicolet model 205 instrument. The NMR spectra were obtained on either a Bruker AMX or a Varian Unity INOVA 500 NMR spectrometer in $CDCl_3$. The EIMS and HRFABMS data were obtained on a JEOL JMS 700 mass spectrometer. The GC data were obtained on a Hewlett-Packard 6890 gas chromatograph-5973 mass selective detector with a HP-5SM column (30 m × 0.25 mm × 0.25 μm). A Knauer preparative HPLC with a refractive index detector, UV detector, and Econosil C_{18} 10 μm column (10 × 250 mm) was used for preparative HPLC. Low-pressure liquid chromatography was carried out using a Merck Lichroprep Lobar-A Si 60 (240 × 10 mm) or Lichroprep Lobar-A RP-18 (240 × 10 mm) column with a FMI QSY-0 pump. Open column chromatography was performed using silica gel (Merck, 70−230 mesh and 230−400 mesh) or Sephadex LH-20 (Pharmacia).

Plant Material. *Aster spathulifolius* Maxim. was collected in Jeju Island, Korea, in August 2001. This plant was identified by Seung Jo Yoo, an Emeritus Professor at Sungkyunkwan University. A voucher specimen (SKK-01-020) is deposited in the College of Pharmacy at Sungkyunkwan University.

Extraction and Isolation. The partially dried and chopped aerial parts of *A. spathulifolius* (8.3 kg) were extracted three times with MeOH at room temperature. The resulting MeOH extract (300 g) followed by successive solvent partition gave hexane (32 g), CH_2Cl_2 (20 g), EtOAc (8 g), and BuOH (30 g) fractions. The hexane fraction (32 g) was chromatographed over a silica gel column by gradient elution with hexane−EtOAc (10:1−0:1) to give four subfractions [S1 (12 g), S2 (6.9 g), S3 (6.5 g), and S4 (4 g)]. 7α-Hydroxymanool (10 mg) and compounds **4** (20 mg) and **5** (15 mg) were isolated by repeated silica gel normal-phase column chromatography using *n*-hexane−EtOAc from the S1, S2, and S3 subfractions, respectively, and compounds **1** (12 mg), **2** (8 mg), **6** (500 mg), and 13-*epi*-sclareol (8 mg) were obtained from subfraction S4. The CH_2Cl_2 fraction (20 g) was chromatographed on a silica gel column (hexane−EtOAc, 3:1 and 1:1) to provide five subfractions (M1−M5). Successive column chromatography using a Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1), a silica gel (hexane−EtOAc, 1:1), and a Lobar-A RP-18 (100% MeCN) column led to the isolation of 13-*epi*-*ent*-manoyl oxide 18-oic acid (18 mg) from subfraction M3 (1.3 g), and compound **7** (8 mg) and (13*R*)-labda-14(15)-ene-8,13-diol 13-*O*-α-L-6′-deoxyidopyranoside (12 mg) were obtained from subfraction M5 (6.1 g). The EtOAc fraction (8 g) was chromatographed over a silica gel column ($CHCl_3$−EtOAc−MeOH, 3:2:1) to give five subfractions (E1−E5). Subfraction E5 (2 g) was also purified, in turn, using Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and Lobar-A RP-18 (50% MeOH) columns to yield compound **3** (7 mg).

(13*R*)-Labda-7,14-diene 13-*O*-*β*-D-(4′-*O*-acetyl)fucopyranoside (1): colorless oil; $[\alpha]^{20}_D$ −63.3° (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3446, 1743, 1645, 1366, 1237, 1041 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 501.3187 (calcd for $C_{28}H_{46}O_6Na$, 501.3192).

(13*R*)-Labda-7,14-diene 13-*O*-*β*-D-(3′-*O*-acetyl)-fucopyranoside (2): colorless oil; $[\alpha]^{20}_D$ −51.1° (*c* 0.04, $CHCl_3$); IR (neat) ν_{max} 3461, 1740, 1640, 1355, 1240, 1038 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 501.3190 (calcd for $C_{28}H_{46}O_6Na$, 501.3192).

(13*R*)-Labda-14(15)-en-8,13-diol 13-*O*-*β*-D-fucopyranoside (3): colorless oil; $[\alpha]^{20}_D$ −43.5° (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 3452, 1235, 1040 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 477.2958 (calcd for $C_{26}H_{46}O_6Na$, 477.2963).

Alkaline Hydrolysis of Compounds 1 and 2. A solution of either compound **1** or **2** (each, 2 mg) in 10% dry NaOMe−MeOH (1 mL) was stirred at 40 °C for 2 h. The reaction mixture was neutralized with 2 N HCl and partitioned between H_2O and *n*-hexane. The *n*-hexane layer was purified by silica gel column chromatography (*n*-hexane−EtOAc, 1:1) to afford (13*R*)-labda-7,14-diene 13-*O*-*β*-D-fucopyranoside (**7**) (each, 0.5 mg).

Acidic Hydrolysis. To a solution of each of compounds **3** (2 mg) and **7** (3 mg) in Me_2CO (3 mL) was added concentrated HCl (0.02 mL), and the mixture was stirred for 10 days at room temperature.[14] Each reaction mixture was shaken with 5 mL of water−$CHCl_3$. The $CHCl_3$ layer was evaporated in vacuo and subjected to silica gel column chromatography eluted with $CHCl_3$−MeOH−H_2O (50:10:1) to give the aglycons labda-14(15)-en-8,13-diol and labda-7,14-dien-13-ol (**4**), respectively. The aqueous layer was concentrated to dryness in vacuo, separated over silica gel column chromatography eluted with $CHCl_3$−MeOH−H_2O (30:10:1), and then purified by a Sephadex LH-20 (MeOH) to afford a sugar.

Preparation of Trimethylsilyl Ether of the Methyl 2-(Fucotetrahydroxybutyl)thiazolidine-4(*R*)-carboxylate. To a solution of the sugar (2.2 mg, 0.007 mol/L) in pyridine (2 mL) was added L-cysteine methyl ester hydrochloride (3.4 mg, 0.01mol/L), and the mixture was stirred for 3 h at room temperature.[15] Excess pyridine was removed with a vacuum pump and the reaction mixture dissolved in CH_3CN (3 mL) and MeOH (2 mL). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (20 μL) and CH_3CN (20 μL) were added, and the solution was kept for 30 min at 60 °C. The supernatant was subjected to GC analysis: column DB-5 MS (15 m × 0.25 mm × 0.25 μm), detector MS (ionization EI), temperature 100 °C (1 min, 10 °C/min) to 300 °C (5 min), injector temperature 100 °C, detector temperature 300 °C, carrier gas He (1.0 mL/min).

Cytotoxicity Evaluation. Cytotoxicity testing was performed in vitro using the SRB (sulforhodamine B) method[18] against five human tumor cell lines, A549 (non-small-cell lung carcinoma), SK-OV-3 (adenocarcinoma, ovarian malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nervous system tumor), and HCT15 (colon adenocarcinoma), at the Korea Research Institute of Chemical Technology. Etoposide and doxorubicin were used as positive controls.

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Supporting Information Available: ^{1}H and ^{13}C NMR data for compounds **1**, **2**, **3**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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