Five New Labdane Diterpenes from *Aster oharai*

Sang Zin Choi,[†] Hak Cheol Kwon,[†] Sang Un Choi,[‡] and Kang Ro Lee*[,†]

Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, Suwon 440-746, Republic of Korea, and Korea Research Institute of Chemical Technology, Taejeon 305-600, Republic of Korea

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Five new labdane diterpenes (**1**−**5**) together with seven known diterpenes have been isolated from a methanol extract of the aerial parts of *Aster oharai*. Five new structures were determined as 7α-hydroperoxylabda-8(17),14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (**1**), 7α-hydroperoxylabda-8,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (**2**), labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-rhamnopyranoside (**3**), labda-7,14-dien-13(*R*)-ol-3-*O*-acetyl-α-L-rhamnopyranoside (**4**), and labda-7,14-dien-13(*R*)-ol-2-*O*-acetyl-α-L-rhamnopyranoside (**5**), on the basis of spectroscopic methods. Compound **1** showed moderate cytotoxicity against four cultured human tumor cell lines with ED_{50} values ranging from 1.1 to 7.7 μg/mL.

Aster oharai Nakai (Asteraceae), a perennial herb, is distributed mainly in the eastern part of South Korea, and its aerial parts have been used to treat asthma and diuresis in Korean traditional medicine.[1] We have reported cytotoxic sesquiterpene peroxides and antiviral quinic acid derivatives from *Aster scaber*.[2,3] In continuation of our research on Korean *Aster* species, 12 labdane diterpenes, including five new compounds (**1**−**5**), were isolated from the hexane-soluble fraction of the methanol extract from the aerial parts of *A. oharai*. The cytotoxic effects of the isolated diterpenes were investigated against eight cultured human cancer cell lines in vitro, and compound **1** showed moderate cytotoxicity against four of the cell lines. The present paper describes the isolation, structural characterization, and biological activity of these diterpenes.

Results and Discussion

Six known compounds, 8,13-epoxy-14-labdane-18-ol,[4] labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside,[5,6] *ent*-13-epi-manoyloxide-18-oic acid,[7] labda-14-en-8,-13-diol,[8,9] labda-7,14-dien-13(*R*)-ol-α-L-6-deoxyidopyranoside,[5,6] and *ent*-manoyloxide-18-oic acid,[7] were characterized by comparing their physical and spectroscopic data with the reported values. Although labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (**6**)[6,10] was previously reported, no ^{1}H and ^{13}C NMR data were available.

Compound **1** was obtained as a colorless oil and gave a positive reaction with peroxide reagent.[11] The molecular formula was assigned as $C_{28}H_{46}O_8$ on the basis of the sodiated molecular ion peak $[M + Na]^+$ that appeared at *m*/*z* 533.3080 in the HRFABMS. The IR spectrum showed the presence of a hydroxyl group at 3421 cm^{-1}. An anomeric signal and a methyl group at C-5′ arising from the sugar moiety appeared at δ 5.04 (1H, s) and 1.17 (3H, d, J = 6.5 Hz), respectively. The signals from the sugar unit appeared at δ_H 1.17 (3H, d, J = 6.5 Hz), 3.58 (1H, br s), 3.86 (1H, br s), 4.50−4.53 (1H, m), 4.90 (1H, m), and 5.04 (1H, s) and δ_C 16.1, 61.6, 68.6 (×2), 73.0, and 96.3 in the ^{1}H and ^{13}C NMR spectra, respectively. ^{1}H and ^{13}C NMR spectra also showed signals for an acetyl group at δ_H 2.15 (3H, s) and at δ_C 20.8 and 171.0, respectively. These data were very similar to those of labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside, which was isolated from *Aster spathulifolius*,[12] suggesting that the sugar in **1** is 4-*O*-acetyl-α-L-6-deoxyidopyranose. In addition, the ^{1}H NMR spectrum showed signals for four methyl groups at δ 0.68 (3H, s), 0.80 (3H, s), 0.87 (3H, s), and 1.42 (3H, s), a carbinol proton at δ 4.50−4.53 (1H, s), an exomethylene group at δ 4.81 (1H, s) and 5.18 (1H, s), and the ABM coupling system of three olefinic protons at δ 5.25 (1H, dd, J = 0.5, 18.0 Hz), 5.30 (1H, dd, J = 0.5, 11.0 Hz), and 5.80 (1H, dd, J = 11.0, 18.0 Hz). In the ^{13}C NMR spectrum, 20 carbon signals appeared besides those of the sugar unit, which included four olefinic carbons at δ 113.2, 117.0, 141.0, and 146.2 and two oxygenated carbons at δ 82.1 and 87.2. ^{1}H NMR signals of the aglycon of **1** were very similar to those of labda-8(17),14-diene-7α,13-diol (7α-hydroxymanool).[13] The major difference was the chemical shift of H-7 (**1**, δ 4.50; 7α-hydroxymanool, δ 5.01). Cambie et al. reported that the coplanar exomethylene group induced a downfield shift of the H-7 signal in 7α-hydroxymanool.[13] The hydroperoxy group at C-7 in **1** may reduce the anisotropic effect of the C-8(17) exomethylene group and the upfield shift induced on H-7. Enzymatic hydrolysis and treatment with triphenylphosphine of **1** yielded labda-8(17),14-diene-7α,13-diol (**1a**), whose ^{1}H NMR spectrum was in good agreement with values reported previously,[13] and a sugar. The sugar was confirmed as L-6-deoxyidose by the comparison of a optical rotation and GC analysis with those of a sugar from the enzymatic hydrolysis of labda-7,14-dien-13(*R*)-ol-α-L-6-deoxyidopyranoside. Martin et al. reported that the β-methyl group (axial) at C-4 in labdane diterpenes was shifted upfield when compared with the α-methyl group (equatorial) in the ^{13}C NMR spectrum.[14] Accordingly, C-19 (δ 21.1) was assigned as being β-oriented (axial), and C-18 (δ 33.4) α-oriented (equatorial). The NOESY spectrum of **1** showed correlation signals of H-19 (δ 0.80)/H-20 (δ 0.68) and H-7 (δ 4.50−4.53)/H-17a (δ 5.18) (Figure 2). The absence of any NOE correlation between H-20 and H-5 indicated their *trans* configuration,[15] So, H-5 could be assigned with the α-orientation (axial). A NOE correlation [H-5 (δ 1.39)/H-9 (δ 1.98)] showed that the relative stereochemistry of H-9 was also α-oriented (axial). The NOE correlation between H-7 and H-17a confirmed their coplanar relationship and the β-orientation (equatorial) of H-7.[13] The C-13 stereochemistry in the known diterpene glycosides, labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside, labda-7,14-dien-13(*R*)-ol-α-L-6-deoxyidopyrano-

* To whom correspondence should be addressed. Tel: 82-31-290-7710. Fax: 82-31-292-8800. E-mail: krlee@yurim.skku.ac.kr.
† SungKyunKwan University.
‡ Korea Research Institute of Chemical Technology.

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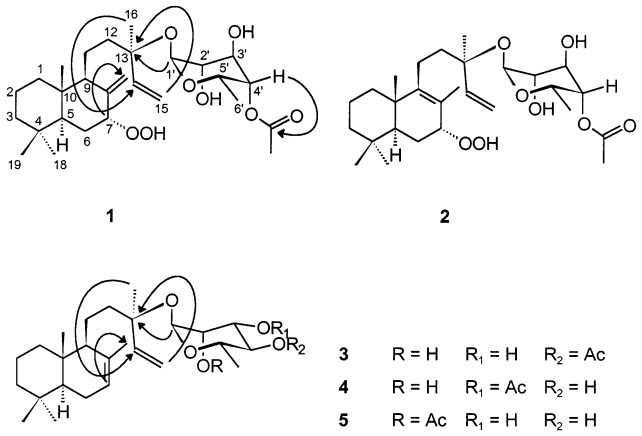

Figure 1. Structures of compounds **1**−**5** and selected HMBC correlations of compounds **2** and **3**.

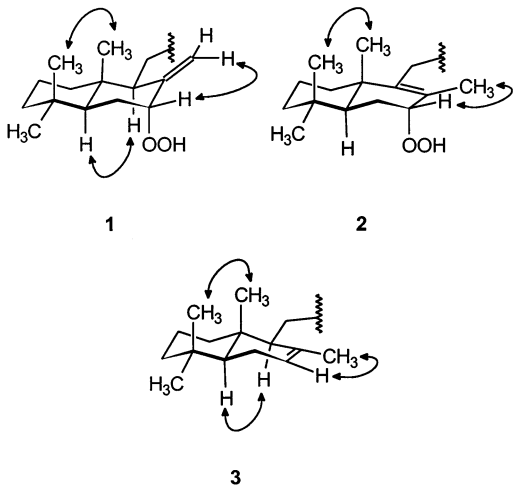

Figure 2. Key NOESY correlations for compounds **1**, **2**, and **3**.

side, and labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (**6**) from *A. oharai*, all have been assigned with the 13*R*-configuration. The signals of C-12, C-13, C-14, and C-16 in the ^{13}C NMR spectrum of **1** were very similar to those of these three known diterpenes. The C-13 stereochemistry in **1** was suggested to be in the *R*-configuration by comparison of the chemical shift in the ^{13}C NMR spectrum and also from biogenetic considerations. Analysis of the HMBC and HMQC spectra led to the assignment of all proton and carbon signals for **1** (Tables 1 and 2). Thus, the structure of **1** was determined as 7α-hydroperoxylabda-8(17),14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside.

Compound **2** was obtained as colorless oil and gave a positive reaction with peroxide reagent.[11] The molecular formula was assigned as $C_{28}H_{46}O_8$ on the basis of the sodiated molecular ion peak $[M + Na]^+$ that appeared at *m*/*z* 533.3071 in the HRFABMS. The IR spectrum showed the presence of a hydroxyl group at 3446 cm^{-1}. The NMR spectra of **2** were very similar to those of compound **1**, but the major difference was the absence of signals for an exomethylene group in **2**. A methyl group signal at δ 1.71 was observed in the ^{1}H NMR spectrum of **2** instead of the exomethylene group signals in **1**. In the ^{13}C NMR spectrum of **2**, two quaternary olefinic carbon signals (δ 122.6 and 149.6) were observed instead of signals for an exomethylene group (δ 113.2 and 146.2) in **1**. These observations suggested that the structure of **2** was 7-hydroperoxylabda-8,-14-dien-13-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside. Analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra permitted the assignment of all proton and carbon signals for **2** and the location of the two double bonds, the hydroperoxy group, and the glycosyl linkage (Tables 1 and 2). Enzymatic hydrolysis of **2** and reaction with triphenylphosphine yielded labda-8,14-dien-7,13-diol (**2a**), which was identified by the comparison of ^{1}H NMR data of **1a** and labda-8,14-dien-13-ol,[16] and a sugar. The sugar was confirmed to be L-6-deoxyidose by comparison of the optical rotation and by GC analysis with those of a sugar from the enzymatic hydrolysis of labda-7,14-dien-13(*R*)-ol-α-L-6-deoxyidopyranoside. The NOESY spectrum of **2** supported the relative stereochemistry of C-20 and C-7 as having the same orientations as those of **1** (Figure 2). The structure of compound **2**, therefore, was determined as 7α-hydroperoxylabda-8,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside.

Compound **3** was obtained as a colorless oil, and its molecular formula was determined as $C_{28}H_{46}O_6$ from the HREIMS (M^+ *m*/*z* 478.3300). The IR spectrum showed the presence of hydroxyl and ester groups at 3446 and 1740 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectra were very similar to those of the known diterpene, labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (**6**). ^{1}H and ^{13}C NMR spectra of **3** showed also acetyl group signals at δ_H 2.15 (3H, s) and at δ_C 20.9 and 172.0, respectively. The H-4′ (δ 4.77) and C-4′ (75.9) signals of **3** appeared more downfield than those of **6** (**6**, H-4′, δ 3.42; C-4′, δ 74.0). This indicated the presence of an acetyl group at C-4′ in **3**. Alkaline hydrolysis[18] of **3** afforded labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (**3a**), which was identified by co-TLC (R_f; 0.25, *n*-hexane−EtOAc, 1:2) with **6** and from its ^{1}H NMR spectrum. The enzymatic hydrolysis[19] of labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside yielded the aglycon, labda-7,14-dien-13-ol, and a sugar. The aglycon was confirmed by comparison of the optical rotation, ^{1}H NMR, and EIMS data with literature values,[17] and the sugar was identified by co-TLC with authentic L-rhamnose (R_f: 0.31, $CHCl_3$−MeOH−H_2O, 30:10:1) and GC analysis.[20] The glycosyl and acetyl linkages were confirmed by HMBC data, which showed the correlation of H-1′ (δ 4.99) to C-13 (δ 80.0) and the acetyl group (δ 2.15) to C-4′ (δ 75.9), respectively (Figure 1). The NOESY spectrum of **3** supported the relative stereochemistry at C-5, C-9, C-10, and C-13 of **3** as being the same in labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (**6**) (Figure 2). The structure of **3**, therefore, was determined as labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-rhamnopyranoside.

Compound **4** was obtained as a colorless oil, and its molecular formula was determined as $C_{28}H_{46}O_6$ from the HREIMS (M^+ *m*/*z* 478.3313). The IR, UV, and NMR spectra of **4** were almost the same as those of **3**. The only difference was the position of an acetyl group, whose location was determined to be at C-3′ by comparison with the NMR data of the α-L-rhamnopyranoside sugar unit in **6**. The signals of H-3′ (δ 5.08) and C-3′ (δ 75.2) in **4** appeared in more downfield regions than those of **6** (**6**, H-3′, δ 3.80; C-3′, δ 72.0). The HMBC spectrum of **4** showed a correlation of H-3′ (δ 5.08) with an acetyl carbon (δ 171.7). The analysis of the ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra of **4** allowed the assignment of all proton and carbon NMR signals in **4** (Tables 1 and 2). Thus, the structure of **4** was determined as labda-7,14-dien-13(*R*)-ol-3-*O*-acetyl-α-L-rhamnopyranoside.

Compound **5** was obtained as a colorless oil, and its molecular formula was determined as $C_{28}H_{46}O_6$ from the HREIMS (M^+ *m*/*z* 478.3307). The IR, UV, and NMR spectra were almost the same as those of compound **3**. The major

Table 1. ^{1}H NMR Data (δ in ppm, J in Hz) for Compounds **1**−**6** (500 MHz, in $CDCl_3$)

H	1	2	3	4	5	6
1α	1.07 td (3.5, 12.5)	1.12 td (3.5, 12.5)	0.96 td (3.5, 12.5)	0.95 td (3.5, 12.5)	0.95 td (3.5, 12.5)	0.95 td (3.5, 12.5)
1*β*	1.71 br d (12.5)	1.73 br d (12.5)	1.81 br d (12.5)	1.81 br d (12.5)	1.82 br d (12.5)	1.82 br d (12.5)
2	1.46−1.54 m	1.57 m	1.46−1.54 m	1.46−1.54 m	1.46−1.54 m	1.46−1.54 m
3α	1.19 m	1.19 m	1.16 m	1.18 m	1.18 m	1.16 m
3*β*	1.40 m	1.45 m	1.42 m	1.41 m	1.42 m	1.43 m
5α	1.39 m	1.40 m	1.17 m	1.17 m	1.18 m	1.17 m
6α	2.04 m	2.21−2.23 m	1.95 br d (17.5)	1.96 br d (17.5)	1.96 br d (17.5)	1.96 br d (17.5)
6*β*	1.59 m	1.44 m	1.85 m	1.84 m	1.86 m	1.83 m
7	4.50−4.53 br m	4.23 br d (3.0)	5.39 br s	5.39 br s	5.39 br s	5.39 br s
9α	1.98 br d (9.5)		1.56 m	1.55 m	1.56 m	1.56 m
11	1.52 m	1.92 m	1.46−1.54 m	1.46−1.54 m	1.46−1.54 m	1.46−1.54 m
12	1.40/1.79 m	1.63/1.97 m	1.51/1.81 m	1.52/1.81 m	1.55/1.81 m	1.50/1.82 m
14	5.80 dd (11.0, 18.0)	5.79 dd (11.0, 17.5)	5.75 dd (11.0, 17.5)	5.74 dd (11.0, 18.0)	5.75 dd (11.0, 17.5)	5.75 dd (10.5, 17.0)
15	5.25 dd (0.5, 18.0)	5.25 dd (0.5,17.5)	5.21 dd (1.0, 17.5)	5.20 dd (1.0, 18.0)	5.21 dd (1.0, 17.5)	5.20 dd (1.0, 17.0)
16	5.30 dd (0.5, 11.0)	5.30 dd (0.5,11.0)	5.25 dd (1.0, 11.0)	5.25 dd (1.0, 11.0)	5.25 dd (1.0, 11.0)	5.24 dd (1.0, 10.5)
17	1.42 s	1.43 s	1.36 s	1.36 s	1.36 s	1.36 s
18	4.81/5.18 s	1.71 s	1.68 s	1.69 s	1.69 s	1.68 s
19	0.87 s	0.95 s	0.86 s	0.86 s	0.87 s	0.86 s
20	0.80 s	0.87 s	0.89 s	0.88 s	0.89 s	0.89 s
	0.68 s	0.92 s	0.76 s	0.76 s	0.76 s	0.76 s
sugar						
1′	5.04 s	5.03 s	4.99 s	4.93 d (2.0)	4.95 d (2.0)	4.95 s
2′	3.58 br s	3.56 br d (3.0)	3.81 br s	3.88 dd (2.0, 3.0)	4.94 dd (2.0, 3.0)	3.80 br s
3′	3.86 br s	3.85 m	3.90 dd (3.0, 9.5)	5.08 dd (3.0, 9.5)	3.98 dd (3.0, 9.5)	3.80 br s
4′	4.90 m	4.90 m	4.77 t (9.5)	3.59 t (9.5)	3.45 t (9.5)	3.42 t (9.0)
5′	4.50−4.53 br m	4.50 dq (2.0, 6.5)	3.97 dq (6.0, 9.5)	3.91 dq (6.0, 9.5)	3.86 dq (6.0, 9.0)	3.86 dq (6.0, 9.0)
6′	1.17 d (6.5)	1.16 d (6.5)	1.18 d (6.0)	1.18 d (6.0)	1.30 d (6.0)	1.29 d (6.0)
Ac	2.15 s	2.14 s	2.15 s	2.17 s	2.17 s	

Table 2. ^{13}C NMR Data (δ in ppm) for Compounds **1**−**6** (125 MHz, in $CDCl_3$)

C	1	2	3	4	5	6
1	38.9	36.2	39.2	39.2	39.2	39.2
2	17.8	18.8	18.8	18.8	18.8	18.8
3	42.1	41.4	42.3	42.3	42.3	42.3
4	33.4	33.0	32.9	32.9	32.9	32.9
5	48.6	45.6	50.2	50.2	50.2	50.2
6	27.6	22.9	23.8	23.8	23.8	23.8
7	87.2	84.6	122.2	122.2	122.3	122.3
8	146.2	122.6	135.2	135.3	135.3	135.3
9	52.3	149.6	55.2	55.3	55.2	55.2
10	40.0	40.1	37.0	37.1	37.0	37.0
11	19.5	22.4	21.0	21.1	21.0	21.8
12	41.0	41.0	44.5	44.5	44.6	44.6
13	82.1	81.4	80.2	80.4	80.6	80.2
14	141.0	140.6	141.7	141.6	141.5	141.8
15	117.0	117.0	116.0	116.2	116.2	116.1
16	22.5	21.7	21.9	21.8	22.0	21.8
17	113.2	17.3	22.1	22.1	22.2	22.1
18	33.4	32.9	33.1	33.1	33.1	33.1
19	21.1	21.5	21.8	21.8	21.8	21.8
20	13.7	18.4	13.6	13.6	13.6	13.6
sugar						
1′	96.3	96.2	94.4	94.5	92.8	94.8
2′	68.6	68.4	72.1	71.0	73.8	72.2
3′	68.6	68.4	70.4	75.2	70.6	72.0
4′	73.0	72.8	75.9	72.0	73.8	74.0
5′	61.6	61.3	65.4	68.4	67.7	67.5
6′	16.1	15.9	17.4	17.5	17.5	17.5
Ac	20.8	18.8	20.9	21.0	21.0	
	171.0	169.4	172.0	171.7	171.0	

difference was a downfield shift of H-2′ and an upfield shift of H-4′ in the ^{1}H NMR spectrum of **5** (**5**, H-2′, δ 4.94, H-4′, δ 3.45; **3**, H-2′, δ 3.81, H-4′ δ 4.77). The HMBC spectrum showed a correlation of H-2′ (δ 4.94) with an acetyl carbon (δ 171.0) in **5**. Thus, the structure of **5** was determined as labda-7,14-dien-13(*R*)-ol-2-*O*-acetyl-α-L-rhamnopyranoside.

Twelve isolates were evaluated for their cytotoxicity against eight human tumor cell lines. Compound **1** showed moderate activity against human colorectal cancer cells (HCT15, 1.1 μg/mL), human central nervous system cancer cells (SNB-19, 1.3 μg/mL), and human skin cancer cells (SK-MEL-2 and LOX IMVI, 1.3 and 1.5 μg/mL). The other compounds showed no significant cytotoxic activity against any of the human cancer cell lines tested (ED_{50} >10 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were recorded with a Shimadzu UV 1601 spectrophotometer. IR spectra were recorded with a Nicolet model 205 instrument. NMR spectra were recorded on either a Bruker AMX or a Varian UNITY INOVA 500 NMR spectrometer in $CDCl_3$. EIMS, HREIMS, and HR-FABMS data were obtained on a JEOL JMS700 mass spectrometer, and ESIMS data were taken on a Micro Mass Quattro II mass spectrometer. Preparative HPLC used a JAI LC-908 instrument with refractive index detector, UV detector, and Econosil C_{18} 10 μm column (250 mm × 22 mm). Open column chromatography was carried out over silica gel (Merck, 70−230) or Sephadex LH-20 (Pharmacia). Low-pressure liquid chromatography was carried out over Merck Lichroprep Lobar-A Si 60 (240 × 10 mm) or a Lichroprep Lobar-B RP-18 (240 × 10 mm) column with a FMI QSY-0 pump (ISCO).

Plant Material. *Aster oharai* was collected on Ullung Island, Kyongsangbuk-Do, Korea, in July 1999. A voucher specimen (SKK-99-002) is deposited at the College of Pharmacy in SungKyunKwan University.

Extraction and Isolation. The partially dried and chopped aerial parts of *A. oharai* (7 kg) were extracted with MeOH three times at room temperature. The resultant MeOH extract (400 g) was subjected to successive solvent partitioning to give *n*-hexane (46 g), CH_2Cl_2 (5 g), EtOAc (12 g), and *n*-BuOH (20 g) fractions. The *n*-hexane fraction (46 g) was chromatographed over a silica gel column using a gradient solvent system of *n*-hexane−EtOAc (10:1−0:1) to give six subfractions (H1−H6). Subfraction H2 (2 g) was subjected to silica gel column chromatography eluting with *n*-hexane−EtOAc (3:1) to give three subfractions (H21−H23). Subfraction H23 (300 mg) was further purified using a RP-18 Lobar-A column (70% MeCN) to afford 8,13-epoxy-14-labdane-18-ol (12 mg). Subfraction H3

(0.7 g) was chromatographed over silica gel and then Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) to give two subfractions (H31 and H32), and the H31 (260 mg) was purified over a silica gel Lobar-A column (*n*-hexane−EtOAc, 3:1) to afford labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (80 mg). In turn, subfraction H32 (160 mg) was purified with a RP-18 Lobar-A column (85% MeCN) to afford *ent*-13-epi-manoyloxide-18-oic acid (18 mg). Next, subfraction H4 (2.8 g) was chromatographed over a silica gel column (CH_2Cl_2−MeOH, 25:1) to give four subfractions (H41−H44). Subfraction H41 (900 mg) was subjected to passage over a Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and purified over a silica gel Lobar-A column (*n*-hexane−EtOAc, 2:1) to give a mixture. This mixture was separated by HPLC (3 mL/min; $CHCl_3$) to afford **1** (6 mg, t_R 30 min) and **2** (6 mg, t_R 24 min). Subfraction H43 (750 mg) was chromatographed on a RP-18 Lobar-B column (80% MeCN) to give two subfractions. The first subfraction (200 mg) was passed over Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and then silica gel Lobar-A column (*n*-hexane−EtOAc, 1:1) to afford labda-14-en-8,13-diol (11 mg) and labda-7,14-dien-13(*R*)-ol-α-L-6-deoxyidopyranoside (85 mg). The second subfraction (180 mg) was further purified on a silica gel Lobar-A column (hexane−EtOAc, 1:1) to afford **3** (30 mg), labda-7,14-dien-13-(*R*)-ol-α-L-rhamnopyranoside (**6**) (17 mg), and a mixture. This mixture was separated by HPLC ($CHCl_3$) to afford **4** (5 mg) and **5** (6 mg). Finally, subfraction H44 (150 mg) was purified over silica gel (CH_2Cl_2−MeOH, 40:1) and Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) to afford *ent*-manoyloxide-18-oic acid (18 mg).

7α-Hydroperoxylabda-8(17),14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (1): colorless oil; $[\alpha]^{20}_D$ −21.3° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (2.62) nm; IR (neat) ν_{max} 3421, 1737, 1647, 1377, 1244, 1039 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 533 $[M + Na]^+$ (100); HRFABMS *m*/*z* 533.3080 (calcd for $C_{28}H_{46}O_8Na$, 533.3090).

7α-Hydroperoxylabda-8,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (2): colorless oil; $[\alpha]^{20}_D$ −36.5° (*c* 0.08, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (2.66) nm; IR (neat) ν_{max} 3446, 1739, 1647, 1377, 1244, 1038 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 533 $[M + Na]^+$ (100); HRFABMS *m*/*z* 533.3071 (calcd for $C_{28}H_{46}O_8Na$, 533.3090).

Enzymatic Hydrolysis of 1 Using β-Glucosidase. Compound **1** (2 mg) with 5 mL of acetate buffer (0.1 M, pH 5.0) and 2 mg of β-glucosidase[21] (ICN, 1150 U/MG) was shaken for 5 days at 35 °C. To the mixture was added 10 mL of water−$CHCl_3$, and triphenylphosphine[22] (3 mg) was added to the $CHCl_3$ layer. After 10 min, the $CHCl_3$ layer was evaporated in vacuo and subjected to silica gel (1 g) column chromatography with the eluent hexane−EtOAc (1:2) to give 1 mg of an aglycon (**1a**). The aqueous layer was concentrated to dryness in vacuo and eluted with $CHCl_3$−MeOH−H_2O (30:10:1) over a silica gel (1 g) column and then purified by Sephadex LH-20 (MeOH) to give a sugar {$[\alpha]^{20}_D$ −15.0° (*c* 0.01, H_2O)}. Compound **2** (2 mg) and labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside (10 mg) were treated by the same method. The optical rotation of L-6-deoxyidose from the enzymatic hydrolysis of labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside was −14.5° (*c* 0.05, H_2O). An aglycon (**2a**) obtained from the enzymatic hydrolysis of **2** was confirmed with the optical rotation and ^1H NMR data.

Labda-8(17),14-dien-7,13-diol (1a): colorless oil; $[\alpha]^{20}_D$ −2.1° (*c* 0.02, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.69 (3H, s, H-20), 0.81 (3H, s, H-19), 0.89 (3H, s, H-18), 1.29 (3H, s, H-16), 4.36 (1H, s, H-17), 4.65 (1H, s, H-17), 5.02 (1H, br s, H-7), 5.06 (1H, dd, *J* = 11.0, 1.0 Hz, H-15), 5.20 (1H, dd, *J* = 17.5, 1.0 Hz, H-15), 5.91 (1H, dd, *J* = 17.5, 11.0 Hz, H-14).

Labda-8,14-dien-7,13-diol (2a): colorless oil; $[\alpha]^{20}_D$ −3.4° (*c* 0.02, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86 (3H, s, H-19), 0.91 (3H, s, H-20), 0.95 (3H, s, H-18), 1.30 (3H, s, H-16), 1.55 (3H, s, H-17), 5.03 (1H, br d, *J* = 3.5 Hz, H-7), 5.08 (1H, dd, *J* = 11.0, 1.0 Hz, H-15), 5.23 (1H, dd, *J* = 17.5, 1.0 Hz, H-15), 5.84 (1H, dd, *J* = 17.5, 11.0 Hz, H-14).

Labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-rhamnopyranoside (3): colorless oil; $[\alpha]^{20}_D$ −53.3° (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (2.37) nm; IR (neat) ν_{max} 1740, 1646, 1376, 1240, 1038 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 478.3300 (calcd for $C_{28}H_{46}O_6$, 478.3294).

Labda-7,14-dien-13(*R*)-ol-3-*O*-acetyl-α-L-rhamnopyranoside (4): colorless oil; $[\alpha]^{20}_D$ −21.8° (*c* 0.104, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 202 (2.60) nm; IR (neat) ν_{max} 1722, 1647, 1375, 1246, 1050 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 478.3313 (calcd for $C_{28}H_{46}O_6$, 478.3294).

Labda-7,14-dien-13(*R*)-ol-2-*O*-acetyl-α-L-rhamnopyranoside (5): colorless oil; $[\alpha]^{20}_D$ −17.3° (*c* 0.132, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 201 (2.57) nm; IR (neat) ν_{max} 1728, 1647, 1376, 1244, 1044 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 478.3307 (calcd for $C_{28}H_{46}O_6$, 478.3294).

Alkaline Hydrolysis of Compound 3. A solution of **3** (3 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*-hexanes−EtOAc, 1:2) to afford **3a** (1 mg).

Enzymatic Hydrolysis of Labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside Using Naringinase. Labda-7,14-dien-13(*R*)-ol-α-L-rhamnopyranoside (10 mg) was mixed with 25 mL of 10% aqueous ethanol and 100 mg of naringinase (ICN, 150 U/G) from a *Penicillium* species, adjusted to pH 4.5 with dilute HCl, and shaken for 7 days at 40 °C. The mixture was shaken with 25 mL of water−$CHCl_3$. The $CHCl_3$ layer was evaporated in vacuo and subjected to silica gel column chromatography with the eluent $CHCl_3$−MeOH−H_2O (50:10:1) to give an aglycon, labda-7,14-dien-13(*R*)-ol (2 mg). The aqueous layer was concentrated to dryness in vacuo and separated on silica gel column chromatography eluted with $CHCl_3$−MeOH−H_2O (30:10:1) and then was purified by Sephadex LH-20 (MeOH) to afford a sugar (1 mg). The optical rotation of L-rhamnose obtained from the enzymatic hydrolysis of labda-7,14-dien-13-(*R*)-ol-α-L-rhamnopyranoside was +7.8° (*c* 0.02, H_2O).

Labda-7,14-dien-13(R)-ol: colorless oil; $[\alpha]^{20}_D$ −2.3° (*c* 0.04, $CHCl_3$); EIMS *m*/*z* 290 $[M]^+$; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.75 (3H, s, H-20), 0.84 (3H, s, H-18), 0.87 (3H, s, H-19), 1.29 (3H, s, H-16), 1.66 (3H, H-17), 5.06 (1H, dd, *J* = 11.0, 1.0 Hz, H-15), 5.21 (1H, dd, *J* = 17.5, 1.0 Hz, H-15), 5.37 (1H, br s, H-7), 5.91 (1H, dd, *J* = 17.5, 11.0 Hz, H-14).

Identification of the Sugars. A solution of each sugar in pyridine (0.8 mL) and Ac_2O (0.5 mL) was stirred overnight at room temperature. The reaction mixture was added to H_2O and concentrated in vacuo. Each residue was extracted with *n*-hexane−H_2O (1:1), and the organic layer was analyzed by GC/MS; column HP-5SM (30 m × 0.25 mm × 0.25 μm), detector MS (ionization EI), temperature 70 °C (2 min, 25 °C/min)−180 °C (4 °C/min)−230 °C (25 °C/min)−320 °C, 1 min and He carrier gas (1.0 mL/min). The retention times of these products were compared with those of authentic L-rhamnose and L-6-deoxyidose obtained from the enzymatic hydrolysis of labda-7,14-dien-13(*R*)-ol-4-*O*-acetyl-α-L-6-deoxyidopyranoside.

Biological Evaluation. The cytotoxic activity was performed by the sulforhodamine B method[23] against eight human tumor cell cultures in vitro, LOX IMVI, SK-MEL-2 (human skin cancer cells), SNB-19, U251 (human central nervous system cancer cells), SW620, HCT15 (human colorectal cancer cells), A549 (human non-small cell lung cancer cells), and SK-OV-3 (human ovarian cancer cells) at Korea Research Institute of Chemical Technology. Doxorubicin hydrochloride was used as a positive control. The cytotoxicities of doxorubicin against LOX IMVI, SK-MEL-2, SNB-19, U251, SW620, HCT15, A549, and SK-OV-3 cell lines were ED_{50} 0.001, 0.002, 0.002, 0.001, 0.003, 0.006, 0.001, and 0.010 μg/mL, respectively.

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References and Notes

(1) Kim, T. J. *Wild Flowers of Korea*; Kugilmedia: Seoul, 1996; p 232.

(2) Jung, C. M.; Kwon, H. C.; Seo, J. J.; Ohizumi, Y.; Matsunaga, K.; Saito, S.; Lee, K. R. *Chem. Pharm. Bull.* **2001**, *49*, 912−914.
(3) Kwon, H. C.; Jung, C. M.; Shin, C. G.; Lee, J. K.; Choi, S. H.; Lee, K. R. *Chem. Pharm. Bull.* **2000**, *48*, 1796−1798.
(4) Gonzalez, A. G.; Artega, M.; Breton, J. L.; Fraga, B. M. *Phytochemistry* **1977**, *16*, 107−110.
(5) Uchio, Y.; Nagsaki, M.; Eguchi, S.; Matasuo, A.; Nakayama, M.; Hayashi, S. *Tetrahedron Lett.* **1980**, *21*, 3775−3778.
(6) Eguchi, S.; Uchio, Y.; Nakayama, M. *Biomed. Mass Spectrom.* **1983**, *10*, 363−368.
(7) Zdero, C.; Bohlmann, F.; King, R. M. *Phytochemistry* **1992**, *31*, 1631−1638.
(8) Bohlmann, F.; Zdero, C.; Hoffmann, E.; Mahanta, P. K.; Dormer, W. *Phytochemistry* **1978**, *17*, 1917−1922.
(9) Stierle, D. B.; Stierle, A. A.; Larsen, R. D. *Phytochemistry* **1988**. *27*, 517−522.
(10) Uchio, Y.; Nagasaki, S. *Koen Yoshishu-Koryo, Terupen oyobi Seiyu Kagaku ni Kansuru Toronkai* **1979**, *23rd*, 53−55; *Chem. Abstr.* **1980**, *92*, 177405.
(11) Ruecker, G.; Mayer, R.; Lee, K. R. *Arch. Pharm. (Weinheim, Ger.* **1989**, *322*, 821−826).
(12) Uchio, Y.; Nagsaki, M.; Eguchi, S.; Matasuo, A.; Nakayama, M.; Hayashi, S. *Tetrahedron Lett.* **1980**, *21*, 3775−3778.
(13) Cambie, R. C.; Grant, P. K.; Huntrakul, C.; Weston, R. J. *Aust. J. Chem.* **1969**, *22*, 1691−1697.
(14) Martin, A. S.; Rovirosa, J.; Becker, R.; Castillo, M. *Phytochemistry* **1980**, *19*, 1985−1987.
(15) Anjaneyulu, A. S. R.; Rao, V. L. *Phytochemistry* **2000**, *55*, 891−901.
(16) Wu, C. L.; Lin, H. R. *Phytochemistry* **1997**, *44*, 101−105.
(17) Sigeru, T.; Kenji, U.; Ichiro, K.; Manabu, K. *Chem. Lett.* **1978**, *4*, 455−456.
(18) Jung, J. H.; Lee, H. G.; Kang, S. S. *Phytochemistry* **1996**, *42*, 447−452.
(19) Bader, G.; Wray, V.; Just, U.; Hiller, K. *Phytochemistry* **1998**, *49*, 153−156.
(20) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−506.
(21) Ono, M.; Masuoka, C.; Odake, Y.; Ito, Y.; Nohara, T. *Phytochemistry* **2000**, *53*, 479−484.
(22) Bohlmann, F.; Gupta, R. K. *Phytochemistry* **1982**, *21*, 2595−2597.
(23) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

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