Regular Article

Eight *ent*-Kaurane Diterpenoid Glycosides Named Diosmariosides A–H from the Leaves of *Diospyros maritima* and Their Cytotoxic Activity

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From the leaves of *Diospyros maritima*, collected from Okinawa Island, eight new glycosides based on *ent*-kaurane-type diterpenoids, entitled diosmariosides A–H, were isolated. The absolute structure of diosmarioside E (5) was determined by X-ray crystallographic analysis. The structure of diosmarioside H was elucidated to be a dimeric compound between diosmarioside A and a sugeroside through a ketal bond. An assay of cytotoxicity towards the lung adenocarcinoma (A549) cell line was performed. Among the compounds isolated, only diosmarioside D (4) and sugeroside 9 showed strong activity. The anti-microbial activity toward multi-drug resistant strains was also determined, but no activity was observed.

Key words *Diospyros maritima*; Ebenaceae; *ent*-kaurane; *ent*-kaurane glycoside; dimeric *ent*-kaurane glycoside

Diospyros maritima Blume (Ebenaceae) is an evergreen tall tree with a height of *ca.* 10 m, distributed in Okinawa, Taiwan, Malaysia, Micronesia and Australia.[1] In summer, it bears green sap fruits of 2 to 3 cm in diameter, which then turn to a dark orange color in autumn. It is known that the fruits contain a toxic naphthoquinone derivative, plumbagin, and their constituents were extensively investigated by Higa *et al.*[2–4] Recently, from the leaves and branches of a related Thai medicinal plant, *D. mollis*, the isolation of naphthoquinone glycosides was reported.[5] In our continuing work on Okinawan resource plants, the constituents of the leaves of *D. maritima* were investigated to give eight *ent*-kaurane diterpenoid glycosides, entitled diosmariosides A–H (**1–8**), along with a known *ent*-kaurane glucoside, sugeroside **9**, isolated from *Ilex sugerokii* var. *brevipedanculata*[6] and *Rubus suaviussmus*,[7] and an *ent*-kaurane diterpenoid, (4*R*,16*R*)-16,17,19-trihydroxy-*ent*-kaur-3-one (**10**),[8] isolated from *Flickingeria fimbriata* (Fig. 1). The structure and stereochemistry of sugeroside **9**, isolated from *R. suaviussmus*, was confirmed by X-ray crystallographic analysis.[7]

Results and Discussion

Eight new compounds (**1–8**) and two known ones (**9** and **10**) were isolated from the MeOH extract of leaves of *D. maritima*, using various kinds of chromatographic techniques. The structures of the new compounds were elucidated by intensive one- and two-dimensional NMR spectroscopic analyses and chemical conversion. The absolute structure of diosmarioside E (**5**) was determined by X-ray crystallographic analysis. The structures of the known compounds were identified by the comparison of spectroscopic data with those reported in the literature.[6,7]

Diosmarioside A (**1**), $[\alpha]_D^{26}$ −63.8, was isolated as colorless plates and its elemental composition was determined to be $C_{31}H_{50}O_{12}$ by the observation of a quasi-molecular ion peak $[M+Na]^+$ using high-resolution (HR) electrospray ionization (ESI) MS. The IR spectrum exhibited strong absorption bands at 3460 and 3341, and 1693 cm^{-1} ascribable to hydroxy groups and a carbonyl functional group, respectively. In the ^{1}H-NMR spectrum, signals for three singlet methyls, methylene protons on an isolated primary alcohol [δ_H 3.95 (1H, d, J=10.8 Hz, H-17b) and 4.47 (1H, d, J=10.8 Hz, H-17a)] and two anomeric protons (δ_H 4.99 and 5.80) were observed. Thus, **1** was expected to be a glycosidic compound and sugar analysis of its hydrolysate using a chiral detector revealed the presence of D-apiose and D-glucose. The ^{13}C-NMR spectrum displayed 31 signals and eleven signals were expected to be the result of sugar moieties. The remaining 20 signals comprised of three methyls, nine methylenes, one of which carried an oxygen atom, three methines, and three quaternary, carbonyl and oxygenated tertiary carbons. These functionalities implied that diosmarioside A (**1**) was a diterpene with five degrees of unsaturation and the comparison of ^{13}C-NMR data with those of sugeroside (**9**) indicated that **1** was an apiofuranosyl sugeroside (Table 1). The relative orientation of the C-17 primary carbinol group was expected to be the same as that of **9** from the phase sensitive (PS)-rotating frame nuclear Overhauser effect spectroscopy (ROESY) correlations between H_2-17 [δ_H 4.47 (d, J=10.8 Hz) and 3.95 (d, J=10.8 Hz)], and H-11a [δ_H 1.60 (m)]. The positions of the sugar linkage were determined by heteronuclear multiple-bond correlation spectroscopy (HMBC), in which the anomeric proton of D-apiofuranoside was correlated with the C-6 of glucopyranose and then that of glucopyranose with C-17 of the aglycone. The mode of linkage of D-glucopyranoside was determined to be β from the coupling constant of anomeric proton (J=7.6 Hz) and that

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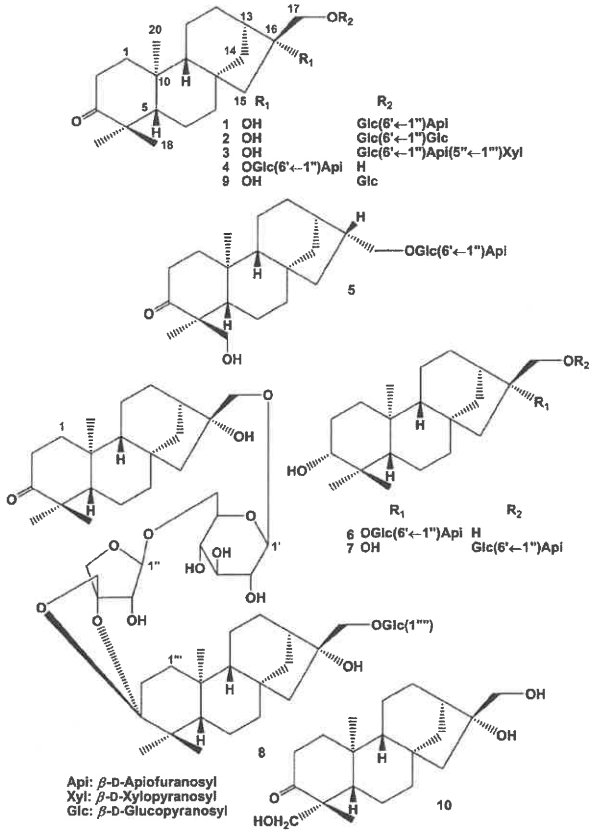

Fig. 1. New (**1**–**8**) and Known (**9** and **10**) Compounds Isolated

of D-aiofuranoside was also to be β by comparison of the ^{13}C-NMR data of authentic samples prepared in our laboratory [methyl β-D-apiofuranoside: δ_C 111.6 (C-1), 77.9 (C-2), 80.5 (C-3), 74.9 (C-4), 65.5 (C-5) and methyl α-D-apiofuranoside: δ_C 104.6 (C-1), 73.4 C-2), 77.9 (C-3), 75.1 (C-4), 65.6 (C-5)][9,10] Diosmarioside A (**1**) was found to be in the *enantio* series from the negative Cotton effect at 289 nm ($\Delta\varepsilon$ −2.87) in the circular dichroism (CD) spectrum.[11,12] Therefore, the structure of diosmarioside A (**1**) was elucidated, as shown in Fig. 1.

Diosmarioside B (**2**), $[\alpha]_D^{23}$ −65.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{32}H_{52}O_{13}$. The physicochemical data for **2** were similar to those of **1**, and HPLC analysis of its hydrolysate showed only one peak for D-glucose, though NMR spectra indicated the presence of two anomeric signals [δ_H 4.97 (d, J=7.8 Hz) on δ_C 106.4 and δ_H 5.08 (d, J=7.9 Hz) on δ_C 105.4]. In the HMBC spectrum, one (δ_H 5.08) of the anomeric protons correlated with C-6′ of the inner glucose unit and the other (δ_H 4.97) with C-17 of the aglycone. From a similar negative Cotton effect at 289 nm in the CD spectrum, **2** was also expected to be in the *ent*-series. Therefore, the structure of diosmarioside B (**2**) was elucidated to be 6′-*O*-β-D-glucopyranoside of the sugeroside, as shown in Fig. 1.

Diosmarioside C (**3**), $[\alpha]_D^{23}$ −92.6, was isolated as an amorphous powder and its elemental composition was determined to be $C_{36}H_{58}O_{16}$. From the NMR spectroscopic data, diosmarioside C (**3**) was found to be an analogous compound to **1** and **2** with three sugar units. Sugar analysis of the hydrolysate of **3** revealed the presence of D-apiose, D-xylose and D-glucose. D-Xylose was expected to be the terminal sugar from typical five ^{13}C-NMR signals (δ_C 105.8, 74.7, 78.2, 71.1 and 67.2) and its anomeric proton (δ_H 4.82) was correlated with C-5″ (δ_C 72.8) of apiofuranoside in the HMBC spectrum. The anomeric proton of apiofuranoside was then correlated with C-6′ (δ_C 69.0) of the inner glucose moiety. From a negative Cotton effect at 289 nm in the CD spectrum, **3** was also expected to be in the *ent*-series. Therefore, the structure of **3** was elucidated to be the 5″-*O*-β-D-xylopyranoside of diosmarioside A, as shown in Fig. 1.

Diosmarioside D (**4**), $[\alpha]_D^{25}$ −83.6, was isolated as colorless needles and its elemental composition was determined to be $C_{31}H_{50}O_{12}$, which was the same as that of **1**. All the functionalities available from the NMR spectral data were also the same as those of **1** and sugar analysis showed the presence of D-apiose and D-glucose. However, the ^{13}C-NMR chemical shifts of oxymethylene (C-17) and oxygenated tertiary (C-16) carbons appeared at δ_C 63.0 and δ_C 90.2, respectively, which were shifted by −12.5 and +9.6 ppm, when compared with those of **1**. Thus, the sugar moiety must be attached at the hydroxy group at the C-16 position. HMBC correlation also supported this fact, with the anomeric proton of the glucose moiety showing a cross peak with C-16 carbon. The relative orientation of the C-17 primary carbinol group was expected to be the same as that of **9** from the PS-ROESY correlations between H-17a [4.12, (d, J=13.0 Hz)] and H-11a [(1.56, (m)]. From a negative Cotton effect at 290 nm in the CD spectrum, **4** was also expected to be in the *ent*-series. Therefore, the structure of **4** was elucidated, as shown in Fig. 1.

Diosmarioside E (**5**), $[\alpha]_D^{22}$ −96.0, was isolated as colorless fine needles and its elemental composition was determined to be $C_{31}H_{50}O_{12}$, which was the same as that of **1**. Diosmarioside E (**5**) was also analogous to the aforementioned compounds, however, the oxygenated tertiary carbon disappeared, instead of which, an isolated primary alcohol [3.94 (1H, brd, J=10.1 Hz) and 3.64 (1H, brd, J=10.1 Hz)] was newly formed. Since these methylene protons showed a correlation cross peak with the carbonyl carbon, the newly formed oxymethylene was placed on either C-18 or C-19. The significant correlation signal between H_3-19 (δ_H 1.03) and H_3-20 (δ_H 0.91) in the ROESY enabled us to place the oxymethylene functional group at the 18-position (Fig. 2). Diosmarioside E (**5**) was found to be in the *enantio* series from the negative Cotton effect at 294 nm ($\Delta\varepsilon$ −0.71) in the CD spectrum[11,12] and the geometry at the 16-position was explored by the PS-ROESY experiment. Significant ROESY correlations between H-17b (δ_H 3.47) and H-14b (δ_H 1.06), and H-16 (δ_H 2.18) and both of H-11a (δ_H 1.53) and H-12b (δ_H 1.32) suggested that the C-17 primary alcohol was in the α-face, namely, the absolute configuration of the 16-position to be *R* (Fig. 2). Enzymatic hydrolysis of **5** gave an aglycone (**5a**) and the correlations were found to be the same as those of glycosidic form, **5**. ^{13}C-NMR chemical shifts of C-16 and its neighboring carbons were reported for the 16*R* and 16*S* congeneric compounds in the literature.[13] However, the data showed some discrepancy between those of **5a** and the 16*S* congener, as well as **5a** and the 16*R* one. Finally, the structure of **5a** was confirmed by the X-ray crystallographic analysis (Fig. 3) and the results obtained from the ROESY experiment were verified, namely, that C-16 had the 16*R* configuration. The Flack parameter [χ=−0.03(3)] verified the absolute configuration of **5a**, as shown in Fig. 3, and the result from the CD experiment was also confirmed.

Table 1. ^{13}C-NMR Spectroscopic Data for Diosmariosides A–H (**1–8**), Sugeroside (**9**) and **5a** (150 MHz, Pyridine-d_5)

	1	**9**[a]	**2**	**3**	**4**	**5**	**5a**[b]	**6**	**7**	**8**			
C										Ca		Cb	
1	39.2	39.2	39.2	39.2	39.3	38.1	38.7	39.1	39.0	1	39.3	1‴	37.2
2	34.2	34.3	34.2	34.2	34.2	36.7	35.4	28.2	28.2	2	34.3	2‴	27.3
3	216.7	216.7	216.7	216.7	216.6	217.4	219.0	78.2	78.2	3	216.6	3‴	114.4
4	47.1	47.3	47.1	47.1	47.1	52.6	52.3	39.4	39.4	4	47.2	4‴	42.3
5	54.3	54.3	54.2	54.2	54.2	46.7	48.7	55.4	55.4	5	54.3	5‴	53.2
6	21.9	21.9	21.9	21.9	21.8	22.3	21.5	20.6	20.7	6	21.9	6‴	20.7
7	41.3	41.3	41.2	41.3	41.1	40.4	40.5	42.4	42.5	7	41.3	7‴	42.3
8	44.5	44.5	44.5	44.5	44.6	44.6	44.5	44.8	44.7	8	44.5	8‴	44.6
9	55.5	55.5	55.5	55.5	55.6	54.5	54.8	57.0	56.9	9	55.6	9‴	56.7
10	38.6	38.6	38.5	38.6	38.6	38.2	38.4	39.4	39.3	10	38.7	10‴	39.1
11	18.9	19.0	19.0	18.9	19.1	19.3	19.1	18.8	18.6	11	19.0	12‴	18.6
12	26.5	26.6	26.5	26.5	25.9	31.2	31.3	26.2	26.9	12	26.0	12‴	26.9
13	46.2	46.3	46.1	46.2	43.2	38.6	38.2	43.4	46.4	13	46.2	13‴	46.5
14	37.2	37.1	37.2	37.2	36.8	36.9	37.3	37.2	37.6	14	37.2	14‴	37.4
15	53.0	52.9	53.4	52.9	51.6	45.5	44.8	52.0	53.4	15	53.1	15‴	53.2
16	80.8	80.8	80.7	80.8	90.2	40.9	43.3	90.3	80.9	16	80.8	16‴	80.7
17	75.5	75.5	76.4	75.5	63.0	74.7	67.5	63.1	75.7	17	75.4	17‴	75.7
18	27.3	27.3	27.3	27.3	27.2	68.6	67.3	28.9	28.9	18	27.3	18‴	23.5
19	21.1	21.1	21.1	21.1	21.0	17.6	16.7	16.3	16.3	19	21.1	19‴	20.3
20	17.7	17.8	17.8	17.7	17.7	17.7	17.3	18.1	18.0	20	17.8	20‴	17.8
1′	106.3	106.6	106.4	106.3	98.4	104.9		98.5	106.4	1′	106.1	1‴	106.7
2′	75.4	75.5	75.2	75.4	75.3	75.1		75.3	75.4	2′	75.4	2‴	75.5
3′	78.6	78.8	78.5	78.6	78.9	78.6		78.9	78.6	3′	78.6	3‴	78.7
4′	71.9	71.7	71.7	71.7	72.1	71.9		72.1	71.9	4′	71.6	4‴	71.7
5′	77.3	78.7	77.2	77.3	77.1	77.2		77.1	77.3	5′	76.9	5‴	78.5
6′	69.0	62.9	70.2	69.0	69.1	69.0		69.2	68.9	6′	67.7	6‴	62.8
1″	111.2		105.4	110.9	111.1	111.2		111.2	111.2	1″	109.5		
2″	77.8		75.4	78.3	77.9	77.8		77.9	77.8	2″	79.0		
3″	80.5		78.3	79.3	80.5	80.5		80.5	80.5	3″	86.4		
4″	75.1		71.6	74.9	75.1	75.1		75.2	75.1	4″	74.5		
5″	65.7		78.5	72.8	65.8	65.6		65.9	65.7	5″	73.9		
6″			62.7										
1‴				105.8									
2‴				74.7									
3‴				78.2									
4‴				71.1									
5‴				67.2									

a) Data were taken from ref. 6 (100 MHz, pyridine-d_5). *b*) Data for $CDCl_3$.

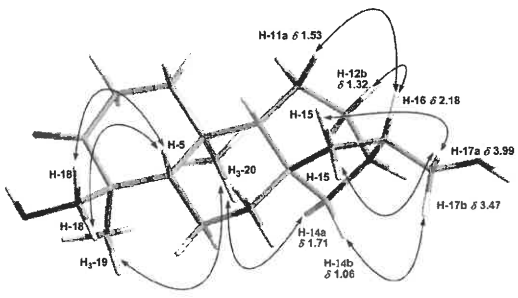

Fig. 2. ROESY Correlations of Dismarioside E (**5**) Sugar Portion Was Omitted for Clearness

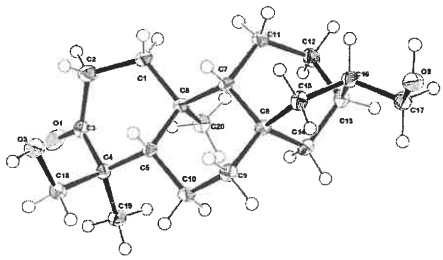

Fig. 3. An ORTEP Drawing of the Crystal Structure of the Aglycone of Dismarioside E (**5a**)

Diosmarioside F (**6**), $[\alpha]_D^{23}$ −57.8, was isolated as colorless needles and its elemental composition was determined to be $C_{31}H_{52}O_{12}$. The ketone absorption band in the IR spectrum observed for compounds **1–5** disappeared, and oxygenated methine proton [δ_H 3.42 (1H, dd, J=10.7, 5.3 Hz)] and carbon (δ_C 78.2) signals were found in the NMR spectra. Since the HMBC correlations are between the *gem*-dimethyl protons (δ_H 0.96 and 1.16) and the oxygenated methine carbon, this carbon must be at the 3-position. The ^{13}C-NMR data of the B, C and D rings were essentially the same as those of diosmarioside E (**4**) (Table 1). Therefore, to confirm the structure of **6**, includ-

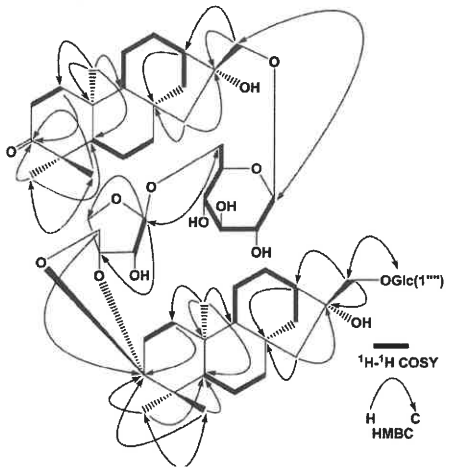

Fig. 4. ^{1}H–^{1}H COSY and HMBC Correlations of Diosmarioside H (**8**)

Dual arrow curves denote that HMBC correlations were observed in both directions.

ing the absolute stereochemistry, diosmarioside E (**4**) was reduced with $NaBH_4$. Hydride must be introduced from the less hindered *si*-face of the carbonyl carbon to give a sole product, **4a**, which was spectroscopically identical as diosmarioside F (**6**).

Diosmarioside G (**7**), $[\alpha]_D^{24}$ −42.2, was isolated as an amorphous powder and its elemental composition was determined to be $C_{31}H_{52}O_{12}$. D-Apiose and D-glucose were analyzed to be present in the hydrolysate of **7** and their connectivity was confirmed from the data of HMBC spectrum. The ^{13}C-NMR chemical shifts of C-16 and C-17 suggested that the sugar moiety was attached at the hydroxy group of C-17. The secondary alcohol was at the C-3 position and then diosmarioside A (**1**) was reduced with $NaBH_4$ to give **1a** (=**7**) as a sole product. Therefore, the structure of **7** was elucidated, as shown in Fig. 1.

Diosmarioside H (**8**), $[\alpha]_D^{24}$ −49.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{57}H_{90}O_{19}$. The IR spectrum exhibited a carbonyl absorption peak (1703 cm^{-1}) along with strong bands for hydroxy groups (3444 cm^{-1}). Three anomeric signals [δ_H 5.00 (d, J=7.9 Hz) on δ_C 106.1, δ_H 5.04 (d, J=7.8 Hz) on δ_C 106.7 and δ_H 5.61 (brs) on δ_C 109.5] were observed in the NMR spectra, while only two sugars, D-apiose and D-glucose, were detected in its hydrolysate. In the HMBC spectrum, one of the anomeric proton (δ_H 5.00) of D-glucopyranoside was correlated with one of the primary alcohol carbon, another anomeric proton (δ_H 5.04) of D-glucopyranoside with the other primary alcohol carbon and the anomeric proton (δ_H 5.61) of D-apiofuranoside with the 6′-position of D-glucopyranoside (Fig. 4). The remaining 40 carbon signals appeared as 19 sets of close peaks, except for carbonyl (δ_C 216.6) and ketal (δ_C 114.4) carbons. HMBC correlation of the methylene protons (δ_H 4.38 and 4.45) of the 5″-position of D-apiofuranoside with the ketal carbon suggested that diosmarioside H (**8**) was a dimeric compound of two kaurane units with 13 degrees of unsaturation. Upon inspection of the ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC spectra (Fig. 4), the bond connectivity was trailed from C-5″ of the D-apiofuranoside to establish that one of the kaurane units possessed diosmarioside A (**1**)-like (unit A) scaffold and that was followed from C-1‴ of the other D-glucopyranoside to the ketal carbon to conclude that the ketal derivative of sugeroside (**9**) (unit B) comprised of the rest of the structure (Fig. 1). Thus, the ketone of sugeroside (**9**) must form a cyclic ketal with hydroxy groups at C-3″ and C-5″ of D-apiofuranoside of the unit A. The ^{13}C-NMR chemical shifts of C-3″ and C-5″ in the unit A were apparently shifted downfield [C-3″: δ_C 80.5 (**1**)→δ_C 86.4 (**8**) and C-5″: δ_C 65.7 (**1**)→73.9 (**8**)], when compared with those of **1**, proving that these groups were involved in the cyclic ketal formation. The negative Cotton effect ($\Delta\varepsilon$: −0.73) at 289 nm in the CD spectrum confirmed that the unit A possessed an *ent*-series kaurene skeleton[11,12] and the stereochemistry of the ketal region was substantiated using the PS-nuclear Overhauser effect spectrum. Significant cross peaks of H_3-18‴ (δ_H 0.96) with H_2-4″ (δ_H 4.21 and 4.44) were able to assign the absolute configuration at the C-3‴ position to be *R* (Fig. 5), as well as. Since ^{13}C-NMR chemical shifts of C-13‴, C-14‴, C-15‴, C-16‴ and C-17‴ were indistinguishable from those of sugeroside (**9**) (Table 1), the unit B was also expected to have an *ent*-type framework. The structure of diosmarioside H (**8**) was elucidated to be a dimeric compound between diosmarioside A (**1**) and sugeroside (**9**).

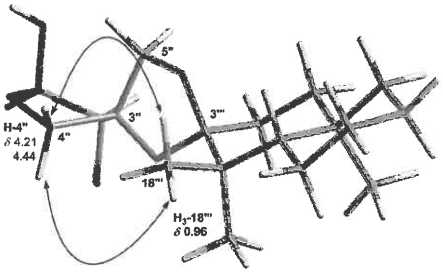

Fig. 5. ROESY Correlations of Diosmarioside H (**8**)

Table 2. Cytotoxicity toward A549 Cells (IC_{50}: μM)

1	>100
2	>100
3	>100
4	5.11±0.23
5	>100
6	>100
7	>100
8	>100
9	2.39±0.27
10	>100
Etoposide	36.5±7.84

Etoposide: Positive control. Each value represents the mean±standard deviation (S.D.) with triplicate experiments.

A considerable amount of diosmarioside A (**1**) and sugeroside **9** were isolated as the mother compounds of **8** from the title plant. Although these compounds were treated with *d*-camphor sulfonic or *p*-toluene sulfonic acids in dehydrated dioxne, no reaction was observed.

Assay of cytotoxicity of compounds isolated toward adenocarcinomic human alveolar basal epithelial cell line, A549 was performed. Among them, only diosmarioside D (**4**) and sugeroside (**9**) showed strong activity at IC_{50} of 5.11 μM ±0.23 and 2.38 μM ±0.26, respectively (Table 2). At least, a ketonic functional group was required at the 3-position for the expres-

sion of activity, however, further structure–activity relationships are currently uncertain.

The anti-microbial activity toward the following strains, *Acinetobactoer baumannii* NBRC 110492 (opportunistically infectious), *Enterococcus faecalis* NBRC 100481 (opportunistically infectious), *Klebsiella pneumoniae* NBRC 14441 (opportunistically infectious), *Pseudomonas aeruginosa* MDRP610 (multi-drug resistant and opportunistically infectious), *Serratia marcescens* NBRC 110513 (opportunistically infectious), *Staphylococcus aureus* MS-29772 (methicillin resistant) and *Acinetobacter* sp. 160 (drug-resistant) was assayed. However, none of the compounds isolated were found to be active, suggesting that the cytotoxicity of compounds **4** and **9** might be specific to mammalian cells. In future work, we will comprehensively investigate the biological properties of these compounds, including the mode of action to the A549 tumor cells.

Experimental

General Experimental Procedures Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-2200 digital polarimeter. IR spectra were measured on JASCO FT/IR-6100 spectrophotometers. ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker Avance III at 600 MHz and 150 MHz, respectively, with tetramethylsilane as an internal standard. CD spectra were obtained with a JASCO J-720 spectropolarimeter. Positive-ion HR-ESI-MS were measured with a Thermo Fisher Scientific LTQ Orbitrap XL. Silica gel column chromatography (CC) was performed on silica gel 60 (70-230 mesh) (E. Merck, Darmstadt, Germany) and reversed-phase octadecylsilanized (ODS) open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) (ϕ=50 mm, L=25 cm). HPLC was performed on an ODS column (Inertsil ODS-3; GL Science, Tokyo, Japan; ϕ=6 mm, L=25 cm, 1.6 mL/min, Cosmosil Cholester; Nacarai Tesque; ϕ=6 mm, L=25 cm, 1.6 mL/min, Cosmosil πNAP; Nacalai Tesque; ϕ=10 mm, L=25 cm, 4.0 mL/min), and the eluate was monitored with photo diode array (200–400 nm) and refractive index monitors. β-Glucosidase was a generous gift from Shin Nihon Chemical Co., Ltd. (Anjo, Aichi, Japan).

Plant Material Leaves of *D. maritima* were collected in Taketomi-cho, Yaeyama-gun, Okinawa, Japan, in November, 2003 and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University (03-DM-Okinawa-1105).

Extraction and Isolation Air-dried leaves of *D. maritima* (7.80 kg) were extracted with methanol (MeOH) (45 L) three times. The MeOH extract was concentrated to 6 L and then washed with *n*-hexane (6 L, 245 g). The methanolic layer was concentrated to a viscous gum. The gummy mass was suspended in H_2O (6 L), and then partitioned with ethyl acetate (EtOAc) (6 L) and 1-BuOH (6 L), successively, to give 397 g and 216 g of EtOAc and 1-butanol (BuOH)-soluble fractions, respectively. The remaining water-layer was concentrated to give a H_2O-soluble-fraction (245 g). The 1-BuOH-soluble fraction was subjected to a Diaion HP-20 CC (ϕ=80 mm, L=50 cm), and eluted with H_2O–MeOH (4:1, 5 L), (3:2, 5 L), (2:3, 5 L), and (1:4, 5 L), and MeOH (5 L), 1 L-fractions being collected. The residue (29.2 g) in fractions 11–14 of Diaion HP-20 CC was subjected to silica gel CC (ϕ=50 mm, L=54.5 cm), and eluted with $CHCl_3$ (3 L), $CHCl_3$–MeOH (99:1, 3 L), (49:1, 3 L), (97:3, 3 L), (19:1, 3 L), (37:3, 3 L), (9:1, 3 L), (7:1, 3 L), (17:3, 3 L), (33:7, 3 L), (4:1, 3 L), (3:1, 3 L), and (7:3, 3 L), 500 mL-fractions being collected. The residue (3.00 g out of 9.08 g) in fractions 50–56 was separated by ODS CC (ϕ=50 mm, L=25 cm), and eluted with a linear solvent system from MeOH–H_2O (1:9, 2 L) to MeOH–H_2O (9:1, 2 L) linear gradient, 10 g-fractions being collected. Ninety three point two milligram of **7** was afforded as crystals in fraction 220–241.

The residue (64.5 g) in fractions 15–19 of Diaion HP-20 CC was subjected to silica gel CC (ϕ=80 mm, L=40 cm), and eluted with $CHCl_3$ (6 L), $CHCl_3$–MeOH (99:1, 6 L), (49:1, 6 L), (97:3, 6 L), (19:1, 6 L), (37:3, 6 L), (9:1, 6 L), (7:1, 6 L), (17:3, 6 L), (33:7, 6 L), (4:1, 6 L), (3:1, 6 L), and (7:3, 6 L), 1 L-fractions being collected. Compounds **10** (56.4 mg), **9** (114 mg) and **4** (344 mg) were obtained as crystals in fractions 28, 42 and 44, respectively.

The residue (3.00 g out of 12.3 g) in fractions 53–62 was separated by ODS CC (ϕ=50 mm, L=25 cm), and eluted with MeOH–H_2O (1:9, 2 L) to MeOH–H_2O (9:1, 2 L) linear gradient, 10 g-fractions being collected. The residue (524 mg) in fractions 216–226 was purified by HPLC (ODS-3, H_2O–MeOH, 2:3) and then the residue (111.0 mg) from the peak at 9.0 min was further purified by HPLC (Cholester, H_2O–MeOH, 11:9) to give 22.1 mg of **5** from the peak at 26.5 min. The residue (104 mg) from the peak at 10.2 min was further purified by HPLC (Cholester, H_2O–MeOH, 11:9) to give 37.9 mg of **2** and 12.4 mg of **3** from the peaks at 25.7 min and 33.2 min, respectively. ODS CC also afforded 9.7 mg of **6** and 449 mg of **1** as crystals in fractions 199–200 and 227–242, respectively. The residue (13.6 mg) in fractions 311–315 was purified by HPLC (ODS-3, H_2O–MeOH, 3:7) and then the residue (8.0 mg) from the peak at 31.0 min was further purified by HPLC (πNAP, H_2O–MeOH, 1:3) to give 5.4 mg of **8** from the peak at 35.4 min.

Diosmarioside A (**1**) Colorless plates, mp 203–204°C, $[\alpha]_D^{26}$ −63.8 (c=0.60, MeOH); IR ν_{max} (KBr) cm^{-1}: 3460, 3341, 2940, 2867, 1693, 1452, 1174, 1082; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.80 (1H, d, J=2.1 Hz, H-1″), 4.99 (1H, J=7.6 Hz, H-1′), 4.78 (1H, J=2.1 Hz, H-2″), 4.77 (1H, brd, J=11.3 Hz, H-6′a), 4.61 (1H, d, J=9.4 Hz, H-4″a), 4.47 (1H, d, J=10.8 Hz, H-17a), 4.37 (1H, d, J=9.4 Hz, H-4″b), 4.21 (2H, m, H-3′ and 6′b), 4.19 (2H, m, H_2-5″), 4.13 (1H, m, H-5′), 4.08 (1H, m, H-2′), 4.05 (1H, m, H-4′), 3.95 (1H, d, J=10.8 Hz, H-17b), 2.47 (2H, dd, J=8.3, 6.4 Hz, H_2-2), 2.42 (1H, brs, H-13), 1.94 (1H, brd, J=11.7 Hz, H-14a), 1.91 (1H, m, H-12a), 1.81 (1H, m, H-1a), 1.80 (1H, brd, J=11.7 Hz, H-14b), 1.76 (1H, d, J=14.2 Hz, H-15a), 1.61 (1H, m, H-7a), 1.61 (1H, d, J=14.2 Hz, H-15b), 1.60 (1H, m, H-11a), 1.44 (2H, m, H-11b and 12b), 1.40 (1H, m, H-7b), 1.30 (3H, m, H-5 and H_2-6), 1.24 (1H, ddd, J=13.1, 8.6, 8.6 Hz, H-1b), 1.08 (3H, s, H_3-18), 1.01 (3H, s, H_3-19), 0.96 (1H, brd, J=8.4 Hz, H-9), 0.92 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −2.87 (289) (c=9.77×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 637.3198 $[M+Na]^+$ (Calcd $C_{31}H_{50}O_{12}Na$: 637.3194).

Diosmarioside B (**2**) Colorless amorphous powder, $[\alpha]_D^{23}$ −65.4 (c=0.50, MeOH)); IR ν_{max} (film) cm^{-1}: 3368, 2933, 2869, 1702, 1456, 1162, 1045; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.08 (1H, d, J=7.9 Hz, H-1″), 4.97 (1H, d, J=7.8 Hz, H-1′),

4.94 (1H, dd, J=11.0, 1.6 Hz, H-6′a), 4.78 (1H, dd, J=8.6, 7.9 Hz, H-2″), 4.54 (1H, dd, J=11.6, 1.6 Hz, H-6″a), 4.41 (1H, d, J=11.0 Hz, H-17a), 4.40 (1H, dd, J=11.6, 5.1 Hz, H-6″b), 4.27 (3H, m, H-6′b, 3″ and 4″), 4.22 (1H, dd, J=8.8, 8.6 Hz, H-3′), 4.15 (1H, m, H-5′), 4.12 (1H, dd, J=9.5, 8.8 Hz, H-4′), 4.08 (1H, dd, J=8.6, 7.8 Hz, H-2′), 4.05 (1H, d, J=11.0 Hz, H-17b), 3.95 (1H, m, H-5″), 2.48 (2H, dd, J=8.6, 6.6 Hz, H_2-2), 2.39 (1H, brs, H-13), 1.97 (1H, dd, J=11.0, 3.6 Hz, H-14a), 1.84 (1H, m, H-1a), 1.82 (1H, m, H-12a), 1.79 (1H, brd, J=11.0 Hz, H-14b), 1.78 (1H, d, J=14.3 Hz, H-15a), 1.71 (1H, d, J=14.3 Hz, H-15b), 1.64 (1H, m, H-7a), 1.61 (1H, m, H-11a), 1.45 (1H, m, H-12b), 1.44 (1H, m, H-11b), 1.38 (1H, m, H-7b), 1.30 (3H, m, H-5 and H_2-6), 1.26 (1H, ddd, J=13.3, 8.6, 8.6 Hz, H-1b), 1.08 (3H, s, H_3-18), 1.01 (3H, s, H_3-19), 0.96 (1H, brd, J=8.6 Hz, H-9), 0.92 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −1.01 (289) (c=7.27×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 667.3305 $[M+Na]^+$ (Calcd $C_{32}H_{52}O_{13}Na$: 667.3300).

Diosmarioside C (**3**) Colorless amorphous powder, $[\alpha]_D^{23}$ −92.6 (c=0.31, MeOH); IR ν_{max} (film) cm^{-1}: 3369, 2932, 2869, 1698, 1457, 1159, 1049; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.78 (1H, d, J=2.8 Hz, H-1″), 5.00 (1H, d, J=7.9 Hz, H-1′), 4.82 (1H, d, J=7.6 Hz, H-1‴), 4.80 (1H, d, J=2.8 Hz, H-2″), 4.75 (1H, dd, J=11.2, 1.6 Hz, H-6′a), 4.55 (1H, d, J=9.5 Hz, H-4″a), 4.49 (1H, d, J=10.7 Hz, H-17a), 4.45 (1H, d, J=10.4 Hz, H-5″a), 4.32 (1H, dd, J=11.3, 5.2 Hz, H-5‴a), 4.31 (1H, d, J=9.5 Hz, H-4″b), 4.21 (1H, dd, J=9.0, 8.9 Hz, H-3′), 4.20 (1H, m, H-6′b), 4.19 (1H, m, H-4‴), 4.11 (1H, m, H-5′), 4.11 (1H, d, J=10.4 Hz, H-5″b), 4.11 (1H, dd, J=8.6, 8.4 Hz H-3‴), 4.09 (1H, dd, J=8.9, 7.9 Hz, H-2′), 4.05 (1H, dd, J=9.2, 9.0 Hz, H-4′), 3.96 (1H, d, J=10.7 Hz, H-17b), 3.98 (1H, dd, J=8.4, 7.6 Hz, H-2‴), 3.65 (1H, dd, J=11.3, 10.3 Hz, H-5‴b), 2.48 (2H, dd, J=8.6, 6.2 Hz, H_2-2), 2.43 (1H, brs, H-13), 1.94 (1H, dd, J=11.3, 4.2 Hz, H-14a), 1.90 (1H, m, H-12a), 1.82 (1H, ddd, J=13.2, 13.0, 6.2 Hz, H-1a), 1.80 (1H, brd, J=11.3 Hz, H-14b), 1.76 (1H, d, J=14.2 Hz, H-15a), 1.62 (1H, d, J=14.2 Hz, H-15b), 1.61 (2H, m, H-7a and 11a), 1.43 (2H, m, H-11b and 12b), 1.42 (1H, m, H-7b), 1.30 (3H, m, H-5 and H_2-6), 1.26 (1H, ddd, J=13.2, 8.8, 8.6 Hz, H-1b), 1.08 (3H, s, H_3-18), 1.01 (3H, s, H_3-19), 0.97 (1H, brd, J=8.4 Hz, H-9), 0.91 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −0.86 (289) (c=5.16×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 769.3626 $[M+Na]^+$ (Calcd $C_{36}H_{58}O_{16}Na$: 769.3617).

Diosmarioside D (**4**) Colorless needles, mp 238–239°C, $[\alpha]_D^{25}$ −83.6 (c=0.25, MeOH); IR ν_{max} (KBr) cm^{-1}: 3341, 2936, 2876, 1704, 1458, 1360, 1160, 1056; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.73 (1H, d, J=1.9 Hz, H-1″), 5.04 (1H, d, J=7.8 Hz, H-1′), 4.76 (1H, d, J=1.9 Hz, H-2″), 4.67 (1H, d, J=9.1 Hz, H-6′a), 4.61 (1H, d, J=9.4 Hz, H-4″a), 4.38 (1H, d, J=9.4 Hz, H-4″b), 4.24 (1H, dd, J=8.9, 8.9 Hz, H-3′), 4.22 (1H, d, J=12.2 Hz, H-5″a), 4.20 (1H, d, J=12.2 Hz, H-5″b), 4.12 (1H, d, J=13.0 Hz, H-17a), 4.08 (2H, m, H-5′ and 6′b), 4.06 (1H, d, J=13.0 Hz, H-17b), 4.03 (1H, dd, J=8.9, 7.8 Hz, H-2′), 3.96 (1H, dd, J=9.1, 8.9 Hz, H-4′), 2.71 (1H, brs, H-13), 2.51 (2H, m, H_2-2), 2.15 (1H, brd, J=11.3 Hz, H-14a), 2.09 (1H, d, J=14.4 Hz, H-15a), 1.95 (1H, brd, J=11.3 Hz, H-14b), 1.81 (1H, ddd, J=12.6, 6.4, 6.4 Hz, H-1a), 1.72 (2H, m, H-7a and 12a), 1.70 (1H, d, J=14.4 Hz, H-15b), 1.56 (2H, m, H-11a and 12b), 1.42 (2H, m, H-7b and 11b), 1.30 (1H, m, H-5), 1.28 (2H, m, H_2-6), 1.25 (1H, m, H-1b), 1.06 (3H, s, H_3-18), 0.99 (1H, brd, J=7.4 Hz, H-9), 0.96 (3H, s, H_3-19), 0.96 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −2.14 (290) (c=8.14×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 637.3198 $[M+Na]^+$ (Calcd $C_{31}H_{50}O_{12}Na$: 637.3194).

Diosmarioside E (**5**) Colorless fine needles, mp 129–131°C, $[\alpha]_D^{22}$ −96.0 (c=0.25, MeOH)); IR ν_{max} (KBr) cm^{-1}: 3257, 2922, 2854, 1703, 1454, 1217, 1069; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.84 (1H, d, J=2.4 Hz, H-1″), 4.83 (1H, d, J=7.7 Hz, H-1′), 4.80 (1H, m, H-2″), 4.80 (1H, brd, J=11.3 Hz, H-6′a), 4.62 (1H, d, J=9.4 Hz, H-4″a), 4.38 (1H, d, J=9.4 Hz, H-4″b), 4.25 (1H, dd, J=9.1, 8.6 Hz, H-3′), 4.25 (1H, dd, J=11.3, 6.5 Hz, H-6′b), 4.18 (2H, m, H_2-5″), 4.14 (1H, m, H-5′), 4.08 (1H, dd, J=9.2, 9.1 Hz, H-4′), 4.05 (1H, dd, J=8.6, 7.7 Hz, H-2′), 3.99 (1H, dd, J=9.1, 7.0 Hz, H-17a), 3.94 (1H, brd, J=10.1 Hz, H-18a), 3.64 (1H, brd, J=10.1 Hz, H-18b), 3.47 (1H, dd, J=9.1, 8.9 Hz, H-17b), 2.71 (1H, ddd, J=17.2, 8.8, 3.7 Hz, H-2a), 2.53 (1H, ddd, J=17.2, 8.8, 8.6 Hz, H-2b), 2.29 (1H, m, H-5), 2.28 (1H, m, H-13), 2.18 (1H, m, H-16), 1.84 (1H, ddd, J=13.2, 8.6, 3.7 Hz, H-1a), 1.71 (1H, brd, J=11.6 Hz, H-14a), 1.53 (1H, m, H-11a), 1.50 (1H, m, H-15a), 1.47 (1H, m, H-6a), 1.44 (1H, m, H-1b), 1.41 (1H, m, H-11b), 1.37 (3H, m, H_2-7 and 12a), 1.32 (1H, m, H-12b), 1.31 (1H, m, H-6b), 1.06 (1H, dd, J=11.6, 3.8 Hz, H-14b), 1.04 (1H, m, H-9), 1.03 (3H, s, H_3-19), 1.00 (1H, dd, J=13.4, 5.2 Hz, H-15b), 0.91 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −0.71 (294) (c=3.26×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 637.3198 $[M+Na]^+$ (Calcd $C_{31}H_{50}O_{12}Na$: 637.3194).

Diosmarioside F (**6**) Colorless needles, mp 272–273°C, $[\alpha]_D^{23}$ −57.8 (c=0.32, MeOH); IR ν_{max} (KBr) cm^{-1}: 3412, 2930, 2869, 1169, 1047; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.75 (1H, d, J=2.1 Hz, H-1″), 5.04 (1H, d, J=7.9 Hz, H-1′), 4.78 (1H, d, J=2.1 Hz, H-2″), 4.70 (1H, d, J=8.6 Hz, H-6′a), 4.62 (1H, d, J=9.4 Hz, H-4″a), 4.38 (1H, d, J=9.4 Hz, H-4″b), 4.23 (1H, d, J=13.4 Hz, H-5″a), 4.22 (1H, m, H-3′), 4.21 (1H, d, J=13.4 Hz, H-5″b), 4.15 (1H, d, J=12.7 Hz, H-17a), 4.10 (3H, m, H-17b, 5′ and 6′b), 4.03 (1H, dd, J=8.8, 7.9 Hz, H-2′), 3.96 (1H, dd, J=8.6, 8.3 Hz, H-4′), 3.42 (1H, dd, J=10.7, 5.3 Hz, H-3), 2.74 (1H, brs, H-13), 2.16 (1H, brd, J=11.2 Hz, H-14a), 2.13 (1H, d, J=14.8 Hz, H-15a), 2.05 (1H, brd, J=11.2 Hz, H-14b), 1.87 (2H, m, H_2-2), 1.77 (1H, m, H-7a), 1.74 (3H, m, H-11a and H-15b), 1.65 (1H, m, H-12b), 1.54 (2H, m, H_2-11), 1.53 (1H, m, H-7b), 1.49 (1H, m, H-6a), 1.30 (1H, m, H-6b), 1.16 (3H, s, H_3-18), 1.01 (3H, s, H_3-20), 0.96 (1H, m, H-9), 0.96 (3H, s, H_3-19) 0.88 (1H, ddd, J=12.7, 12.6, 4.3 Hz, H-1b), 0.75 (1H, brd, J=11.6 Hz, H-5); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; HR-ESI-MS (positive-ion mode): m/z: 639.3355 $[M+Na]^+$ (Calcd $C_{31}H_{52}O_{12}Na$: 639.3351).

Diosmarioside G (**7**) Colorless amorphous powder, $[\alpha]_D^{24}$ −42.2 (c=0.32, MeOH)); IR ν_{max} (film) cm^{-1}: 3421, 2930, 2847, 1167, 1048; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.80 (1H, d, J=2.2 Hz, H-1″), 4.99 (1H, d, J=7.5 Hz, H-1′), 4.79 (1H, d, J=2.2 Hz, H-2″), 4.70 (1H, d, J=11.5 Hz, H-6′a), 4.61 (1H, d, J=9.3 Hz, H-4″a), 4.48 (1H, d, J=10.8 Hz, H-17a), 4.37 (1H, d, J=9.3 Hz, H-4″b), 4.21 (2H, m, H-3′ and 6′b), 4.20 (1H, d, J=13.4 Hz, H-5″a), 4.18 (1H, d, J=13.4 Hz, H-5″b), 4.13 (1H, m, H-5′), 4.08 (1H, dd, J=8.2, 7.5 Hz, H-2′), 4.04 (1H, dd, J=9.3, 8.6 Hz, H-4′), 3.95 (1H, d, J=10.8 Hz, H-17b), 3.42 (1H, dd, J=11.1, 5.0 Hz, H-3), 2.42 (1H, brs, H-13), 1.93 (1H, m, H-14a), 1.91 (2H, m, H-12a and 14b), 1.86 (2H, m, H_2-2), 1.78 (1H, d, J=14.3 Hz, H-15a), 1.74 (1H, brd, J=13.3 Hz, H-1a), 1.67 (1H, brd, J=12.5 Hz, H-7a), 1.64 (1H, d, J=14.3 Hz, H-15b), 1.56 (2H, m, H_2-11), 1.50 (2H, m, H-6a and 12b), 1.47

(1H, ddd, J=12.5, 12.5, 3.4 Hz, H-7b), 1.31 (1H, m, H-6b), 1.19 (3H, s, H_3-18), 1.00 (3H, s, H_3-19), 0.97 (3H, s, H_3-20), 0.92 (1H, brd, J=7.5 Hz, H-9), 0.86 (1H, ddd, J=13.3, 12.7, 3.7 Hz, H-1b), 0.75 (1H, brd, J=11.5 Hz, H-5); ^{13}C-NMR (pyridine-d_5, 150 MHz,): Table 1; HR-ESI-MS (positive-ion mode): m/z: 639.3355 $[M+Na]^+$ (Calcd $C_{31}H_{52}O_{12}Na$: 639.3351).

Diosmarioside H (**8**) Colorless amorphous powder, $[\alpha]_D^{24}$ −49.4 (c=0.36, MeOH); IR ν_{max} (KBr) cm^{-1}: 3444, 2932, 2869, 1703, 1456, 1054; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 5.61 (1H, brs, H-1″), 5.04 (1H, d, J=7.8 Hz, H-1‴), 5.00 (1H, d, J=7.9 Hz, H-1′), 4.61 (1H, dd, J=11.1, 1.7 Hz, H-6′a), 4.59 (1H, dd, J=11.7, 2.3 Hz, H-6‴a), 4.54 (1H, d, J=10.3 Hz, H-17a), 4.49 (1H, d, J=10.7 Hz, H-17‴a), 4.45 (1H, d, J=8.9 Hz, H-5″a), 4.44 (1H, d, J=8.6 Hz, H-4″a), 4.43 (1H, dd, J=11.7, 5.3 Hz, H-6‴b), 4.38 (1H, d, J=8.9 Hz, H-5″b), 4.33 (1H, brs, H-2″), 4.26 (1H, m, H-4‴), 4.25 (2H, m, H-3′ and 3‴), 4.21 (1H, d, J=8.6 Hz, H-4″b), 4.16 (1H, dd, J=11.1, 5.8 Hz, H-6′b), 4.10 (2H, m, H-2′ and 2‴), 4.09 (1H, m, H-4′), 4.05 (1H, m, H-5′), 4.00 (1H, d, J=10.3 Hz, H-17b), 4.00 (1H, m, H-5‴), 3.93 (1H, d, J=10.7 Hz, H-17‴b), 2.52 (2H, dd, J=8.6, 6.4 Hz, H_2-2), 2.47 (1H, brs, H-13), 2.44 (1H, brs, H-13‴), 1.99 (1H, m, H-12a), 1.97 (1H, m, H-14a), 1.94 (1H, m, H-14‴a), 1.89 (2H, m, H-1a and 14‴b), 1.88 (1H, m, H-12‴a), 1.86 (1H, m, H-14b), 1.84 (1H, m, H-2‴a), 1.80 (1H, d, J=14.3 Hz, H-15a), 1.78 (1H, d, J=14.3 Hz, H-15‴a), 1.77 (1H, m, H-2‴b), 1.67 (1H, m, H-11a), 1.66 (2H, m, H-7a and 7‴a), 1.66 (1H, d, J=14.3 Hz, H-15b), 1.61 (1H, d, J=14.3 Hz, H-15‴b), 1.56 (1H, m, H-1‴a), 1.53 (1H, m, H-12b), 1.52 (2H, m, H-11b and 11‴a), 1.50 (2H, m, H-7‴b and H-12‴b), 1.47 (1H, m, H-11‴b), 1.45 (1H, m, H-7b), 1.43 (1H, m, H-6‴a), 1.35 (3H, m, H-5 and H_2-6), 1.33 (1H, m, H-5‴), 1.30 (1H, m, H-1b), 1.28 (1H, m, H-6‴b), 1.16 (1H, ddd, J=13.2, 13.2, 3.4 Hz, H-1‴b), 1.11 (3H, s, H_3-18), 1.03 (3H, s, H_3-19), 1.02 (1H, m, H-9), 1.00 (1H, m, H-9‴), 0.98 (3H, s, H_3-20‴), 0.96 (3H, s, H_3-18‴), 0.95 (3H, s, H_3-19‴), 0.91 (3H, s, H_3-20); ^{13}C-NMR (pyridine-d_5, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −0.73 (289) (c=1.86×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 1101.5973 $[M+Na]^+$ (Calcd $C_{57}H_{90}O_{19}Na$: 1101.5969).

Sugar Analysis About 500 μg each of **1**–**8** was hydrolyzed with 1 M HCl (0.1 mL) at 90°C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed by HPLC with a chiral detector (JASCO OR-4090) on an amino column [InertSustain NH_2, CH_3CN–H_2O (4 : 1), 1 mL/min]. A hydrolyzate of **2** gave a peak for D-glucose at 10.9 min, ones of **1**, **4**–**8** gave peaks for D-apiose and D-glucose at 6.1 min and 10.9 min, respectively, and one of **3** gave peaks for D-apiose, D-xylose and D-glucose at 6.1, 7.3 and 10.9 min, respectively, with positive optical rotation signs. The peaks were identified by co-chromatography with authentic samples.

$NaBH_4$ Reduction of Diosmariosides D (4) and A (1) to Diosmariosides F (6) and G (7) To a solution of diosmarioside D (**4**) (20.3 mg) in 1.0 mL of MeOH was added 10.4 mg of $CeCl_3 \cdot 7H_2O$ and then 1.6 mg of $NaBH_4$, the reaction mixture being stirred for 5 min at 25°C. Excess $NaBH_4$ was quenched by the addition of 1 mL of $(CH_3)_2CO$ and then the reaction mixture was evaporated to dryness. The residue was purified by preparative TLC (developed with $CHCl_3$–MeOH–H_2O, 15 : 6 : 1, and then eluted with $CHCl_3$–MeOH, 1 : 1) to afford 2.7 mg of **4a** (=**6**). Diosmarioside A (**1**) (20.0 mg) was similarly reduced to give 6.7 mg of **1a** (=**7**). **4a**: $[\alpha]_D^{22}$ −52.2 (c=0.27, MeOH); NMR data were identical with those of **6**; HR-ESI-MS (positive-ion mode): m/z: 639.3351 $[M+Na]^+$ (Calcd $C_{31}H_{52}O_{12}Na$: 639.3351). **1a**: $[\alpha]_D^{24}$ −37.3 (c=0.34, MeOH); NMR data were identical with those of **7**; HR-ESI-MS (positive-ion mode): m/z: 639.3352 $[M+Na]^+$ (Calcd $C_{31}H_{52}O_{12}Na$: 639.3351).

Enzymatic Hydrolysis of Diosmarioside E (5) Diosmarioside E (**5**) (8.2 mg) was treated with β-glucosidase (4.0 mg) in 1 mL of H_2O at 37°C for 48 h. The reaction mixture was partitioned with 2 mL of EtOAc and the aglycone (**5a**) (2.0 mg) was recovered in the organic layer. Aglycone (**5a**): colorless crystals, mp 168–170°C, $[\alpha]_D^{26}$ −73.0 (c=0.10, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 3391, 2927, 2857, 1694, 1456, 1375, 1044; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.94 (1H, d, J=11.2 Hz, H-18a), 3.42 (2H, m, H_2-17), 3.40 (1H, d, J=11.2 Hz, H-18b), 2.62 (1H, ddd, J=16.6, 12.4, 7.2 Hz, H-2a), 2.34 (1H, ddd, J=16.6, 6.2, 2.9 Hz, H-2b), 2.12 (1H, m, H-13), 2.10 (1H, ddd, J=13.4, 7.2, 2.9 Hz, H-1a), 1.97 (1H, ddddd, J=13.4, 7.9, 7.9, 7.9, 7.9 Hz, H-16), 1.87 (1H, brd, J=11.7 Hz, H-14a), 1.66 (1H, m, H-5), 1.64 (2H, m, H_2-11), 1.59 (2H, m, H-12a and 15a), 1.57 (1H, m, H-7a), 1.49 (1H, m, H-12b), 1.48 (1H, m, H-6a), 1.47 (1H, m, H-7b), 1.45 (1H, m, H-6b), 1.34 (1H, ddd, J=13.4, 12.4, 6.2 Hz, H-1b), 1.19 (3H, s, H_3-20), 1.14 (1H, brd, J=7.6 Hz, H-9), 0.96 (1H, brd, J=11.7 Hz, H-14b), 1.01 (3H, s, H_3-19), 0.95 (1H, dd, J=13.5, 5.4 Hz, H-15b); ^{13}C-NMR ($CDCl_3$, 150 MHz): Table 1; CD $\Delta\varepsilon$ (nm): −0.84 (290) (c=3.16×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode): m/z: 343.2245 $[M+Na]^+$ (Calcd $C_{20}H_{32}O_3Na$: 343.2244).

X-Ray Crystallographic Analysis of 5a The colorless plate (0.200×0.150×0.010 mm^3), obtained from methanol, was immersed in Paraton-N oil and placed in the N_2 cold stream at 100 K. The diffraction experiment was performed on a Bruker D8VENTURE system (PHOTON-100 CMOS detector, Cu$K\alpha$: λ=1.54178 Å). Absorption correction was performed by an empirical method implemented in SADABS.[14] Structure solution and refinement were performed by using SHELXT-2014/5[15] and SHELXL-2018/3.[16]

$C_{20}H_{32}O_3$, M_r=320.45; Orthorhombic, space group $P2_12_12_1$, Z=4, D_{calc}=1.254 g·cm^{-3}, a=7.2244(6), b=11.0155(9), c=21.3219(17) Å, V=1696.8(2) Å^3, 22546 observed and 3556 independent [$I>2\sigma(I)$] reflections, 335 parameters, final R_1=0.0329, wR_2=0.0912, S=0.972 [$I>2\sigma(I)$]. Flack parameter: χ=−0.03(3). All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were refined isotropically on the calculated positions using a riding model except for hydroxy hydrogen. The largest difference peak and hole were 0.525 and −0.367 eÅ^{-3}, respectively. Supplementary X-ray crystallographic data for **5a** (CCDC 1851583) can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223–336–033; or deposit@ccdc.cam.ac.uk).

Cytotoxic Activity toward Lung Adenocarcinoma, A549 Cells Cytotoxic activity toward lung adenocarcinoma cells was determined by colorimetric cell viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Lung adenocarcinoma cell line A549 was purchased from the JCRB Cell Bank, Japan. A549 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated fetal calf serum, and kanamycin (100 μg/mL) and amphotericin B (5.6 μg/mL).

In a 96-well plate, 1 μL aliquots of sample solutions and the cancer cells (5×10^3 cells/well) in 100 μL medium were added to each well, and then the plate incubated at 37°C under a 5% CO_2 atmosphere for 72 h. A solution (100 μL) of MTT (0.5 mg/mL) was then added to each well and the incubation was continued for a further 1 h. The absorbance of each well was measured at 540 nm using a Molecular Devices Versamax tunable microplate reader. Dimethyl sulfoxide (DMSO) was used as a negative control and etoposide as a positive control. The cytotoxic activity was calculated as:

$$\% \text{ inhibition} = [1-(A_{test}-A_{blank})/(A_{control}-A_{blank})]\times100$$

where $A_{control}$ is the absorbance of the control (DMSO) well, A_{test} the absorbance of the test wells, and A_{blank} the absorbance of the cell-free wells.

Anti-microbial Activity Anti-microbial activity toward bacteria was determined by the conventional paper disk (8 mm in diameter; ADVANTEC, Japan) diffusion method. Assay plates were prepared by pouring 25 mL of Müller–Hinton agar (Difco, U.S.A.) inoculated with a 250-μL aliquot of an overnight broth culture of the test organisms into a culture dish. *Acinetobactoer baumannii* NBRC 110492, *Enterococcus faecalis* NBRC 100481, *Klebsiella pneumoniae* NBRC 14441, *Serratia marcescens* NBRC 110513, *Pseudomonas aeruginosa* MDRP610, *Staphylococcus aureus* MS-29772, and *Acinetobacter* sp. 160 were used as test organisms. NBRC strains were obtained from the National Biological Resource Center, Japan. Other strains were provided by Laboratory of Bacterial Drug Resistance, Gunma University Graduate School of Medicine. Compounds **1**–**10** were dissolved in MeOH (1 mg/mL) and a paper disk containing each of the compounds (70 μg) was placed on the assay plate seeded with each of the test organisms. Growth inhibition was examined after 24 h incubation at 37°C. Arbekacin, levofloxacin and piperacillin were used as positive controls.

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Conflict of Interest The authors declare no conflict of interest.

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