A Monoterpene Glucoside and Three Megastigmane Glycosides from *Juniperus communis* var. *depressa*

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A new monoterpene glucoside (1) and three new natural megastigmane glycosides (2—4) were isolated along with a known megastigmane glucoside (5) from twigs with leaves of *Juniperus communis* var. *depressa* (Cupressaceae) collected in Oregon, U.S.A., and their structures were determined on the basis of spectral and chemical evidence. In addition, the antibacterial activities of the isolated components against *Helicobacter pylori* were also investigated.

Key words *Juniperus communis* var. *depressa*; monoterpene glucoside; megastigmane glycoside; *Helicobacter pylori*; Cupressaceae

In a survey of chemical components from useful plants grown in western North America, we have identified a number of various types of phenolic compounds (nine phenylpropanoids, six neolignans, and seven flavonoids) in their glycoside form from the aerial parts of *Juniperus communis* var. *depressa* (Cupressaceae) and those results were reported in our previous papers.[1,2] Upon continued chemical investigation of the same plant material, a new monoterpene glucoside (**1**) and three new natural megastigmane glycosides (**2**—**4**) were isolated together with a known megastigmane, corchoionoside C (**5**).[3] In the present paper, we describe the isolation, structure elucidation, and biological activity of these constituents.

The *n*-BuOH-soluble part obtained from the MeOH extract was separated by a combination of silica gel, octadecyl silica gel (ODS), and Sephadex LH-20 column chromatographies, followed by HPLC separation to afford each of the four new natural terpenic compounds (**1**—**4**) and the known megastigmane.

Compound **1**, a white powder, $[\alpha]_D$ −36.3°, gave the $[M-H]^-$ ion peak at *m*/*z* 345 in the FAB-MS (negative mode). The high-resolution (HR) spectrum in the same mode revealed the molecular formula to be $C_{16}H_{26}O_8$. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) suggested **1** to be a β-D-glucopyranoside.[4,5] On the aglycone, proton signals due to two *tert* methyls (δ 1.35, 1.39, both s) and an olefinic proton (δ 7.27, br dd, J=3.0, 3.0 Hz) and 10 carbon signals including two *tert* methyls (δ_C 23.3, 24.8) and a carboxyl group (δ_C 169.9) were observed. The two-dimensional (2D) study using ^{1}H–^{1}H shift-correlation spectroscopy (COSY) indicated that a menthane-type monoterpene is assigned to the aglycone. The position of the carboxyl group was determined to be at C-1 based on the presence of a cross peak between H-2 and the carboxylic carbon (C-7) in heteronuclear multiple-bond correlation spectroscopy (HMBC), suggesting that the planar

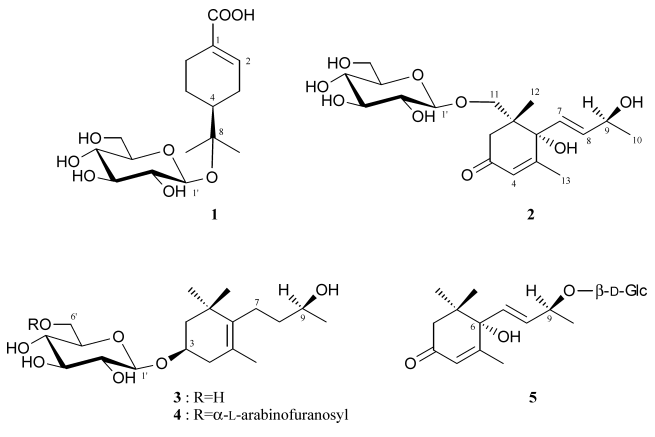

Chart 1

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** in C_5D_5N[a)]

NO.	**1**	
	δ_H	δ_C
1		131.8
2	7.27 (br dd, 3.0, 3.0)	139.1
3α	2.47 (br dd, 12.0, 16.0)	27.8
β	2.08 (br d, 16.0)	
4α	1.85 (dddd, 12.0, 12.0, 4.8, 1.8)	43.7
5α	2.22 (br d, 12.0)	23.8
β	1.26 (dddd, 12.0, 12.0, 12.0, 4.8)	
6α	2.42 (m)	26.0
β	2.80 (br d, 16.0)	
7		169.9
8		79.0
9	1.39 (s)[b)]	23.3[b)]
10	1.35 (s)[b)]	24.8[b)]
Glc 1′	5.02 (d, 7.8)	98.6
2′	3.98 (dd, 9.0, 7.8)	75.3
3′	4.26 (dd, 9.0, 9.0)	78.8
4′	4.24 (dd, 9.0, 9.0)	71.9
5′	3.91 (ddd, 9.0, 5.2, 2.5)	78.0
6′	4.35 (dd, 11.8, 5.2)	63.0
	4.49 (dd, 11.8, 2.5)	

a) Measured at 600 MHz (^{1}H-NMR) and 150 MHz (^{13}C-NMR). *b*) Interchangeable in each column.

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Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for **2**, **2a**, **3**, and **4** in CD_3OD

No.	**2**[a]		**2a**[b]		**3**[a]		**4**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		46.3		49.7		38.8		38.8
2α	2.64 (dd, 17.3, 0.7)	45.5	2.35 (dd, 18.0, 2.4)	53.3	1.83 (ddd, 12.0, 3.6, 2.4)		1.85 (ddd, 12.0, 3.5, 2.4)	
β	2.38 (br d, 17.3)		2.65 (dd, 18.0, 2.8)		1.49 (dd, 12.0, 12.0)	47.5	1.49 (dd, 12.0, 12.0)	47.6
3		200.9		211.3	4.05 (m)	73.3	4.02 (m)	73.8
4	5.90 (dd, 1.3, 0.7)	127.8	2.43 (dd, 17.6, 2.4, α)	54.0	2.34 (br dd, 16.8, 5.8, α)	39.8	2.33 (br dd, 16.8, 5.8, α)	39.9
			2.78 (d, 17.6, β)		2.02 (β)[c]		2.03 (β)[c]	
5		167.2	6.73 (d, 8.1)	87.5		125.1		125.1
6		79.4	6.85 (dd, 8.1, 2.0)	82.4		138.6		138.6
7	5.75 (dd, 16.0, 1.2)	129.8	6.02 (dd, 15.2, 1.2)	125.7	2.02[c]	25.5	2.03[c]	25.6
					2.12 (dt, 13.2, 5.1)		2.12 (dt, 13.2, 5.1)	
8	5.83 (dd, 16.0, 5.6)	137.2	6.17 (dd, 15.2, 5.4)	140.7	1.45 (m)	40.7	1.45 (m)	40.7
					1.53 (m)		1.53 (m)	
9	4.33 (m)	68.7	4.38 (m)	68.9	3.71 (m)	69.2	3.71 (m)	69.2
10	1.24 (d, 6.4)	23.8	1.28 (d, 6.4)	24.0	1.17 (d, 6.3)	23.2	1.17 (d, 6.3)	23.3
11	3.59 (d, 10.0)	74.6	3.64 (d, 8.0)	78.4	1.05 (s)	28.8	1.06 (s)	28.9
	3.96 (d, 10.0)		3.91 (dd, 8.0, 2.8)					
12	1.06 (s)	20.1	0.95 (s)	15.7	1.07 (s)	30.3	1.07 (s)	30.3
13	1.92 (d, 1.3)	19.5	1.18 (s)	19.2	1.64 (s)	20.0	1.64 (s)	20.0
Glc 1′	4.14 (d, 7.8)	104.6			4.42 (d, 7.8)	102.4	4.41 (d, 7.8)	102.6
2′	3.14 (dd, 9.0, 7.8)	75.1			3.15 (dd, 9.0, 7.8)	75.2	3.14 (dd, 9.0, 7.8)	75.2
3′	3.31 (dd, 9.0, 9.0)	78.0			3.36 (dd, 9.0, 9.0)	78.1	3.35 (dd, 9.0, 9.0)	78.1
4′	3.27 (dd, 9.0, 9.0)	71.5			3.30 (dd, 9.0, 9.0)	71.7	3.28 (dd, 9.0, 9.0)	72.0
5′	3.21 (ddd, 9.0, 5.6, 2.4)	78.0			3.26 (ddd, 9.0, 5.6, 2.4)	77.9	3.45 (ddd, 9.0, 5.6, 2.4)	76.6
6′	3.65 (dd, 12.0, 5.6)	62.7			3.67 (dd, 12.0, 5.6)	62.8	3.60 (dd, 12.0, 5.6)	68.0
	3.84 (dd, 12.0, 2.4)				3.86 (dd, 12.0, 2.4)		4.02 (dd, 12.0, 2.4)	
Ara 1″							4.95 (d, 1.0)	109.9
2″							3.98 (dd, 3.4, 1.0)	83.2
3″							3.82 (dd, 5.4, 3.4)	79.0
4″							3.97 (ddd, 5.4, 5.4, 3.4)	86.1
5″							3.64 (dd, 12.0, 5.4)	63.1
							3.73 (dd, 12.0, 3.4)	

a) At 600 MHz (^{1}H-NMR) and 150 MHz (^{13}C-NMR). *b*) At 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR). *c*) Overlapping with other signals.

structure of the aglycone is the same as that of a known monoterpene, oleuropeic acid.[6] The absolute configuration of the aglycone was decided as follows. On enzymatic hydrolysis with emulsin from almond (β-glucosidase), **1** gave the corresponding genuine aglycone which showed a negative-signed optical rotation of −51.4°. A comparison of this observed rotation with the reported values for (−)-*S*-oleuropeic acid (−114°) and its (+)-*R*-isomer (+46.5°) prepared synthetically from (−)-β-pinene and (+)-α-pinene, respectively, *via* the microbial transformations[6] indicated the aglycone to be (−)-*S*-oleuropeic acid. The cross peaks between the anomeric proton and each of the two *tert* methyls in nuclear Overhauser enhancement spectroscopy (NOESY), together with the HMBC correlation between the anomeric proton and C-8, demonstrated that the glucosyl moiety is bonded to the alcoholic group (8-OH) of the aglycone through the glycosidic linkage. In conclusion, **1** is defined as (−)-oleuropeic acid 8-*O*-β-D-glucopyranoside [=(4*S*)-4-(1-β-D-glucopyranosyloxy-1-methyl)ethyl-1-cyclohexene-1-carboxylic acid].

Compound **2**, a white powder, $[\alpha]_D$ +85.9°, gave a quasimolecular ion peak, $[M-H]^-$, at *m*/*z* 401 in the FAB-MS (negative mode). The HR-FAB-MS in the same mode revealed the molecular formula to be $C_{19}H_{30}O_9$. In addition, the FAB-MS (negative mode) also afforded a significant fragment peak at *m*/*z* 239, arising from the loss of a hexosyl unit from the quasimolecular ion. The ^{1}H- and ^{13}C-NMR spectral data were analyzed with the aid of 2D ^{1}H–^{1}H COSY and heteronuclear multiquantum coherence (HMQC) experiments (Table 2). Based on Table 2, the presence of the β-D-glucopyranosyl moiety was demonstrated as the sugar part of **2**.[4,5] On the other hand, the aglycone part contained the following structural fragments: a *tert* methyl [δ 1.06 (3H, s, H_3-12)], a *sec* methyl [δ 1.24 (3H, d, J=6.4 Hz, H_3-10)], a vinyl methyl [δ 1.92 (3H, d, J=1.3 Hz, H_3-13)], an oxymethylene [δ 3.59, 3.96 (both 1H, d, J=10.0 Hz, H_2-11)], a four-carbon chain involving a *trans*-disubstituted olefin [CH=CHCH(OH)CH_3], which is bonded to a quarternary carbon atom [δ 5.75 (1H, dd, J=16.0, 1.2 Hz, H-7), δ 5.83 (1H, dd, J=16.0, 5.6 Hz, H-8), δ 4.33 (1H, m, H-9), δ 1.24 (3H, d, J=6.4 Hz, H_3-10)], an α,β-unsaturated carbonyl group [δ_H 5.90 (1H, dd, J=1.3, 0.7, H-4), δ_C 200.9 (C-3)]. Based on the detailed study of the HMBC correlation of **2** (Fig. 1), a megastigmane-type planar structure as shown in Fig. 1 constituted the aglycone part structure. This planar structure contained three asymmetric carbons of C-1, C-6, and C-9, among which the absolute configuration at C-6 was first determined as follows. The CD spectrum of **2** exhibited a positive-signed Cotton effect ($\Delta\varepsilon$ +9.88) at 245 nm, consistent with the β-orientation of the side chain including a 7(8)-double bond (thus the 6*R* configuration) according to the reported evidence.[7] The NOESY spectrum of **2** (Fig. 1) gave three significant cross peaks (H-7/H_3-12, H-7/H-2β, and H_3-12/H-2β), suggesting that the *tert* methyl (C-12) attached at C-1 is β-oriented (thus the 1*R*

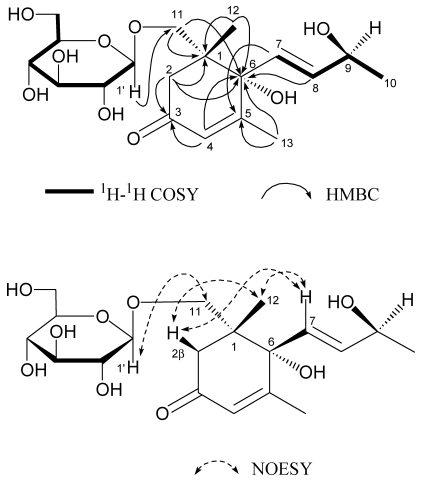

Fig. 1. Selected Correlations in COSY, HMBC and NOESY of **2**

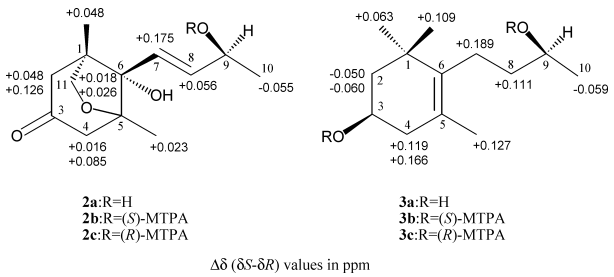

Fig. 2. Data of Modified Mosher's Method for **2a** and **3a**

configuration). Finally, the absolute configuration of the 9-*sec* OH group on the side chain of **2** was determined as follows. Enzymatic hydrolysis of **2** with cellulase afforded an artificial aglycone (**2a**), instead of the desired genuine aglycone. The HR-EI-MS spectrum of **2a** showed that the molecular formula of **2a** is $C_{13}H_{20}O_4$, consistent with that of the genuine aglycone. To elucidate the structure of **2a**, the ^{13}C-NMR chemical shifts of **2a** were compared with those of the aglycone part of **2** as follows (Table 2). Characteristic signals due to a methylene carbon (δ 54.0, C-4) and a quarternary carbon bearing an ethereal oxygen (δ 87.5, C-5) were observed in **2a** instead of those due to the 4(5)-double bond carbons (δ 127.8, C-4 and δ 167.2, C-5) observed in **2**. Furthermore, the carbonyl carbon (C-3) in **2a** resonated downfield by 10.4 ppm (δ 211.3) compared with that (C-3; δ 200.9) in **2**, indicative of the presence of a saturated carbonyl group in **2a** instead of the α,β-unsaturated carbonyl in **2**. This spectral evidence suggested that the primary alcohol (11-OH) liberated by the enzymic hydrolysis of **2** is linked to one (C-5) of the double bond carbons to form the five-membered ether ring in **2a**. The ^{1}H-NMR data of **2a** (Table 2) were also consistent with the established structure of **2a** shown in Fig. 2 (at this stage, the absolute configuration at C-9 in **2a** is uncertain). The remaining structural problem, *i.e.*, the absolute configuration at C-9 of **2a** was determined according to the modified Mosher's method.[8] Treatment of **2a** with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA chloride) gave the corresponding 9-(*S*)-MTPA ester (**2b**) and 9-(*R*)-MTPA ester (**2c**), respectively. Differences between **2b** and **2c** in ^{1}H-NMR chemical shifts, *i.e.*, $\Delta\delta$ values of $\delta S-\delta R$, depicted in Fig. 2, suggested that the absolute configuration at C-9 in **2a** (accordingly, in **2**) is *S*.[8] Finally, the position of the glucosyl residue on the aglycone was made clear on the basis of the following NOESY and HMBC experiments (Fig. 1). The NOESY cross peak between H-1′ and H_2-11 and the HMBC correlation between H-1′ and C-11 indicated that the glucose is connected to the hydroxy group at C-11 on the aglycone. Based on the accumulated evidence, **2** is defined as (1*R*,6*R*,9*S*)-6,9,11-trihydroxy-4,7-megastigmadien-3-one 11-*O*-β-D-glucopyranoside. Recently, a megastigmane glucoside designated 6-hydroxy-junipeionoloside, with the same planar structure as **2**, was isolated from *Juniperus phoenicea*.[9] However, the stereostructure of 6-hydroxy-junipeionoloside was not established and no chiroptical data (optical rotation, CD evidence, *etc.*) on this compound was provided in that report.[9] Therefore it is impossible to discuss the identity between 6-hydroxy-junipeionoloside and **2** at present.

Compound **3**, a white powder, $[\alpha]_D$ −48.5°, gave the molecular formula of $C_{19}H_{34}O_7$ based on the $[M-H]^-$ ion peak at *m/z* 373.2217 in the HR-FAB-MS (negative mode). The ^{1}H- and ^{13}C-NMR analysis was performed with the aid of 2D techniques such as ^{1}H–^{1}H COSY, NOESY, HMQC, and HMBC and as a result, all protons and carbons were assigned as shown in Table 2. These established assignments (Table 2) suggested that **3** is constituted of a β-D-glucopyranosyl residue[4,5] and a megastigmane aglycone with a planar stucture shown for **3a** in Fig. 2. Furthermore, the presence of the NOESY cross peak between H-1′ and H-3 and HMBC correlation between H-1′ and C-3 demonstrated the glucosyl moiety to be connected to the 3-OH group of the aglycone through a glycosidic linkage. The absolute configurations at C-3 and C-9 of the aglycone were determined as follows. On enzymic hydrolysis, **3** gave the corresponding genuine aglycone (**3a**), which was subjected to the Mosher's procedure in the same manner as in the case of **2a**. A difference between the 3,9-di-(*S*)-MTPA ester (**3b**) and 3,9-di-(*R*)-MTPA ester (**3c**) in chemical shifts was indicative of the 3*R*, 9*S* configurations (Fig. 2).[8] Based on the above-mentioned combined evidence, **3** is now defined as (3*R*,9*S*)-megastigman-5-en-3,9-diol 3-*O*-β-D-glucopyranoside. Recently, synthetic (3*R*,9*S*)-megastigman-5-en-3,9-diol 3-*O*-β-D-glucopyranoside has been reported and its ^{1}H- and ^{13}C-NMR data coincided with those of **3**.[10] However, **3** was the first isolation from a natural source.

Compound **4**, a white powder, $[\alpha]_D$ −47.1° had the molecular formula of $C_{24}H_{42}O_{11}$, which was decided based on the $[M-H]^-$ ion at *m/z* 505.2651 in HR-FAB-MS (negative mode). In a similar manner as in the case of **3**, the ^{1}H- and ^{13}C-NMR spectra were analyzed in detail and as a result, all protons and carbons of **4** were assigned as shown in Table 2. Based on the assignments, the presence of β-D-glucopyranosyl and α-L-arabinofuranosyl moieties[4,5] as the sugar part of **4** were revealed along with megastigmane-type aglycone with the same plane structure as the aglycone (**3a**) of **3**. The subsequent NOESY and HMBC studies suggested that α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl moiety is bonded to the 3-quasi-equatorial hydroxy group of the aglycone (Fig. 3). Due to a shortage in the isolated amounts of **4**, Mosher's procedure could not be applied to determine the absolute configurations at C-3 and C-9 of the aglycone. However, a detailed comparison of the ^{1}H- and ^{13}C-NMR data of **4** with those of **3** (Table 2) suggested that **4** must correspond

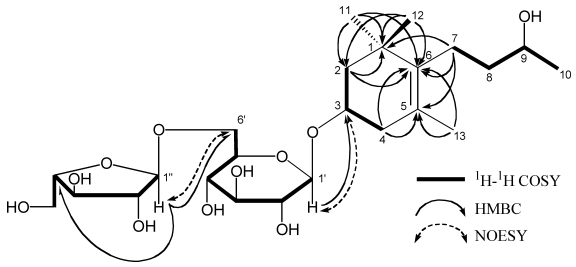

Fig. 3. Selected Correlations in COSY, HMBC and NOESY of **4**

Table 3. MIC Values of **1**, **2**, **3**, and **5** against *Helicobacter pylori*

Compound	MIC (μg/ml) *H. pylori*		
	NCTC11637	NCTC11916	OCO1
1	100	100	100
2	100	100	100
3	50	50	50
5	>200	>200	>200
Nat. hinokitiol	100	100	50
Syn. hinokitiol	100	100	50

to 6′-*O*-α-L-arabinofuranoside of **3**. Furthermore, the ^{1}H-chemical shifts of the methylene signals due to C-7 of **4** (δ 2.03, 2.12 ppm, 1H each) were compared with those of synthetic (3*R*,9*R*)- and (3*R*,9*S*)-megastigman-5-en-3,9-diol 3-*O*-β-D-glucopyranosides [δ 1.92, 2.21 ppm, 1H each in the (3*R*,9*R*)-isomer and δ 2.02 and 2.12 ppm, 1H each in the (3*R*,9*S*)-isomer, respectively],[10] suggesting that **4** has the absolute configurations of (3*R*,9*S*). In conclusion, **4** is defined as (3*R*,9*S*)-megastigman-5-en-3,9-diol 3-*O*-[α-L-arabinofuranosyl-(1→6)]-β-D-glucopyranoside.

Compound **5** was defined as formula **5** based on our structural elucidation using HR-FAB-MS, ^{1}H- and ^{13}C-NMR, and CD spectral analyses. Furthermore, it was identical with a known megastigmane, corchoionoside C [=(6*S*,9*S*)-roseoside A] by comparison of the optical rotation and ^{1}H- and ^{13}C-NMR spectral data.[3]

Antibacterial activities of **1**, **2**, **3**, **5**, and hinokitiol against three strains of *Helicobacter pylori* (NCTC11637, NCTC11916, and OCO1) were evaluated by measurement of the minimum inhibitory concentration (MIC value),[11] and the results are shown in Table 3. One (**3**) of the new megastigmane glucosides showed potent inhibition (MIC value=50 μg/ml) comparable to those of natural and synthetic hinokitiol as positive controls. Both the new monoterpene glucoside (**1**) and the other new megastigmane glucoside (**2**) also had mild anti-*H. pylori* activity (MIC value=100 μg/ml). However, the MIC value of the known megastigmane glucoside (**5**) was greater than 200 μg/ml, indicative of no activity. The inhibitory activities of natural products against *H. pylori* have been investigated in earnest for about the last 10 years, but it is reported here for the first time that the glucosides of lower terpenes such as **1**, **2**, and **3** show potent or mild anti-*H. pylori* activity. Furthermore, we expect that the present results may contribute to development of new anti-*H. pylori* drugs.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a GE-Omega 600 or JEOL JNM-ECA 600 (^{1}H at 600 MHz and ^{13}C at 150 MHz) or a JEOL JNM-GX 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometer. Chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. FAB- and HR-FAB-MS spectra in negative mode (matrix, triethanolamine or glycerin), along with EI- and HR-EI-MS spectra, were obtained with a JEOL JMS-700T spectrometer. Optical rotations were determined on a JASCO DIP-140 polarimeter and CD spectra were recorded on a JASCO J-820 spectropolarimeter. GLC was carried out on a Shimadzu GC-7AG under the following conditions: capillary column, TC-1 (0.32 mm i.d.×30 m, GL Sciences Inc.); detector, hydrogen flame ionization detector; column temperature, 230 °C; injection temperature, 250 °C; and carrier gas, N_2. For column chromatography, silica gel 60 (230—400 mesh, Merck), Chromatorex ODS DM1020T (100—200 mesh, Fuji Silysia), and Sephadex LH-20 (Amersham Biosciences) were used. Kiesel gel 60 F_{254} (Merck) and RP-18 F_{254} (Merck) were used for analytical TLC. Preparative HPLC was performed on a JAI LC-918 instrument with an RI-50 differential refractometer and a JAIGEL-ODS or a JAIGEL-GS 310 column, and also on a JASCO PU-2086 instrument with an RI-2031 differential refractometer and a TSK gel ODS-80 T_S column. The β-glucosidase (almond emulsin) and cellulase used in this work were commercially obtained from Sigma-Aldrich Japan Co. and Nagase Co. Ltd., respectively.

Plant Material Twigs with leaves of *J. communis* var. *depressa* were collected in July 1997, in Oregon, U.S.A. A voucher specimen (Murata J. *et al*., No. 053) was deposited in the Herbarium, Botanical Gardens, The University of Tokyo (TI), Japan.

Extraction and Isolation The dried and cut materials (2.4 kg) were extracted three times with MeOH (18 l×weekly) at room temperature. The MeOH solution was evaporated *in vacuo* to afford a dark greenish extract (488 g), an aliquot (202 g) of which was partitioned between *n*-hexane and MeOH. The MeOH-soluble part (130 g) was further partitioned between *n*-BuOH and water. The resulting *n*-BuOH extract (76 g) was chromatographed on silica gel and eluted with $CHCl_3$–MeOH–H_2O (7 : 3 : 1, a lower phase) to give 10 fractions (for each fraction, the abbreviations from A to J are used). Fraction F (6.2 g) was subsequently fractionated with silica gel column chromatography [$CHCl_3$–MeOH–H_2O (9 : 3 : 1, a lower phase)] into two fractions. The latter fraction obtained was further separated with Sephadex LH 20 column chromatography (eluted with MeOH) and subsequent ODS column chromatography (eluted with 50% MeOH) into three subfractions. Subfraction 2 was further purified with HPLC (JAIGEL-ODS column; 50% MeOH as an eluent) to give **3** (40.8 mg). Subfraction 3 was purified by repeated HPLC separation [JAIGEL-GS column (eluted with 50% MeOH), followed by a TSK gel ODS-80Ts column (50% MeOH)] to yield known **5** (56.2 mg). Fraction H (10.8 g) was divided into 13 fractions (from H-1 to H-13) with ODS column chromatography eluted with 50% MeOH. Fraction H-6 (1.96 g) was applied to a Sephadex LH-20 column eluted with 80% MeOH to afford two fractions. The first fraction obtained from fraction H-6 was further separated on an ODS column (eluted with 50% and 70% MeOH, successively) to give four subfractions. Subfraction 1 was further purified by repeated HPLC separation using JAIGEL-ODS (eluted with 50% MeOH) and JAIGEL-GS (eluted with 50% MeOH) columns to give **1** (124.7 mg). Subfraction 3 was purified on a Sephadex LH-20 column (eluted with MeOH) and HPLC separation (JAIGEL-GS column; 50% MeOH as an eluting agent) to afford **4** (4.2 mg). Fraction H-8 (1.77 g) was subjected to ODS column chromatography eluted with 50% MeOH to be divided into six fractions. The first fraction obtained was purified by HPLC separation using JAIGEL ODS (eluted with 50% MeOH) and subsequent JAIGEL-GS (eluted with 50% MeOH) columns to give **2** (62.9 mg).

1: A white powder, $[\alpha]_D$ −36.3° (c=0.54, MeOH). HR-FAB-MS (negative mode) *m/z*: 345.1544 (Calcd for $C_{16}H_{25}O_8$, $[M-H]^-$: 345.1550). ^{1}H- and ^{13}C-NMR data are given in Table 1.

2: A white powder, $[\alpha]_D$ +85.9° (c=0.32, MeOH). FAB- and HR-FAB-MS (negative mode) *m/z*: 401.1805 (Calcd for $C_{19}H_{29}O_9$, $[M-H]^-$: 401.1811), 239 $[M-H-162]^-$. CD (c=1.08×10^{-4} mol/l, MeOH) $\Delta\varepsilon$ (λ nm): +9.88 (245). ^{1}H- and ^{13}C-NMR data are given in Table 2.

3: A white powder, $[\alpha]_D$ −48.5° (c=1.05, MeOH). HR-FAB-MS (negative mode) *m/z*: 373.2217 (Calcd for $C_{19}H_{33}O_7$, $[M-H]^-$: 373.2226). ^{1}H- and ^{13}C-NMR data are given in Table 2.

4: A white powder, $[\alpha]_D$ −47.1° (c=0.23, MeOH). HR-FAB-MS (negative mode) *m/z*: 505.2651 (Calcd for $C_{24}H_{41}O_{11}$, $[M-H]^-$: 505.2649). ^{1}H- and ^{13}C-NMR data are given in Table 2.

5: A white powder, $[\alpha]_D$ +64.1° (c=1.01, MeOH). HR-FAB-MS (negative mode) *m/z*: 385.1858 (Calcd for $C_{19}H_{29}O_8$, $[M-H]^-$: 385.1862). CD (c=9.3×10^{-5} mol/l, MeOH) $\Delta\varepsilon$ (λ nm): +9.94 (242). The ^{1}H- and ^{13}C-

NMR spectral data of **5** were in agreement with those reported for corchoionoside C [=(6*S*,9*S*)-roseoside A].[3)]

Enzymatic Hydrolysis of 1 A solution of **1** (20.0 mg) in water (2 ml) was incubated with β-glucosidase (almond emulsin; 10 mg) at 37 °C for 17 h. The reaction mixture was poured into a large amount of water and extracted with AcOEt. The solvent of the extract was evaporated to dryness under reduced pressure to give the corresponding aglycone **1a** (8.5 mg), a white powder, $[\alpha]_D$ −51.4° (*c*=0.58, MeOH). HR-FAB-MS (negative mode) *m/z*: 183.1029 (Calcd for $C_{10}H_{15}O_3$, $[M-H]^-$: 183.1021). ^{1}H-NMR (400 MHz, pyridine-d_5) δ: 1.30 (6H, s, H_3-9, H_3-10), 1.40 (1H, m, H-5), 1.71 (1H, m, H-4), 2.20 (2H, m, H-3, H-5), 2.46 (2H, m, H-3, H-6), 2.90 (1H, br d, *J*=16.0 Hz, H-6), 7.38 (1H, br dd, *J*=3.0, 3.0 Hz, H-2). ^{13}C-NMR (100 MHz, pyridine-d_5) δ: 24.0 (C-9 or C-10), 26.2 (C-5), 27.1 (C-10 or C-9), 27.8 (C-6), 28.0 (C-3), 45.1 (C-4), 70.9 (C-8), 131.8 (C-1), 139.4 (C-2), 169.9 (C-7).

Enzymatic Hydrolysis of 2 A solution of **2** (14.9 mg) in water (2 ml) was incubated with cellulase (20 mg) at 37 °C for 43 h. The reaction mixture was poured into a large amount of water and extracted with AcOEt. The solvent of the extract was evaporated to dryness under reduced pressure to give an artifact aglycone **2a** (3.2 mg), a white powder, $[\alpha]_D$ +27.9° (*c*=0.17, MeOH). HR-EI-MS *m/z*: 222.1252 (Calcd for $C_{13}H_{18}O_3$, $[M-H_2O]^+$: 222.1256). ^{1}H- and ^{13}C-NMR data are given in Table 2.

Preparation of (*S*)-MTPA Ester (2b) and (*R*)-MTPA Ester (2c) from 2a To a solution of **2a** (1.6 mg) in pyridine (0.5 ml), (*R*)-(−)-MTPA chloride (40 μl) was added and the reaction mixture was allowed to stand for 25 h at room temperature. After the solvent was evaporated *in vacuo*, the resulting residue was extracted with a mixture of ether and water. The ether layer was washed with brine and dried over $MgSO_4$. After evaporation of ether, the residue was purified with silica gel column chromatography eluted with a mixture of *n*-hexane and acetone (2 : 1) to give the corresponding 9-(*S*)-MTPA ester (**2b**) (3.0 mg), a colorless oil. ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.953 (3H, s, H_3-12), 1.111 (3H, s, H_3-13), 1.416 (3H, d, *J*=6.4 Hz, H_3-10), 2.358 (1H, dd, *J*=18.0, 2.4 Hz, H-2), 2.408 (1H, dd, *J*=17.6, 2.4 Hz, H-4), 2.595 (1H, dd, *J*=18.0, 2.8 Hz, H-2), 2.644 (1H, d, *J*=17.6 Hz, H-4), 3.640 (1H, d, *J*=8.0 Hz, H-11), 3.908 (1H, dd, *J*=8.0, 2.8 Hz, H-11), 5.684 (1H, m, H-9), 6.170 (1H, dd, *J*=15.2, 6.8 Hz, H-8), 6.247 (1H, d, *J*=15.2 Hz, H-7). A similar treatment of **2a** (1.6 mg) with (*S*)-(+)-MTPA chloride (40 μl) gave the corresponding 9-(*R*)-MTPA ester (**2c**) (3.0 mg), a colorless oil. ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.905 (3H, s, H_3-12), 1.088 (3H, s, H_3-13), 1.471 (3H, d, *J*=6.4 Hz, H_3-10), 2.310 (1H, dd, *J*=18.0, 2.4 Hz, H-2), 2.392 (1H, dd, *J*=17.6, 2.4 Hz, H-4), 2.469 (1H, dd, *J*=18.0, 2.8 Hz, H-2), 2.559 (1H, d, *J*=18.0 Hz, H-4), 3.622 (1H, d, *J*=8.0 Hz, H-11), 3.882 (1H, dd, *J*=8.0, 2.8 Hz, H-11), 5.703 (1H, m, H-9), 6.072 (1H, d, *J*=15.2 Hz, H-7), 6.114 (1H, dd, *J*=15.2, 6.8 Hz, H-8).

Enzymatic Hydrolysis of 3 A solution of **3** (6.0 mg) in water (2 ml) was incubated with β-glucosidase (almond emulsin; 10 mg) at 37 °C for 16.5 h. The reaction mixture was poured into a large amount of water and extracted with AcOEt. The usual work-up and purification of the AcOEt layer gave the corresponding genuine aglycone **3a** (3.0 mg), a white powder, $[\alpha]_D$ −54.4° (*c*=0.30, MeOH). HR-EI-MS *m/z*: 212.1779 (Calcd for $C_{13}H_{24}O_2$, M^+: 212.1776). ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.03 (3H, s, H_3-11), 1.06 (3H, s, H_3-12), 1.17 (3H, d, *J*=6.3 Hz, H_3-10), 1.37 (1H, dd, *J*=12.0, 12.0 Hz, H-2β), 1.45 (1H, m, H-8), 1.52 (1H, m, H-8), 1.63 (3H, s, H_3-13), 1.68 (1H, ddd, *J*=12.0, 3.6, 2.4 Hz, H-2α), 1.92 (1H, br dd, *J*=16.6, 10.8 Hz, H-4β), 2.02 (1H, dt, *J*=13.2, 5.1 Hz, H-7), 2.12 (1H, dt, *J*=13.2, 5.1 Hz, H-7), 2.18 (1H, br dd, *J*=16.6, 5.8 Hz, H-4α), 3.70 (1H, m, H-9), 3.84 (1H, m, H-3). ^{13}C-NMR (100 MHz, CD_3OD) δ: 20.0 (C-13), 23.3 (C-10), 25.6 (C-7), 28.9 (C-11), 30.4 (C-12), 38.9 (C-1), 40.7 (C-8), 43.0 (C-4), 49.6 (C-2), 65.7 (C-3), 69.2 (C-9), 125.5 (C-5), 138.4 (C-6).

Preparation of 3,9-Di-(*S*)-MTPA Ester (3b) and 3,9-Di-(*R*)-MTPA Ester (3c) from 3a In a similar manner as in the derivation of **2b** and **2c** from **2a**, **3a** (1.5 mg each) was treated with (*R*)-(−)- and (*S*)-(+)-MTPA chloride (40 μl each) to afford **3b** (2.0 mg) and **3c** (2.2 mg), respectively. **3b**: a colorless oil, ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.972 (3H, s, H_3-11), 1.043 (3H, s, H_3-12), 1.291 (3H, d, *J*=6.3 Hz, H_3-10), 1.500 (1H, dd, *J*=12.0, 12.0 Hz, H-2β), 1.618 (3H, s, H_3-13), 1.689 (2H, m, H_2-8), 1.729 (1H, ddd, *J*=12.0, 3.6, 2.4 Hz, H-2α), 2.080 (2H, m, H_2-7), 2.165 (1H, br dd, *J*=16.0, 10.0 Hz, H-4β), 2.369 (1H, br dd, *J*=16.6, 5.8 Hz, H-4α), 5.111 (1H, m, H-9), 5.252 (1H, m, H-3). **3c**: a colorless oil, ^{1}H-NMR (400 MHz, CD_3OD) δ: 0.909 (3H, s, H_3-11), 0.934 (3H, s, H_3-12), 1.350 (3H, d, *J*=6.3 Hz, H_3-10), 1.491 (3H, s, H_3-13), 1.560 (1H, dd, *J*=12.0, 12.0 Hz, H-2β), 1.578 (2H, m, H_2-8), 1.779 (1H, ddd, *J*=12.0, 3.6, 2.4 Hz, H-2α), 1.891 (2H, m, H_2-7), 1.999 (1H, br dd, *J*=16.6, 10.0 Hz, H-4β), 2.250 (1H, br dd, *J*=16.6, 5.8 Hz, H-4α), 5.099 (1H, m, H-9), 5.218 (1H, m, H-3).

Determination of Configurations of Each Component Glucose in 1—4 and the Component Arabinose in 4 A solution of each of **1**—**4** (each 2.0 mg) in 2 M HCl–EtOH (1 : 1; 0.5 ml) was stirred under reflux for 2 h. The solution was extracted with EtOAc and water. The resulting aqueous layer was neutralized with Amberlite IRA-93ZU (Organo Co., Ltd.) and evaporation of the water gave a monosaccharide residue that was subjected to the preparation of the corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure.[5)] The D-configuration for glucose obtained from each of **1**—**4** and the L-configuration for arabinose from **4** were determained, respectively, based on a direct comparison with D- and L-standards of glucose (t_R: D-Glc, 13 min 15 s; t_R: L-Glc, 13 min 54 s) and arabinose (t_R: D-Ara, 8 min 13 s; t_R: L-Ara, 7 min 39 s).

Bioassay. Bacteria and Broth *Helicobacter pylori* NCTC11637, *H. pylori* NCTC11916, and *H. pylori* OCO1 were used in the present assay. Brucella broth and Brucella agar (Becton Dickinson Co., Ltd.) were used for preincubation of *H. pylori* and for the measurement of MIC, respectively.

Measurements of MIC of 1, 2, 3, and 5 MIC values were determined using the agar dilution method.[11)] Each of the samples (**1**, **2**, **3**, and **5**, and both natural and synthetic specimens of hinokitiol as positive standards) was dissolved individually in dimethylsulfoxide (0.5 ml) and added to sterilized distilled water, giving a sample solution of 200 μg/ml that was diluted with sterilized distilled water according to the two-fold dilution method to afford 100, 50, and 25 μg/ml of solutions, respectively. To each sample solution (1 ml) at various concentrations in each Petri dishes, sterilized albumin solution (250 mg/50 ml; 1 ml) and sterilized Brucella agar (8 ml) were added and mixed. *H. pylori* suspension 50 μl (about 1.0×10^7 cells/ml) was inoculated into each agar plate. The agar plates were placed in a cultivation jar equipped with AnaeroPack® Campylo (Mitsubishi Gas Co., Ltd.). After cultivation at 37 °C for 2 d under slightly aerobic conditions, survival or death of the bacteria was judged and the MIC was determined.

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