

Medicinal Flowers. XV.¹⁾ The Structures of Noroleanane- and Oleanane-Type Triterpene Oligoglycosides with Gastroprotective and Platelet Aggregation Activities from Flower Buds of *Camellia japonica*

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The methanolic extract from the flower buds of *Camellia japonica* L. (Theaceae) were found to exhibit potent inhibitory activities on ethanol- or indomethacin-induced gastric mucosal lesions in rats. Through bioassay-guided separation, 28-noroleanane-type triterpene oligoglycosides, camelliosides A, B, and C, and an oleanane-type triterpene oligoglycoside, camellioside D, were isolated from the methanolic extract together with five known compounds. The absolute stereostructures of camelliosides were determined on the basis of chemical and physicochemical evidence, which included the structure revision of the nortriterpene aglycons (camellenodiol and camelledionol). The principal oligoglycosides, camelliosides A and B, showed platelet aggregation activity in addition to the gastroprotective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats.

Key words camellioside; gastroprotective effect; platelet aggregation activity; *Camellia japonica*; camellenodiol; camelledionol

The Theaceae plant, *Camellia* (*C.*) *japonica* L. (Japanese name “Tsubaki”) is widely cultivated as an ornamental or garden tree in Japan. The flower buds of *C. japonica* have been used for the treatment of blood vomiting and bleeding due to internal and external injury, and also as an antiinflammatory, tonic, and stomatic in Japanese folk medicine and Chinese traditional medicine. As chemical constituents of this natural medicine, several triterpenes and flavonoids were reported,^{2,3)} but the constituents responsible for the traditional medicinal uses have not been identified. We have already reported the isolation and structure elucidation of acylated polyhydroxyoleanane-type triterpene oligoglycosides, camelliasaponins A₁, A₂, B₁, B₂, C₁, and C₂, from the seeds of *C. japonica* and these oligoglycosides were found to inhibit alcohol absorption in rats.^{4,5)} During the course of our studies on medicinal flowers,^{1,6–10)} we found that the methanolic extract from the flower buds of *C. japonica* showed potent gastroprotective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats. Through bioassay-guided separation, we isolated three then new 28-noroleanane-type triterpene oligoglycosides, camelliosides A (1), B (2), and C (3), and an oleanane-type triterpene oligoglycoside, camellioside D (4), together with five known constituents. This paper presents a full account of the isolation

and absolute stereostructure elucidations of four then new oligoglycosides (1–4).¹¹⁾ In addition, we describe the gastroprotective effects of principal camelliosides (1, 2) on gastric lesions induced by ethanol or indomethacin in rats as well as the inhibitory effects on rabbit platelet aggregation.

Isolation of Camelliosides The fresh flower buds of *C. japonica* (collected in Idzu-ohshima island, Tokyo, Japan) were extracted with methanol under reflux. The methanolic extract (8.0% from the fresh flower buds) was partitioned into an EtOAc–H₂O mixture to furnish an EtOAc-soluble fraction and aqueous layer. The aqueous layer was further extracted with *n*-BuOH to give *n*-BuOH and H₂O-soluble fractions. As shown in Table 1, the methanolic extract and *n*-BuOH-soluble fraction (the saponin fraction) were found to show potent protective activities on ethanol- or indomethacin-induced gastric lesions in rats, whereas the EtOAc-soluble and H₂O-soluble fractions did not exhibit the activity.

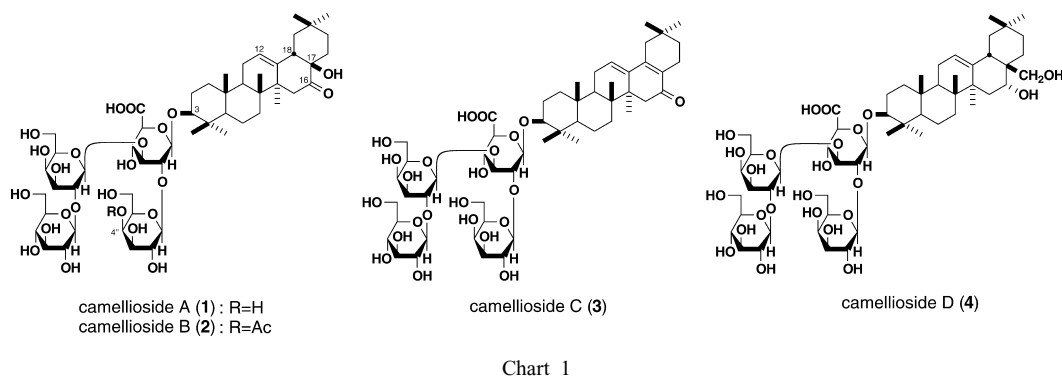
The active fraction (the *n*-BuOH-soluble fraction) was subjected to ordinary and reversed-phase silica-gel column chromatographies and finally HPLC to furnish camelliosides A (1, 0.10%), B (2, 0.067%), C (3, 0.0052%), and D (4, 0.0023%) together with (–)-epicatechin (0.030%). Through the similar procedure, oleanolic acid (0.0006%), benzyl β-D-

Table 1. Effects of the MeOH Extract, AcOEt-, *n*-BuOH, and H₂O-Soluble Fractions from the Flowers of *C. japonica* on Gastric Lesions Induced by EtOH or Indomethacin in Rats

Treatment	Dose (mg/kg, <i>p.o.</i>)	<i>N</i>	EtOH-induced gastric lesions		<i>N</i>	Indomethacin-induced gastric lesions	
			Lesion index (mm)	Inhibition (%)		Lesion index (mm)	Inhibition (%)
Control	—	12	115.8±8.7	—	18	102.0±10.4**	—
MeOH ext.	100	8	71.3±12.6	38.4	8	60.9±10.4**	40.3
	200	8	11.0±5.3**	90.5	8	37.3±5.4**	63.5
AcOEt-soluble fraction	50	8	101.8±27.1	12.1	8	84.8±11.6	16.9
<i>n</i> -BuOH-soluble fraction	25	8	58.7±15.8**	49.3	8	51.4±13.5**	49.6
	50	8	37.6±13.0**	67.6	8	38.9±7.9**	61.9
H ₂ O-soluble fraction	50	8	149.6±12.1	–29.2	8	87.2±6.8	14.6

Each value represents the mean±S.E.M. Significantly different from the control group, ***p*<0.01.

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glucopyranoside¹²⁾ (0.0004%), methyl gallate (0.0003%), (+)-catechin (0.0011%), and (–)-epicatechin (0.038%) were isolated from the EtOAc-soluble fraction.

Structure of Camellioside A (1) Camellioside A (1) was isolated as colorless fine crystals of mp 226–229 °C from aqueous MeOH with negative optical rotation ($[\alpha]_D^{26} -32.1^\circ$). The IR spectrum of **1** showed strong absorption bands at 3453 and 1078 cm^{-1} suggestive of an oligoglycosidic structure and weak bands at 1736, 1719, and 1656 cm^{-1} ascribable to carbonyl, carboxyl, and olefin functions. In the negative-ion FAB-MS of **1**, a quasimolecular ion peak was observed at m/z 1103 $[\text{M}-\text{H}]^-$, together with fragment ion peaks at 941 $[\text{M}-\text{C}_6\text{H}_{11}\text{O}_5]^-$, and 779 $[\text{M}-\text{C}_{12}\text{H}_{21}\text{O}_{10}]^-$, which were derived by cleavage of the glycoside linkages of the terminal hexose and dihexose parts. The positive-ion FAB-MS of **1** showed a quasimolecular ion peak at m/z 1127 $[\text{M}+\text{Na}]^+$ and the molecular formula $\text{C}_{53}\text{H}_{84}\text{O}_{24}$ of **1** was determined by high-resolution MS measurement of the quasimolecular ion peak $[\text{M}+\text{Na}]^+$. Acid hydrolysis of **1** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) liberated D-glucuronic acid, D-galactose, and D-glucose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.^{13–15)} Enzymatic hydrolysis of **1** with glycyrrhizic acid hydrolase gave an aglycon, camellenodiol (**5**),²⁾ which was derived to the 3-O-acetate (**5a**) by acetylation with acetic anhydride (Ac_2O)-pyridine and camelledionol (**6**) by oxidation with chromium trioxide (CrO_3)-pyridine.²⁾ Camellenodiol and camelledionol were isolated from the same plant material and their structures were reported as **5'** and **6'**, respectively, by means of detail ^1H -NMR and CD analysis.²⁾

From the biogenetic consideration on noroleanane-type triterpene aglycons,¹⁶⁾ we had a considerable doubt as to the position of the tertiary hydroxyl group in the previous formula (**5'**, **6'**) of camellenodiol and camelledionol. The previous structures of camellenodiol and camelledionol were finally revised as **5** and **6**, respectively, on the basis of the ^1H - ^1H correlation spectroscopy (^1H - ^1H COSY) and heteronuclear multiple-bond correlations (HMBC) experiments (Fig. 1) and X-ray crystallographic analysis (Fig. 2). Namely, the ^1H -NMR (pyridine- d_5) and ^{13}C -NMR (Table 1) spectra of **5**, which were assigned by various NMR experiment,¹⁷⁾ showed signals due to seven tertiary methyls [δ 0.80, 0.89, 0.95, 0.96, 1.00, 1.04, 1.19 (H_3 -24, 29, 25, 30, 23, 26, 27)], an allylic proton [δ 2.72 (dd, $J=4.0, 14.0$ Hz, H-18)], a methylene adjacent to a ketone group [δ 1.81 (d, $J=14.0$ Hz, H_2 -15)], and an olefin proton [δ 5.51 (dd-like, H-12)]. The

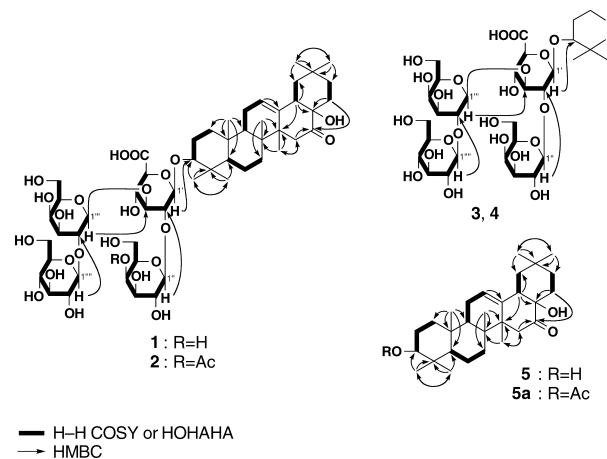


Fig. 1. 2D NMR Correlations of **1**–**5** and **5a**

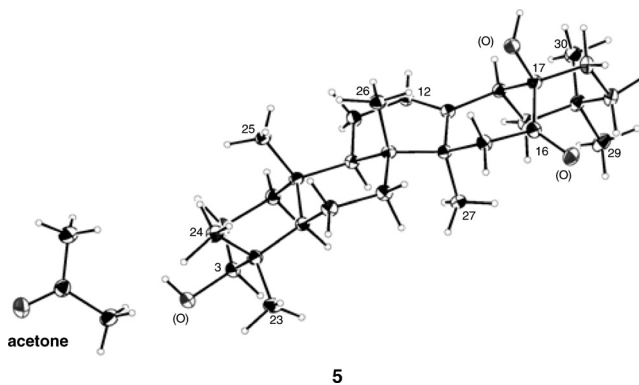
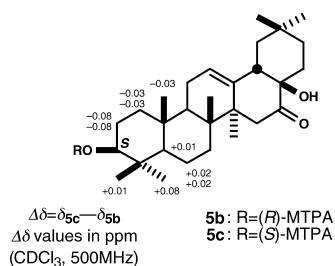
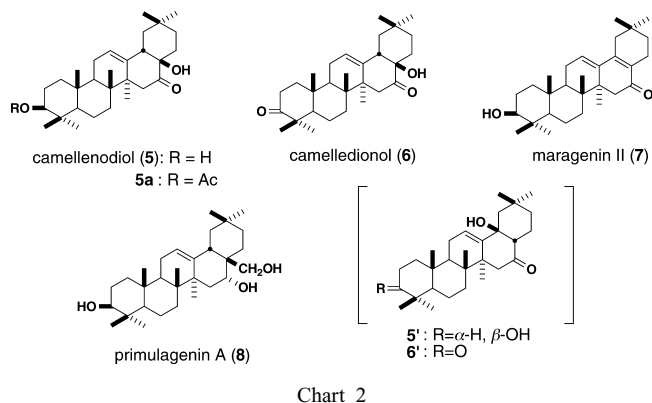


Fig. 2. X-Ray Crystallographic Analysis of **5**

^1H - ^1H COSY experiment on **5** and **5a** indicated the presence of partial structures written in bold lines as shown in Fig. 1. The connectivities of the quaternary carbons were elucidated by HMBC experiment, which showed long-range correlations between the following protons and carbons: H_2 -15 and C-16, 27; H-18 and C-14, 17, 19; H-22 and C-16, 17. This evidence led us to elucidate the position of the tertiary hydroxyl group in **5**. Furthermore, the structure of camellenodiol (**5**) was confirmed by the X-ray crystallographic analysis as shown in Fig. 2. Consequently, the structure of camellenodiol was revised from **5'** to **5**. Next, the absolute stereochemistry of **5** was determined by application of modified Mosher's method.¹⁸⁾ That is, treatment of **5** with (*R*)- or (*S*)- α -methoxy- α -trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylamino-

Fig. 3. Results of the Modified Mosher's Method for **5b** and **5c**

propyl)carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) to yield the (*R*)- and (*S*)-MTPA esters (**5b**, **5c**), respectively. As shown in Fig. 3, the signals due to protons attached to C-5, C-6, C-23 and C-24 in the (*S*)-MTPA ester (**5c**) were observed at lower fields compared with those of the (*R*)-MTPA ester (**5b**) [$\Delta\delta$: positive], while the signals due to protons of C-1, C-2, and C-25 in **5c** were observed at higher fields compared with those of **5b** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 3-position of **5** was determined to be *S* and the absolute stereostructure of **5** was elucidated as shown. Moreover, the structures of related compounds of **5**, 3-acetylcamellenodiols and camelledionol,²⁾ were also revised from **5'** and **6'** to **5a** and **6**, respectively (Chart 2).

The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra¹⁷⁾ of **1** showed signals assignable to a camellenodiols moiety [δ 0.87, 0.90, 0.98, 1.10, 1.27, 1.34, 1.37 (3H each, all s, H₃-25, 29, 30, 24, 26, 23, 27), 3.10 (1H, dd, *J*=3.5, 14.5 Hz, H-18), 3.27 (1H, dd, *J*=4.5, 11.8 Hz, H-3), 5.49 (1H, br s, H-12)] together with a β-D-glucuronopyranosyl moiety [δ 4.90 (1H, d, *J*=7.4 Hz, H-1'), a β-D-glucopyranosyl moiety [δ 5.18 (1H, d, *J*=7.7 Hz, H-1''), and two β-D-galactopyranosyl moieties [δ 5.70 (1H, d, *J*=7.6 Hz, H-1'''), 5.76 (1H, d, *J*=7.6 Hz, H-1'')]. The oligoglycosidic structure of **1** was determined by ¹H-¹H COSY, homo-nuclear Hartmann-Hahn spectroscopy (¹H-¹H HOHAHA) and HMBC experiments (Fig. 1). In the HMBC experiment of **1**, long-range correlations were observed between the following protons and carbons (H-1' and C-3, H-1'' and C-2', H-1''' and C-3', H-1''' and C-2''). On the basis of those findings, the structure of camellioside A (**1**) was characterized as shown.

Structures of Camelliosides B–D (2–4) Camellioside B (**2**) was obtained as colorless fine crystals of mp 221–224 °C from aqueous MeOH with negative optical rotation ($[\alpha]_D^{23}$ –30.0°). The IR spectrum of **2** showed absorption

bands at 1744, 1726, and 1655 cm^{–1} ascribable to carbonyl, carboxyl, and olefin functions and strong broad bands at 3453 and 1078 cm^{–1} suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **2**, quasi-molecular ion peaks were observed at *m/z* 1169 [*M*+Na]⁺ and *m/z* 1145 [*M*–H][–] together with fragment ion peaks at *m/z* 983 [*M*–C₆H₁₁O₅][–] and *m/z* 821 [*M*–C₁₂H₂₁O₁₀][–], and high resolution MS analysis revealed the molecular formula of **2** to be C₅₅H₈₆O₂₅. Acid hydrolysis with 5% H₂SO₄–1,4-dioxane (1 : 1, v/v) of **2** liberated D-glucuronic acid, D-galactose, and D-glucose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.^{13–15)} The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra¹⁷⁾ of **2** showed signals assignable to a β-D-glucuronopyranosyl moiety [δ 4.89 (1H, d, *J*=7.6 Hz, H-1')], a β-D-glucopyranosyl moiety [δ 5.16 (1H, d, *J*=7.6 Hz, H-1''), and two β-D-galactopyranosyl moieties [δ 5.70 (1H, d, *J*=7.6 Hz, H-1'''), 5.87 (1H, d-like, H-1'')] together with an acetyl group [δ 2.13 (3H, s, –OCOCH₃)] and an aglycon moiety [δ 0.87, 0.90, 0.98, 1.10, 1.27, 1.34, 1.37 (3H each, all s, H₃-25, 29, 30, 24, 26, 27, 23), 3.08 (1H, dd-like, H-18), 3.30 (1H, dd, *J*=3.5, 11.0 Hz, H-3), 5.45 (1H, br s, H-12)]. The proton and carbon signals in the ¹H- and ¹³C-NMR spectra of **2** were superimposable on those of **1**, except for the signals due to an acetyl group. Treatment of **2** with 0.1% sodium methoxide (NaOMe)–MeOH liberated **1**, and comparison of the ¹³C-NMR data of **2** with those of **1** indicated the presence of an acetylation shift¹⁹⁾ around the 4''-position. Finally, the HMBC experiment on **2** showed a long-range correlation between the 4''-proton [δ 5.93 (1H, br s)] and acetyl carbonyl carbon (δ_C 170.9). Consequently, the structure of camellioside B was determined as the 4''-acetyl derivative (**2**) of camellioside A.

Camellioside C (**3**), which was isolated as colorless fine crystals of mp 224–227 °C (from aqueous MeOH) with negative optical rotation ($[\alpha]_D^{26}$ –15.8°), showed absorption bands at 3453, 1736, 1665, 1655, and 1078 cm^{–1} ascribable to hydroxyl, carbonyl, carboxyl, olefin, and ether functions in the IR spectrum. In the UV spectrum of **3**, the absorption maximum was observed at 299 (log ϵ 4.0) nm, suggestive of an α,β-unsaturated ketone group with extended conjugation.²⁾ The molecular formula C₅₃H₈₂O₂₃ of **3** was also determined from the quasimolecular ion peaks at *m/z* 1109 [*M*+Na]⁺, *m/z* 1087 [*M*+H]⁺, and *m/z* 1085 [*M*–H][–] in the positive-ion and negative-ion FAB-MS and by high-resolution MS measurement. By the acid hydrolysis of **3**, D-glucuronic acid, D-galactose, and D-glucose were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.^{13–15)} Enzymatic hydrolysis of **3** with glycyrrhizic acid hydrolase furnished maragenin II (**7**) as its aglycon.^{2,20)} The ¹H- and ¹³C-NMR (pyridine-*d*₅, Table 1) spectra¹⁷⁾ of **3** showed the presence of a β-D-glucuronopyranosyl moiety [δ 4.90 (1H, d, *J*=7.4 Hz, H-1')], a β-D-glucopyranosyl moiety [δ 5.17 (1H, d, *J*=8.1 Hz, H-1''), and two β-D-galactopyranosyl moieties [δ 5.69 (1H, d, *J*=7.6 Hz, H-1'''), 5.76 (1H, d, *J*=7.7 Hz, H-1'')] together with a maragenin II part.^{2,20)} The oligoglycosidic structure of **3** was elucidated by the HMBC experiment as shown in Fig. 1, which showed the same long-range correlations as those of **1**. This evidence led us to elucidate the structure of camellioside C (**3**) to be as shown.

Camellioside D (**4**), obtained as colorless fine crystals of

Table 2. ^{13}C -NMR Data for Camelliosides A (**1**), B (**2**), C (**3**), and D (**4**) and Camellenodiols (**5**)

	1 ^{a)}	2 ^{a)}	3 ^{a)}	4 ^{a)}	5 ^{a)}	5 ^{b)}		1 ^{a)}	2 ^{a)}	3 ^{a)}	4 ^{a)}
C-1	38.5	38.7	38.9	38.9	38.9	38.5	GlcA-1'	105.5	105.8	105.6	105.7
C-2	26.4	26.7	26.6	26.6	28.0	27.2	-2'	79.0	78.5	79.1	79.3
C-3	89.5	89.9	89.5	89.5	78.0	79.0	-3'	83.7	84.8	84.0	84.6
C-4	39.4	39.8	39.6	39.6	39.3	38.8	-4'	70.9	71.2	71.0	71.1
C-5	55.7	55.9	55.8	55.9	55.7	55.4	-5'	76.9	77.1	77.0	77.0
C-6	18.3	18.6	18.4	18.6	18.7	18.3	-6'	171.9	171.7	171.7	171.8
C-7	33.2	33.5	33.8	33.3	33.4	32.8					
C-8	40.1	40.3	39.0	40.1	40.2	39.9	Gal-1''	103.0	102.8	103.1	103.3
C-9	46.9	47.1	46.3	47.1	47.1	46.8	-2''	73.7	73.2	73.9	74.0
C-10	36.8	37.1	36.8	36.9	37.3	37.1	-3''	74.7	74.0	74.9	75.0
C-11	23.8	24.1	24.4	23.9	23.9	23.7	-4''	69.8	72.0	69.9	70.0
C-12	124.1	124.2	127.3	122.4	123.8	125.7	-5''	76.3	75.1	76.4	76.4
C-13	142.3	142.6	139.2	145.2	142.4	140.5	-6''	61.8	61.4	61.9	61.8
C-14	48.1	48.4	45.1	42.1	48.3	47.4					
C-15	43.3	43.5	40.4	34.9	43.3	43.0	4''-Ac		21.2		
C-16	214.9	214.8	199.1	74.3	214.8	213.5			170.9		
C-17	76.3	76.5	129.0	41.1	76.4	76.5					
C-18	52.7	53.0	146.3	42.6	52.8	52.6	Gal-1'''	101.4	101.9	101.6	101.8
C-19	48.2	48.3	44.5	48.4	48.1	47.2	-2'''	83.0	83.5	83.2	83.5
C-20	30.9	31.1	29.2	31.3	30.9	30.8	-3'''	74.7	75.1	74.9	75.0
C-21	37.2	37.4	34.7	37.2	37.2	36.5	-4'''	69.8	69.9	69.9	69.9
C-22	31.5	31.8	21.3	30.4	31.5	30.4	-5'''	76.3	76.5	76.4	76.4
C-23	28.1	28.2	28.1	28.2	28.7	28.2	-6'''	61.9	62.5	62.1	62.1
C-24	16.7	16.9	16.9	16.9	16.5	15.6					
C-25	15.3	15.5	15.6	15.8	15.5	15.3	Glc-1'''	106.2	106.8	106.5	106.7
C-26	17.7	17.9	18.0	17.1	17.8	17.3	-2'''	75.8	76.2	76.0	76.1
C-27	27.0	27.3	23.3	27.4	27.1	27.1	-3'''	78.1	78.5	78.3	78.4
C-28				70.2			-4'''	71.0	71.4	71.2	71.2
C-29	32.6	32.9	28.6	33.5	32.7	32.4	-5'''	78.2	78.5	78.4	78.5
C-30	23.6	23.9	28.2	24.9	23.7	23.7	-6'''	62.2	61.8	62.4	62.4

Measured in a) pyridine- d_5 and b) CDCl_3 at 125 MHz. GlcA: β -D-glucopyranosiduronic acid; Gal: β -D-galactopyranosyl; Glc: β -D-glucopyranosyl.

Table 3. Effects of Camelliosides A (**1**) and B (**2**) on Gastric Lesions Induced by EtOH or Indomethacin in Rats

Treatment	Dose (mg/kg, p.o.)	N	EtOH-induced gastric lesions		N	Indomethacin-induced gastric lesions	
			Lesion index (mm)	Inhibition (%)		Lesion index (mm)	Inhibition (%)
Control	—	9	141.4 $\pm$ 9.0	—	5	119.3 $\pm$ 11.5	—
Camellioside A (1)	5	6	93.8 $\pm$ 13.3	33.7	5	106.6 $\pm$ 9.8	10.7
	10	6	85.1 $\pm$ 30.2	39.8	5	84.2 $\pm$ 10.7	29.4
	20	6	32.0 $\pm$ 10.6**	77.3	5	61.1 $\pm$ 12.8**	46.2
Camellioside B (2)	5	6	92.1 $\pm$ 20.0	34.9	5	104.7 $\pm$ 6.5	12.2
	10	6	63.1 $\pm$ 20.6**	55.4	5	68.0 $\pm$ 10.9*	43.0
	20	6	11.6 $\pm$ 3.1**	91.8	5	43.0 $\pm$ 8.2**	63.9
Control	—	7	146.8 $\pm$ 9.7	—	5	88.7 $\pm$ 9.6	—
Omeprazole	2.5				4	80.9 $\pm$ 12.4	8.8
	5				5	38.0 $\pm$ 16.5*	57.2
	10	7	78.3 $\pm$ 15.9**	46.7	5	4.7 $\pm$ 1.8**	94.7
	15	7	31.1 $\pm$ 11.6**	78.8			
	20	7	15.9 $\pm$ 3.1**	89.2			
Control	—	8	136.0 $\pm$ 10.6	—	6	85.2 $\pm$ 2.4	—
Cimetidine	12.5	5	123.4 $\pm$ 9.4	9.3	6	61.2 $\pm$ 5.5**	28.2
	25	7	87.2 $\pm$ 7.9**	35.9	6	34.8 $\pm$ 5.0**	59.2
	50	7	72.9 $\pm$ 10.1**	46.4	6	17.6 $\pm$ 3.2**	79.3
	100	7	64.4 $\pm$ 4.9**	52.6			

Each value represents the mean $\pm$ S.E.M. Significantly different from the control group, * p <0.05, ** p <0.01.

mp 222–225 °C (from aqueous MeOH) with negative optical rotation ($[\alpha]_D^{26}$ -3.2°), showed absorption bands due to hydroxyl, carboxyl, olefin, and ether functions (3453, 1719, 1655, 1078 cm^{-1}) in the IR spectrum. The molecular formula $\text{C}_{54}\text{H}_{88}\text{O}_{24}$ of **4** was also determined from the quasimolecular ion peaks at m/z 1143 $[\text{M}+\text{Na}]^+$ and m/z 1119 $[\text{M}-\text{H}]^-$ in the positive-ion and negative-ion FAB-MS and by high-reso-

lution MS measurement. The acid hydrolysis of **4** liberated D-glucuronic acid, D-galactose, and D-glucose, which were identified by GLC analysis,^{13–15} while enzymatic hydrolysis of **4** with glycyrrhizic acid hydrolase furnished primulagenin A (**8**).²¹ The ^1H - and ^{13}C -NMR (pyridine- d_5 , Table 2) spectra¹⁷ of **4** showed the presence of a β -D-glucuronopyranosyl moiety [δ 4.92 (1H, d, $J=7.3$ Hz, H-1')], a β -D-glu-

Table 4. Platelet Aggregating Activities of Camelliosides A (1) and B (2)

Treatment	Dose ($\mu\text{g/ml}$)	Aggregation (% of control)
Camellioside A (1)	1	15
	10	16
	50	47
	100	83
Camellioside B (2)	1	17
	10	13
	50	43
	100	104

Each values represents mean of two experiments.

copyranosyl moiety [δ 5.18 (1H, d, $J=7.9$ Hz, H-1''), and two β -D-galactopyranosyl moieties [δ 5.64 (1H, d, $J=7.6$ Hz, H-1''), 5.76 (1H, d, $J=7.6$ Hz, H-1'')] together with a primulagenin A part. The carbon signals due to the 3-*O*-tetraglycoside moiety in the ^{13}C -NMR spectrum of **4** were superimposable on those of **1** and **3**. The HMBC experiment of **4** exhibited long-range correlations as shown in Fig. 1, so that the structure of camellioside D (**4**) was characterized to be as shown.

Effects of Principal Saponin Constituents (1, 2) on Gastric Lesions by Ethanol or Indomethacin in Rats Effects of the principal oligoglycosides, camelliosides A (**1**) and B (**2**), on ethanol- or indomethacin-induced gastric lesions were examined.^{22,23} As shown in Table 3, **1** and **2** showed protective effects on both ethanol- and indomethacin-induced gastric lesions [ethanol-induced: **1** ($\text{ED}_{50}=9.7$ mg/kg), **2** (7.4 mg/kg); indomethacin-induced: **1** (21 mg/kg), **2** (13 mg/kg)] and their gastroprotective effects were equivalent or stronger than those of reference compounds, omeprazole (ethanol-induced: 10 mg/kg; indomethacin-induced: 4.7 mg/kg), cimetidine (ethanol-induced: 69 mg/kg; indomethacin-induced: 21 mg/kg).^{24–26}

Platelet Aggregation Activities of Principal Saponin Constituents (1, 2) Furthermore, since this natural medicine has been used as hemostatic, we examined the rabbit platelet aggregation activities (*in vitro*).²⁷ As shown in Table 4, the principal oligoglycosides, camelliosides A (**1**) and B (**2**), concentration-dependently caused the aggregation of the platelets (10–100 $\mu\text{g/ml}$).

The gastroprotective and platelet aggregation effects of this natural medicine and the principal constituents may be important evidence substantiating the traditional effect of this natural medicine such as the treatment effects of blood vomiting and bleeding and stomatic effects.

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^1H -NMR spectra, JNM-LA500 (500 MHz); ^{13}C -NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-GC-MATE mass spectrometer, JEOL JMS-SX 102A mass spectrometer.

The following experimental conditions were used for chromatography: ordinary-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with Silica gel 60F₂₅₄ (Merck, normal-phase) and Silica gel RP-18 F_{254S} (Merck, reversed-phase); HPTLC, pre-

coated TLC plates with Silica gel 60F₂₅₄ (Merck, normal-phase), Silica gel RP-18 WF_{254S} (Merck, reversed-phase). Detection was done by spraying with 1% $\text{Ce}(\text{SO}_4)_2$ –10% aqueous H_2SO_4 followed by heating.

Extraction and Isolation The fresh flower buds of *C. japonica* (6.0 kg, collected in Izu-ohshima island, Tokyo, Japan) was finely cut and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the methanolic extract (480 g, 8.0% from flesh flowers). The methanolic extract (400 g) was partitioned in an EtOAc– H_2O (1 : 1, v/v) mixture. The aqueous layer was extracted with *n*-BuOH and removal of the solvent *in vacuo* from the EtOAc-, *n*-BuOH-, and H_2O -soluble portions yielded 25 g (0.5%), 100 g (2.0%), and 275 g (5.5%) of the residue, respectively. The *n*-BuOH-soluble fraction (83.3 g) was subjected to ordinary-phase silica gel column chromatography {3.0 kg, CHCl_3 – MeOH – H_2O [(7 : 3 : 0.5–6 : 4 : 1–5 : 5 : 1, v/v/v)– MeOH]} to afford 13 fractions [Fr. 1 (1.5 g), Fr. 2 (0.8 g), Fr. 3 (2.3 g), Fr. 4 (2.8 g), Fr. 5 (25.6 g), Fr. 6 (0.8 g), Fr. 7 (15.6 g), Fr. 8 (2.9 g), Fr. 9 (8.4 g), Fr. 10 (4.0 g), Fr. 11 (2.7 g), Fr. 12 (1.6 g), Fr. 13 (14.3 g)]. Fraction 5 (18.6 g) was separated by reversed-phase silica gel column chromatography [560 g, MeOH – H_2O (10 : 90–30 : 70–50 : 50–70 : 30, v/v)– MeOH] to furnish 13 fractions [Fr. 5-1 (0.30 g), Fr. 5-2 (0.12 g), Fr. 5-3 (2.10 g), Fr. 5-4 (1.40 g), Fr. 5-5 (0.58 g), Fr. 5-6 (1.30 g), Fr. 5-7 (0.44 g), Fr. 5-8 (0.34 g), Fr. 5-9 (0.60 g), Fr. 5-10 (4.90 g), Fr. 5-11 (2.00 g), Fr. 5-12 (2.10 g), Fr. 5-13 (2.52 g)]. Fraction 5-4 (1.30 g) was further separated by HPLC [YMC-pack ODS-A, 250 $\times$ 20 mm i.d., MeOH –1% aqueous AcOH (25 : 75, v/v)] to give (–)-epicatechin (431 mg, 0.036%). Fraction 5-10 (1.15 g) was separated by HPLC [MeOH –1% aqueous AcOH (70 : 30, v/v)] to give camelliosides A (**1**, 260 mg, 0.10%) and B (**2**, 175 mg, 0.067%). Fraction 5-11 (1.15 g) was purified by HPLC [MeOH –1% aqueous AcOH (75 : 25, v/v)] to furnish camelliosides C (**3**, 34 mg, 0.052%) and D (**4**, 15 mg, 0.0023%).

The EtOAc-soluble fraction (18.0 g) was subjected to ordinary-phase silica gel column chromatography [600 g, CHCl_3 – MeOH – H_2O (15 : 3 : 1, lower layer–10 : 3 : 1, lower layer–7 : 3 : 0.5, v/v/v)– MeOH] to afford nine fractions [Fr. 1 (1.5 g), Fr. 2 (1.9 g), Fr. 3 (0.7 g), Fr. 4 (1.6 g), Fr. 5 (4.4 g), Fr. 6 (1.1 g), Fr. 7 (1.1 g), Fr. 8 (1.5 g), Fr. 9 (4.2 g)]. Fraction 2 (1.7 g) was purified by reversed-phase silica gel column chromatography [60 g, MeOH – H_2O (50 : 50–70 : 30–90 : 10, v/v)– MeOH] and finally HPLC [MeOH –1% aqueous AcOH (90 : 10, v/v)] to give oleanolic acid (22 mg, 0.0006%). Fraction 4 (1.3 g) was purified by reversed-phase silica gel column chromatography [40 g, MeOH – H_2O (30 : 70–50 : 50–70 : 30, v/v)– MeOH] and finally HPLC [MeOH –1% aqueous AcOH (35 : 65, v/v)] to furnish benzyl β -D-glucopyranoside (14 mg, 0.0004%) and methyl gallate (12 mg, 0.0003%). Fraction 5 (2.0 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH – H_2O (10 : 90–20 : 80–70 : 30, v/v)– MeOH] and finally HPLC [MeOH –1% aqueous AcOH (25 : 75, v/v)] to furnish (–)-epicatechin (30 mg, 0.0017%) and (+)-catechin (19 mg, 0.0011%).

These known constituents were identified by comparison of their physical data with commercially obtained sample or with reported values.¹²⁾

Camellioside A (1): Colorless fine crystals (mp 226–229 °C from aqueous MeOH); [α]_D²⁵ –32.1° ($c=1.80$, pyridine); IR ν_{max} (KBr) 3453, 2962, 1736, 1719, 1656, 1078 cm^{-1} ; ^1H -NMR (pyridine- d_5) δ : 0.87, 0.90, 0.98, 1.10, 1.27, 1.34, 1.37 (3H each, all s, H_3 -25, 29, 30, 24, 26, 23, 27), 3.10 (1H, dd, $J=3.5$, 14.5 Hz, H-18), 3.27 (1H, dd, $J=4.5$, 11.8 Hz, H-3), 4.90 (1H, d, $J=7.4$ Hz, H-1'), 5.18 (1H, d, $J=7.7$ Hz, H-1''), 5.49 (1H, brs, H-12), 5.70 (1H, d, $J=7.6$ Hz, H-1'), 5.76 (1H, d, $J=7.6$ Hz, H-1''); ^{13}C -NMR (pyridine- d_5) δ : given in Table 2; Positive-ion FAB-MS m/z 1127 [$\text{M}+\text{Na}$]⁺; Negative-ion FAB-MS m/z 1103 [$\text{M}-\text{H}$][–], 941 [$\text{M}-\text{C}_6\text{H}_{11}\text{O}_5$][–], 779 [$\text{M}-\text{C}_{12}\text{H}_{21}\text{O}_{10}$][–]; High-resolution MS m/z 1127.5244 (Calcd for $\text{C}_{53}\text{H}_{84}\text{O}_{24}\text{Na}$, 1127.5250).

Camellioside B (2): Colorless fine crystals (mp 221–224 °C from aqueous MeOH); [α]_D²⁵ –30.0° ($c=0.80$, pyridine); IR ν_{max} (KBr) 3453, 2962, 1744, 1726, 1655, 1380, 1078 cm^{-1} ; ^1H -NMR (pyridine- d_5) δ : 0.87, 0.90, 0.98, 1.10, 1.27, 1.34, 1.37 (3H each, all s, H_3 -25, 29, 30, 24, 26, 27, 23), 2.13 (3H, s, $-\text{OCOCH}_3$), 3.08 (1H, dd-like, H-18), 3.30 (1H, dd, $J=3.5$, 11.0 Hz, H-3), 4.89 (1H, d, $J=7.6$ Hz, H-1'), 5.16 (1H, d, $J=7.6$ Hz, H-1''), 5.45 (1H, brs, H-12), 5.70 (1H, d, $J=7.6$ Hz, H-1'), 5.87 (1H, d-like, H-1'), 5.93 (1H, brs, H-4''); ^{13}C -NMR (pyridine- d_5) δ : given in Table 2; Positive-ion FAB-MS m/z 1169 [$\text{M}+\text{Na}$]⁺; Negative-ion FAB-MS m/z 1145 [$\text{M}-\text{H}$][–], 983 [$\text{M}-\text{C}_6\text{H}_{11}\text{O}_5$][–], 821 [$\text{M}-\text{C}_{12}\text{H}_{21}\text{O}_{10}$][–]; High-resolution MS m/z 1169.5352 (Calcd for $\text{C}_{55}\text{H}_{86}\text{O}_{25}\text{Na}$, 1169.5356).

Camellioside C (3): Colorless fine crystals (mp 224–227 °C from aqueous MeOH); [α]_D²⁶ –15.8° ($c=0.10$, MeOH); UV (MeOH) λ_{max} (log ϵ) 299 (4.0 nm); IR ν_{max} (KBr) 3453, 2926, 1736, 1665, 1655, 1078 cm^{-1} ; ^1H -NMR (pyridine- d_5) δ : 0.84, 0.87, 0.88, 0.90, 1.10, 1.20, 1.30 (3H, all s, H_3 -25, 26, 30, 29, 24, 27, 23), 3.30 (1H, dd, $J=4.5$, 11.7 Hz, H-3), 4.90 (1H, d,

$J=7.4$ Hz, H-1'), 5.17 (1H, d, $J=8.1$ Hz, H-1'''), 5.69 (1H, d, $J=7.6$ Hz, H-1'''), 5.76 (1H, d, $J=7.7$ Hz, H-1''), 6.07 (1H, brs, H-12); ^{13}C -NMR (pyridine- d_5) δ_{C} given in Table 2; Positive-ion FAB-MS m/z 1109 [$\text{M} + \text{Na}$] $^+$, 1087 [$\text{M} + \text{H}$] $^+$; Negative-ion FAB-MS m/z 1085 [$\text{M} - \text{H}$] $^-$, 923 [$\text{M} - \text{C}_6\text{H}_{11}\text{O}_5$] $^-$, 761 [$\text{M} - \text{C}_{12}\text{H}_{21}\text{O}_{10}$] $^-$; High-resolution MS m/z 1109.5137 (Calcd for $\text{C}_{53}\text{H}_{82}\text{O}_{23}\text{Na}$, 1109.5145).

Camellioside D (4): Colorless fine crystals (mp 222–225 °C from aqueous MeOH); $[\alpha]_{\text{D}}^{26} -3.2^\circ$ ($c=0.10$, MeOH); IR ν_{max} (KBr) 3453, 2926, 1719, 1655, 1078 cm^{-1} ; ^1H -NMR (pyridine- d_5) δ : 0.85, 0.94, 1.05, 1.09, 1.12, 1.30, 1.81 (3H each, all s, H₃-25, 26, 29, 24, 30, 23, 27), 2.47 (1H, dd-like, H-18), 3.32 (1H, dd, $J=4.3$, 11.9 Hz, H-3), 4.59 (1H, brs, H-16), 4.92 (1H, d, $J=7.3$ Hz, H-1'), 5.18 (1H, d, $J=7.9$ Hz, H-1'''), 5.37 (1H, brs, H-12), 5.64 (1H, d, $J=7.6$ Hz, H-1'''), 5.76 (1H, d, $J=7.6$ Hz, H-1''); ^{13}C -NMR (pyridine- d_5) δ_{C} given in Table 2; Positive-ion FAB-MS m/z 1143 [$\text{M} + \text{Na}$] $^+$; Negative-ion FAB-MS m/z 1119 [$\text{M} - \text{H}$] $^-$, 957 [$\text{M} - \text{C}_6\text{H}_{11}\text{O}_5$] $^-$, 795 [$\text{M} - \text{C}_{12}\text{H}_{21}\text{O}_{10}$] $^-$; High-resolution MS m/z 1143.5538 (Calcd for $\text{C}_{54}\text{H}_{88}\text{O}_{24}\text{Na}$, 1143.5563).

Acid Hydrolysis of Camelliosides A (1), C (3), and D (4) A solution of **1**, **3**, or **4** (5 mg each) in 5% aqueous H_2SO_4 –1,4-dioxane (1 : 1, 2 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was transferred to a Sep-Pak C₁₈ cartridge with H_2O and MeOH. The H_2O -eluted fraction was concentrated *in vacuo* to give a residue, which was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After the reaction, the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant of the reaction mixture was then subjected to GLC analysis to identify the derivatives of D-glucose (i), D-galactose (ii), and D-glucuronic acid (iii) from **1**, **3**, and **4**. GLC conditions: Supelco STBTM-1, 30 m $\times$ 0.25 mm (i.d.) capillary column; injector temperature, 230 °C; detector temperature, 230 °C; column temperature 230 °C; He flow rate 15 ml/min; t_{R} : (i) 24.4 min, (ii) 25.6 min, and (iii) 26.5 min.

Enzymatic Hydrolysis of Camelliosides A (1), C (3), and D (4) A solution of **1** (100 mg) in 0.2 M acetate buffer (pH 4.4, 5.0 ml) was treated with glycyrrhizinic acid hydrolase (Maruzen Pharmaceutical Co., Ltd., Hiroshima, Japan, 5.0 ml) and the mixture was stirred at 44 °C for over night. After treatment of the reaction mixture with EtOH, the solvent was evaporated to dryness under reduced pressure and the residue was purified by ordinary-phase silica gel column chromatography [2.0 g, *n*-hexane–EtOAc (3 : 1, v/v)] to give camellenodiol (**5**, 30 mg, 76%). Using a similar procedure, a solution of **3** (13 mg) or **4** (2 mg) in 0.2 M acetate buffer (pH 4.4, 2.0 or 0.5 ml) was treated with glycyrrhizinic acid hydrolase (2.0 or 0.5 ml) and the mixture was stirred at 44 °C for over night. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by ordinary-phase silica gel column chromatography [1.0 g, *n*-hexane–EtOAc (5 : 1, v/v)] to furnish **7** (from **3**: 4 mg, 78%) or **8** (from **4**: 0.6 mg, 73%), which were identified by comparison of physical data ($[\alpha]_{\text{D}}$, IR, ^1H -NMR, ^{13}C -NMR) with reported values.^{20,21)}

Camellenodiol (5): ^1H -NMR (CDCl_3) δ : 0.75 (1H, d-like, H-5), 0.80, 0.89, 0.95, 0.96, 1.00, 1.04, 1.19 (3H each, all s, H₃-24, 29, 25, 30, 23, 26, 27), 1.00 (1H, m, H-1), 1.30 (1H, m, H-19), 1.32 (2H, m, H-7 and 21), 1.41 (1H, m, H-22), 1.45 (1H, m, H-19), 1.48 (1H, m, H-7), 1.54 (1H, m, H-9 and 21), 1.58 (1H, m, H-2), 1.63 (4H, m, H-1, 2, and H₂-6), 1.81 (1H, d, $J=14.0$ Hz, H-15), 1.98 (2H, m, H₂-11), 2.21 (1H, m, H-22), 2.72 (1H, dd, $J=4.0$, 14.0 Hz, H-18), 3.17 (1H, d, $J=14.0$ Hz, 15-H), 3.22 (1H, dd-like, H-3), 5.51 (1H, dd-like, H-12); ^{13}C -NMR (CDCl_3 and pyridine- d_5) δ_{C} given in Table 2. Camellenodiol (**5**) was identified by comparison of the physical data ($[\alpha]_{\text{D}}$, IR, ^1H -NMR (90 MHz), and MS) with reported values.²⁾

Crystal Data for 5 Colorless prismatic crystals, mp 222.8–223.2 °C (from aqueous acetone), $\text{C}_{29}\text{H}_{46}\text{O}_3 \cdot (\text{CH}_3)_2\text{CO}$, $M=500.76$, crystal dimensions: 0.25 $\times$ 0.18 $\times$ 0.30 mm, crystal system: orthorhombic, lattice type: primitive, lattice parameters: $a=13.237(2)$, $b=28.911(2)$, $c=7.649(2)$ Å, $V=2927.2(8)$ Å³, space group: $P2_12_12_1$ (#19), $Z=4$, $D_{\text{calc}}=1.136$ g/cm³, $F_{000}=1104.00$, $\mu(\text{CuK}\alpha)=5.65$ cm⁻¹, temperature: 23.0 °C, structure solution: TEXSAN (direct method: SAPI91), residuals: $R=0.081$, $R_w=0.140$, $R1=0.046$, goodness of fit indicator: 1.38. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated $\text{CuK}\alpha$ ($\lambda=1.54178$ Å) radiation and a rotating anode generator.

Preparation of the (R)-MTPA Ester (5b) and (S)-MTPA Ester (5c) from 5 A solution of **5** (3.8 mg) in CH_2Cl_2 (0.5 ml) was treated with (R)- α -methoxy- α -trifluoromethylphenylacetic acid [(R)-MTPA, 3.5 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC $\cdot$ HCl, 3.0 mg) and 4-dimethylaminopyridine (4-DMAP, 2.5 mg), and

the mixture was stirred at room temperature for 3.5 h. The reaction mixture was poured into ice-water and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous NaHCO_3 , and brine, then dried over MgSO_4 powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was by ordinary-phase silica gel column chromatography [0.5 g, *n*-hexane–EtOAc (5 : 1, v/v)] to give **5b** (5.4 mg, 95%). Using a similar procedure, (S)-MTPA esters [**5c** (4.6 mg, 91%)] were obtained from **5** (3.9 mg), using (S)-MTPA (3.5 mg), EDC $\cdot$ HCl (3.0 mg), and 4-DMAP (2.5 mg).

Compound 5b: ^1H -NMR (CDCl_3) δ : 0.82, 0.84, 0.89, 0.96, 0.99, 1.04, 1.19 (3H each, all s, H₃-24, 23, 29, 30, 25, 26, 27), 0.87 (1H, d-like, H-5), 1.11 (1H, m, H-1), 1.32 (3H, m, H-7, 19, 21), 1.41 (1H, m, H-22), 1.48 (1H, m, H-19), 1.51 (1H, m, H-7), 1.54 (1H, m, H-21), 1.56 (3H, m, H₂-6), 1.57 (1H, m, H-9), 1.69 (1H, m, H-1), 1.77 (1H, m, H-2), 1.81 (1H, d, $J=14.7$ Hz, H-15), 1.84 (1H, m, H-2), 1.99 (2H, m, H₂-11), 2.10 (1H, m, H-22), 2.72 (1H, dd, $J=4.2$, 14.0 Hz, H-18), 3.17 (1H, d, $J=14.7$ Hz, H-15), 3.56 (3H, s, $-\text{OCH}_3$), 4.72 (1H, dd, $J=4.9$, 11.6 Hz, H-3), 5.51 (1H, dd, $J=3.6$, 3.7 Hz, H-12), 7.38–7.56 (5H, m, Ph-H).

Compound 5c: ^1H -NMR (CDCl_3) δ : 0.83, 0.89, 0.92, 0.96, 0.96, 1.03, 1.19 (3H each, all s, H₃-24, 29, 23, 30, 25, 26, 27), 0.88 (1H, d-like, H-5), 1.08 (1H, m, H-1), 1.32 (3H, m, H-7, 19, 21), 1.41 (1H, m, H-22), 1.48 (1H, m, H-19), 1.51 (1H, m, H-7), 1.54 (1H, m, H-21), 1.56 (1H, m, H-9), 1.57 (2H, m, H₂-6), 1.66 (1H, m, H-1), 1.69 (1H, m, H-2), 1.76 (1H, m, H-2), 1.81 (1H, d, $J=14.4$ Hz, H-15), 1.99 (2H, m, H₂-11), 2.10 (1H, m, H-22), 2.72 (1H, dd, $J=4.2$, 14.0 Hz, H-18), 3.17 (1H, d, $J=14.4$ Hz, H-15), 3.53 (3H, s, $-\text{OCH}_3$), 4.72 (1H, dd, $J=4.9$, 11.6 Hz, H-3), 5.51 (1H, dd, $J=3.3$, 3.7 Hz, H-12), 7.39–7.54 (5H, m, Ph-H).

Acetylation of 5 A solution of **5** (5 mg) in pyridine (1.0 ml) was treated with acetic anhydride (Ac_2O , 0.8 ml) and the mixture was stirred at room temperature for 8 h. The reaction mixture was poured into ice-water and the whole reaction mixture was extracted with EtOAc. Work-up of the EtOAc extract as described above gave a product, which was purified by ordinary-phase silica gel column chromatography [0.5 g, *n*-hexane–EtOAc (5 : 1, v/v)] to furnish **5a** (6 mg, quant.) which was identified by comparison of the physical data ($[\alpha]_{\text{D}}$, IR, ^1H -NMR, MS) with reported values.²⁾

Oxidation of 5 A solution of **5** (5 mg) was treated with chromium trioxide (CrO_3)–pyridine suspension mixture (3.0 mg: 0.5 ml), and the whole mixture was stirred at room temperature for 8 h. The reaction mixture was poured into saturated aqueous NaCl and the whole was extracted with EtOAc. The EtOAc extract was washed with brine then dried over MgSO_4 . Removal of the solvent under reduced pressure gave a crude product, which was purified by ordinary-phase silica gel column chromatography [0.5 g, *n*-hexane–EtOAc (5 : 1, v/v)] to furnish **6** (2 mg, 41%), which was identified by comparison of the physical data ($[\alpha]_{\text{D}}$, IR, UV, ^1H -NMR, MS) with reported values.

Alkaline Hydrolysis of Camellioside B (2) A solution of **2** (20 mg) in 0.1% NaOMe–MeOH (5.0 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was separated by normal-phase silica gel column chromatography [0.4 g, CHCl_3 –MeOH– H_2O (7 : 3 : 1, lower layer, v/v/v)] to give **1** (13 mg, 66%).

Bioassay. Animals Male Sprague-Dawley rats weighing about 230–250 g and male white rabbits weighing about 2.0–3.0 kg were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chows (MF for rats and RC-4 for rabbits, Oriental Yeast Co., Ltd., Japan). The rats were fasted for 24–26 h prior to the beginning of the experiment, but were allowed free access to tap water.

Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats The acute gastric lesions were induced by oral administration of ethanol or indomethacin according to the method described previously with slight modifications.^{22,23)} Briefly, 99.5% ethanol (1.5 ml/rat) or indomethacin (20 mg/kg, dissolved in 5% sodium bicarbonate, and then diluted in water and neutralized with 0.2 M HCl and adjusted to 1.5 ml/rat) was administered to 24–26 h fasted rats using a metal orogastric tube. One hour after administration of ethanol or 4 h after administration of indomethacin, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 10 ml 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of gastric lesions were measured as previously described, and the total length (mm) was expressed as a lesion index.

Test samples were suspended in 5% acacia solution. Cimetidine and omeprazole were suspended in 0.5% CMC-Na. Test samples in vehicle and vehicle only (control group) was administered orally at a dose of 5 ml/kg 1 h prior to the application of ethanol or indomethacin.

Statistical Analysis Values were expressed as means $\pm$ S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (p) values less than 0.05 were considered significant. ED₅₀ values were estimated based on linear regressions of probit-transformed values of inhibition (%).

Platelet Aggregation Activity Wash rabbit platelets were prepared as described previously.²⁵⁾ The platelets suspension (5×10^5 cells/ μ l) in 225 μ l of an assay buffer (137 mM NaCl, 2.7 mM KCl, 3.8 mM HEPES, pH 7.4) and 25 μ l of 12 mM CaCl₂/saline were mixed and incubated at 37 °C for 1 min. One microliter of test sample dissolved in DMSO was added to 24 μ l of the assay buffer, and the solution was dropped into the platelets suspension with stirring. Aggregation responses were recorded by an aggregometer (Hema Tracer 1, Nikko Bioscience, Japan). The aggregation (%) was calibrated with the platelets suspension (0% transmission) and the assay buffer (100% transmission).

References and Notes

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