Medicinal Flowers. XXVI.[1] Structures of Acylated Oleanane-Type Triterpene Oligoglycosides, Yuchasaponins A, B, C, and D, from the Flower Buds of *Camellia oleifera*—Gastroprotective, Aldose Reductase Inhibitory, and Radical Scavenging Effects—

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The methanolic extract and its 1-butanol-soluble fraction from the flower buds of *Camellia oleifera* Abel were found to exhibit inhibitory effects on ethanol- and indomethacin-induced gastric mucosal lesions in rats. The ethyl acetate- and 1-butanol-soluble fractions also showed inhibitory effects on rat lens aldose reductase and scavenging effects on 1,1-diphenylpicryl-2-hydrazyl radical and superoxide. From the 1-butanol-soluble fraction, four new acylated oleanane-type triterpene oligoglycosides, yuchasaponins A, B, C, and D, were isolated and their structures were elucidated on the basis of chemical and physicochemical evidence. On the other hand, quercetin 3-*O*-α-L-rhamnopyranoside and kaempferol 3-*O*-α-L-rhamnopyranoside were isolated from the ethyl acetate- and 1-butanol-soluble fractions as the principal constituents, and their gastroprotective effects were examined.

Key words yuchasaponin; *Camellia oleifera*; acylated oleanane-type triterpene oligoglycoside; medicinal flower; gastroprotective; antioxidative

A *Camellia* plant, *Camellia* (*C.*) *oleifera* Abel, is distributed in the central and south China, such as Jiangsu, Anhui, Zhejiang, Dongting, and Fujian provinces. The flower buds of this plant (Chinese name 茶子木花) have been used for the treatment of blood vomiting and bleeding due to internal and external injury in Chinese traditional medicine. As chemical constituents of *C. oleifera*, tannins,[2] flavonoids,[3] and lignans[4] were isolated from the seeds and leaves. However, the chemical constituents and biological activities of the flower buds of this plant have not yet characterized. Recently, we have reported the isolation and structure elucidation of acylated polyhydroxyoleanane-type triterpene oligoglycosides from the flower buds of *C. sinensis*[1,5—9] and *C. japonica*.[10,11] Furthermore, those oligoglycosides were found to exhibit antiallergic,[6] antidiabetic,[7] antiobesitic,[5,8] gastroprotective,[7,10,11] and platelet aggregation[10,11] activities. In the course of our characterization studies on *Camellia* speicies[12—15] and medicinal flowers,[16—21] we found that the methanolic (MeOH) extract and its 1-butanol (1-BuOH)-soluble fraction from the flower buds of *C. oleifera* showed gastroprotective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats. The 1-BuOH- and ethyl acetate (EtOAc)-soluble fractions exhibited inhibitory activities on rat lens aldose reductase and scavenging effects on 1,1-diphenylpicryl-2-hydrazyl (DPPH) radical and superoxide. From the 1-BuOH-soluble fraction, we isolated four new acylated oleanane-type triterpene oligoglycosides called yuchasaponins A (**1**), B (**2**), C (**3**), and D (**4**) together with 11 known constituents including flavonoids. This paper deals with the isolation and structure elucidations of new yuchasaponins (**1**—**4**) as well as the gastroprotective effects of principal constituents from the flower buds of *C. oleifera*.

Biological Activities of the Extract and Fractions The flower buds of *C. oleifera*, which were cultivated in Fujian province of China, were extracted with MeOH. The MeOH extract (12.1% from the dried flower buds) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (3.8%) and an aqueous phase. The aqueous phase was further extracted with 1-BuOH to give 1-BuOH (0.87%)- and H_2O (7.5%)-soluble fractions. As shown in Tables 1 and 2, the MeOH extract and 1-BuOH-soluble fraction were found to show potent protective effects on ethanol- and indomethacin-induced gastric lesions in rats, whereas the EtOAc- and H_2O-soluble fractions exhibited the weak activity. On the other hand, the EtOAc- and 1-BuOH-soluble fractions showed inhibitory activities on rat lens aldose reductase (Table 3) and scavenging effects on DPPH radical and superoxide (Table 4).

Isolation of Yuchasaponins The 1-BuOH-soluble fraction was subjected to ordinary- and reversed-phase silica gel column chromatographies and finally HPLC to furnish yuchasaponins A (**1**, 0.00012%), B (**2**, 0.00006%), C (**3**, 0.00006%), and D (**4**, 0.00010%) together with jegosaponin B[22] (**5**, 0.00010%), quercetin 3-*O*-α-L-rhamnopyranoside[23]

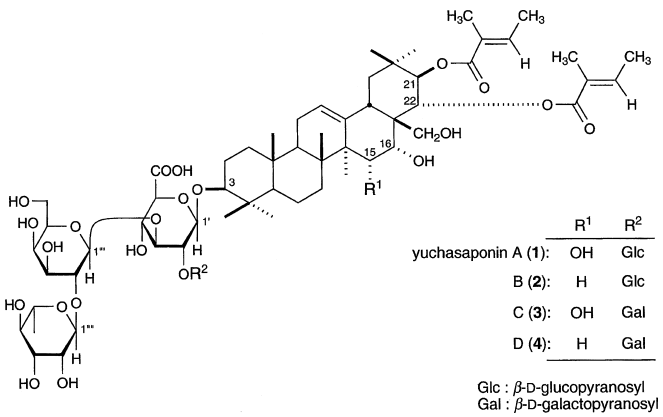

Chart 1

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Table 1. Effects of the MeOH Extract and EtOAc-, 1-BuOH-, and H_2O-Soluble Fractions from the Flower Buds of *C. oleifera* on Gastric Lesions Induced by EtOH in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	10	99.7±3.7	—
MeOH ext.	125	6	69.2±17.6*	30.6
	250	7	16.4±8.0**	83.6
	500	7	0.7±0.5**	99.3
Control	—	13	100.2±4.7	—
EtOAc-soluble fraction	50	7	74.8±13.4	32.1
	100	7	48.1±15.6**	56.3
1-BuOH-soluble fraction	50	8	88.5±15.3	19.7
	100	8	18.6±5.3**	83.1
H_2O-soluble fraction	50	7	80.0±12.9	27.4
	100	7	57.9±11.7**	47.5

Ethanol (1.5 ml/rat) was orally administered to 24 h fasted rats. One hour later, rats were killed and the total length (mm) of gastric lesions was determined. Test sample was administered orally 1 h before administration of ethanol. Values represent the means±S.E.M. Significantly different from the control group, $*p<0.05$, $**p<0.01$. Test sample was suspended in 5% acacia solution.

Table 2. Effects of the MeOH Extract and EtOAc-, 1-BuOH-, and H_2O-Soluble Fractions from the Flower Buds of *C. oleifera* on Gastric Lesions Induced by Indomethacin in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	8	77.8±9.8	—
MeOH ext.	125	6	44.8±10.6	42.4
	250	6	24.3±4.9**	68.8
	500	6	9.7±2.8**	87.6
EtOAc-soluble fraction	50	6	66.8±17.1	14.2
	100	6	48.0±10.8	38.3
1-BuOH-soluble fraction	50	6	51.0±15.2	34.5
	100	6	18.1±7.3**	76.7
H_2O-soluble fraction	50	6	89.1±8.8	−14.5
	100	6	67.0±4.7	14.0

Indomethacin (20 mg/kg) was orally administered 20 24 h fasted rats. Four hours later, rats were killed and the total length (mm) of gastric lesions was determined. Test sample was administered orally 1 h before administration of Indomethacin. Values represent the means±S.E.M. Significantly different from the control group, $**p<0.01$. Test sample was suspended in 5% acacia solution.

Table 3. Inhibitory Effects of the EtOAc-, 1-BuOH-, and H_2O-Soluble Fractions from the Flower Buds of *C. oleifera* on Rat Lens Aldose Reductase

Treatment	IC_{50} (μg/ml)
EtOAc-soluble fraction	1.5
1-BuOH-soluble fraction	4.9
H_2O-soluble fraction	62.8

Table 4. Scavenging Effects of the EtOAc-, 1-BuOH-, and H_2O-Soluble Fractions from the Flower Buds of *C. oleifera* on DPPH Radical and Superoxide ($^{\cdot}O_2^-$)

Treatment	DPPH radical SC_{50} (μg/ml)	$^{\cdot}O_2^-$ IC_{50} (μg/ml)
EtOAc-soluble fraction	4.3	2.2
1-BuOH-soluble fraction	2.4	1.8
H_2O-soluble fraction	13.8	7.2

SC_{50}: 50% scavenging concentration.

(**6**, 0.0067%), kaempferol 3-*O*-α-L-rhamnopyranoside[23] (**7**, 0.0019%), quercetin (0.0004%), and gallic acid (0.016%). From the EtOAc-soluble fraction, **6** (0.73%), **7** (0.72%), quercetin 3-*O*-β-D-glucopyranoside[23] (0.0016%), quercetin (0.39%), kaempferol (0.0007%), (−)-epigallocatechin 3-*O*-β-D-glucopyranoside[24] (0.00007%), oleanolic acid (**8**, 0.17%), 4-aminophenol[25] (0.0002%), 3-(4-hydroxyphenyl)acrylic acid[25] (0.0011%), and gallic acid (0.017%) were isolated by the similar procedure to that of the 1-BuOH-soluble fraction.

The Structures of Yuchasaponins Yuchasaponin A (**1**) was isolated as colorless fine crystals with mp 234.9—235.7 °C (from $CHCl_3$–MeOH) and exhibited a negative optical rotation ($[\alpha]_D^{21}$ −12.1° in MeOH). The IR spectrum of **1** showed strong absorption bands at 3475 and 1078 cm^{-1} suggestive of an oligoglycosidic structure and weak bands at 1719 and 1655 cm^{-1} ascribable to carbonyl and α,β-unsaturated ester functions. In the negative-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1315 $(M-H)^-$ together with fragment ion peaks at *m/z* 1169 $(M-C_6H_{11}O_4)^-$ and *m/z* 1007 $(M-C_{12}H_{21}O_9)^-$, which were derived by cleavage of the glycoside linkages. The positive-ion FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 1339 $(M+Na)^+$ and the molecular formula of **1** was determined to be $C_{64}H_{100}O_{28}$ by high-resolution MS measurement of the quasimolecular ion peak $(M-H)^-$. Alkaline hydrolysis of **1** with 10% aqueous KOH–50% aqueous 1,4-dioxane [1 : 1 (v/v)] liberated desacyl-derivative (**1a**) and an organic acid, tiglic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[26—28] The positive- and negative-ion FAB-MS of **1a** showed quasimolecular ion peaks at *m/z* 1175 $(M+Na)^+$ and *m/z* 1151 $(M-H)^-$, respectively and the molecular formula, $C_{54}H_{88}O_{26}$, of **1a** was determined by high-resolution MS measurement. In the negative-ion FAB-MS of **1a**, fragment ion peaks were observed at *m/z* 1005 $(M-C_6H_{11}O_4)^-$ and *m/z* 843 $(M-C_{12}H_{21}O_9)^-$. Acid hydrolysis of **1a** with 5% aqueous H_2SO_4 in 1,4-dioxane [1 : 1 (v/v)] liberated R_1-barrigenol (**1b**)[5,6,29] and four mucosaccharides, D-galactose, D-glucose, D-glucuronic acid, and L-rhamnose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[30—32] The ^1H- (pyridine-d_5) and ^{13}C-NMR (Table 5) spectra of **1**, which were assigned by various NMR experiments,[33] showed signals assignable to a R_1-barrigenol part {seven methyls [δ 0.83, 1.02, 1.06, 1.13, 1.17, 1.34, 1.84 (all s, 25, 26, 24, 29, 23, 30, 27-H_3)], a methylene bearing an oxygen function [δ 3.46, 3.74 (both d, J=10.3 Hz, 28-H_2)], two methines bearing a tigloyl (*trans*-2-methyl-2-butenoyl) group [δ 6.32, 6.70 (both d, J=12.4 Hz, 22, 21-H)]}, a β-D-glucopyranosiduronic acid moiety [δ 4.89 (d, J=7.2 Hz, 1′-H)], a β-D-glucopyranosyl moiety [δ 5.85 (d, J=7.6 Hz, 1″-H)], a β-D-galactopyranosyl moiety [δ 6.07 (d, J=7.8 Hz, 1‴-H)], and an α-L-rhamnopyranosyl moiety [δ 6.20 (br s, 1⁗-H)], and two tigloyl groups [21-Tig.: δ 1.61 (d, J=6.3 Hz, 4-H_3), 1.90 (br s, 5-H_3), 7.03 (dq, J=1.5, 6.3 Hz, 3-H); 22-Tig.: δ 1.34 (d, J=5.7 Hz, 4-H_3), 1.73 (br s, 5-H_3), 6.79 (dq, J=1.4, 5.7 Hz, 3-H)]. The oligoglycosidic structure and positions of the two tigloyl groups were clarified by heteronuclear multiple bond connectivity (HMBC) experiment on **1**, which showed long-range correlations between the following protons and carbons: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C; 21,22-protons and tigloyl carbonyl carbons. On the basis of this evidence, the structure of

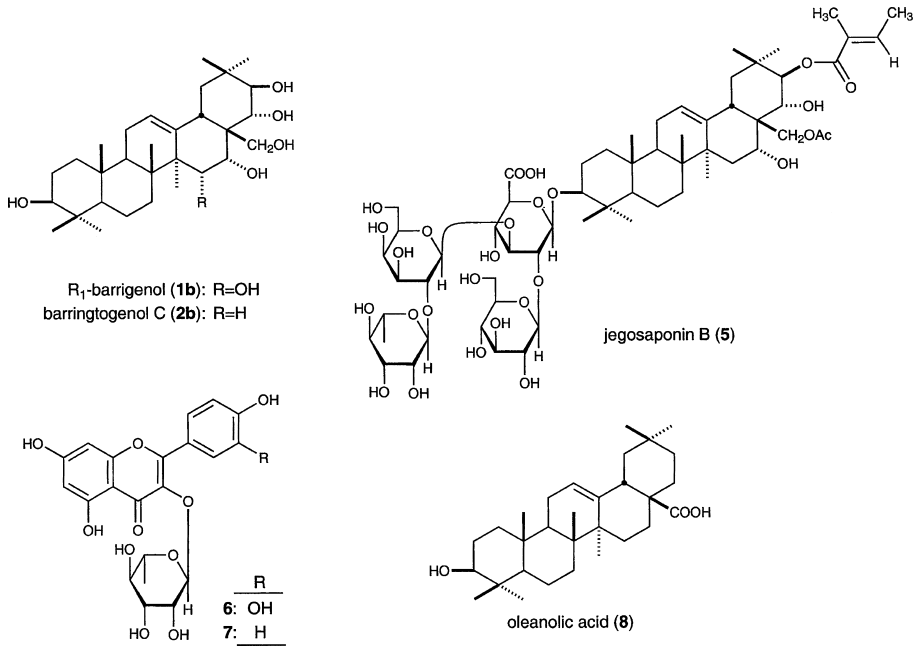

Chart 2

yuchasaponin A was determined to be 21,22-di-*O*-tigloyl R_1-barrigenol 3-*O*-*β*-D-glucopyranosyl(1—2)[*α*-L-rhamnopyranosyl(1—2)-*β*-D-galactopyranosyl(1—3)]-*β*-D-glucopyranosiduronic acid (**1**).

Yuchasaponin B (**2**) was also isolated as colorless fine crystals of mp 236.5—237.0 °C with negative optical rotation ($[\alpha]_D^{18}$ −3.7° in MeOH). The IR spectrum of **2** showed absorption bands due to hydroxyl, carbonyl, and *α*,*β*-unsaturated ester functions. The molecular formula, $C_{64}H_{100}O_{27}$, of **2** was characterized from negative- and positive-ion FAB-MS [*m*/*z* 1299 $(M-H)^-$, *m*/*z* 1323 $(M+Na)^+$] and by high-resolution FAB-MS measurement. Alkaline hydrolysis of **2** with 10% aqueous KOH–50% aqueous 1,4-dioxane [1 : 1 (v/v)] liberated desacyl-jegosaponin (**2a**)[34] and tiglic acid, which was identified by HPLC.[26—28] Acid hydrolysis of **2a** with 5% aqueous H_2SO_4 in 1,4-dioxane [1 : 1 (v/v)] liberated barringtogenol C (**2b**)[35] and four mucosaccharides (D-galactose, D-glucose, D-glucuronic acid, and L-rhamnose), which were identified by GLC.[30—32] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 5) spectra[33] indicated the presence of a desacyl-jegosaponin part [*δ* 3.24 (dd, *J*=4.3, 11.6 Hz, 3-H), 6.27, 6.70 (both d, *J*=10.4 Hz, 22, 21-H), 4.91 (d, *J*=7.7 Hz, 1′-H), 5.87 (d, *J*=7.7 Hz, 1″-H), 6.08 (d, *J*=7.9 Hz, 1‴-H), 6.21 (br s 1⁗-H)] and two tigloyl groups. The positions of two tigloyl groups in **2** were elucidated by HMBC experiment, which showed long-range correlations between the 21,22-protons and tigloyl carbonyl carbons. Consequently, the structure of yuchasaponin B was determined to be 21,22-di-*O*-tigloyl barringtogenol C 3-*O*-*β*-D-glucopyranosyl(1—2)[*α*-L-rhamnopyranosyl(1—2)-*β*-D-galactopyranosyl(1—3)]-*β*-D-glucopyranosiduronic acid (**2**).

Yuchasaponins C (**3**) and D (**4**), which were obtained as colorless fine crystals (**3**: mp 234.4—235.6 °C; **4**: 232.9—233.6 °C) with negative optical rotation (**3**: $[\alpha]_D^{20}$ −21.3°; **4**: $[\alpha]_D^{22}$ −16.6° in MeOH), showed absorption bands due to hydroxyl, carbonyl, and *α*,*β*-unsaturated ester functions in their IR spectra. The positive- and negative-ion FAB-MS of **3** and **4** exhibited quasimolecular ion peaks [**3**: *m*/*z* 1339 $(M+Na)^+$, *m*/*z* 1315 $(M-H)^-$; **4**: *m*/*z* 1323 $(M+Na)^+$, *m*/*z* 1299 $(M-H)^-$] together with fragment ion peaks [**3**: *m*/*z* 1169 $(M-C_6H_{11}O_4)^-$, *m*/*z* 1007 $(M-C_{12}H_{21}O_9)^-$; **4**: *m*/*z* 1153 $(M-C_6H_{11}O_4)^-$, *m*/*z* 991 $(M-C_{12}H_{21}O_9)^-$] and high-resolution FAB-MS analysis indicated their molecular formulas to be $C_{64}H_{100}O_{28}$ and $C_{64}H_{100}O_{27}$, respectively. Alkaline hydrolysis of **3**, and **4** furnished desacyl-derivatives (**3a**, **4a**) and tiglic acid, which was identified by HPLC.[26—28] On the acid hydrolysis, **3a** and **4a** liberated **1b** (from **3a**) and **2b** (from **4a**) together with D-galactose, D-glucuronic acid, and L-rhamnose, which were identified by GLC.[30—32] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 5) spectra of **3** and **4** indicated the presence of a R_1-barrigenol part (for **3**) and barringtogenol C part (for **4**) together with the common tetraglycoside part {two *β*-D-galactopyranosyl moieties [**3**: *δ* 5.71 (d, *J*=7.6 Hz, 1″-H), 6.02 (d, 7.7 Hz, 1‴-H), **4**: *δ* 5.71 (d, *J*=7.5 Hz, 1″-H), 6.01 (d, *J*=7.7 Hz, 1‴-H)], a *β*-D-glucopyranosiduronic acid moiety [**3**: *δ* 4.96 (d, *J*=6.8 Hz, 1′-H), **4**: *δ* 4.97 (d, *J*=7.6 Hz, 1′-H)], an *α*-L-rhamnopyranosyl moiety [**3**: *δ* 6.20 (br s, 1⁗-H), **4**: *δ* 6.19 (br s, 1⁗-H)], and two tigloyl groups. The HMBC experiments on **3** and **4** showed long-range correlations between the following protons and carbons: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C; 21,22-protons and tigloyl carbonyl carbons. This evidence led us to formulate the structures of yuchasaponins C (**3**) and D (**4**) as shown.

Next, we examined the gastroprotective effects of the principal constituents (**6**, **7**, **8**, gallic acid) from the flower buds of *C. oleifera* and the flavonoid constituents (**6**, **7**) were found to show moderate activities as shown in Table 6. On the other hand, we have reported the isolation and structure elucidations of acylated polyhydroxyoleanane-type oligoglycosides with potent gastroprotective activities from several medicinal flowers or medicinal foodstuffs: that is, escins (*Aesculus hippocastanum*, seeds),[36] theasaponins (*Camellia sinensis*, seeds),[27] assamsaponins (*Camellia assamica*, seed

Table 5. ^{13}C-NMR (125, 150 MHz) Data of Yuchasaponins A—D (**1**—**4**)

C-	**1**	**2**	**3**	**4**	C-	**1**	**2**	**3**	**4**
1	39.0	39.0	39.0	38.9	3-*O*-GlcA				
2	26.5	26.5	26.5	26.4	1′	105.3	105.3	105.3	105.2
3	89.6	89.6	89.6	89.7	2′	79.7	79.5	79.8	79.7
4	39.6	39.7	39.7	39.7	3′	83.0	83.0	83.4	83.3
5	55.6	55.8	55.7	55.9	4′	71.1	71.2	71.1	71.1
6	18.9	18.5	18.9	18.5	5′	77.0	77.0	77.0	76.8
7	36.7	33.2	36.5	33.2	6′	172.0	172.0	172.1	172.1
8	41.5	40.1	41.5	40.1	2′-*O*-Glc				
9	47.2	47.0	47.2	46.9	1″	102.8	102.8		
10	37.0	36.8	37.0	36.8	2″	76.4	76.5		
11	24.0	23.9	24.0	23.9	3″	78.5	78.5		
12	125.5	124.1	125.5	123.9	4″	72.7	72.7		
13	143.7	142.8	143.7	142.8	5″	78.0	78.0		
14	47.2	41.8	47.8	41.7	6″	63.0	63.6		
15	73.4	34.9	73.4	34.9	2′-*O*-Gal				
16	67.6	68.7	67.6	68.5	1″			103.7	103.7
17	48.6	48.3	48.6	48.2	2″			73.5	73.5
18	41.0	40.1	41.0	40.1	3″			75.3	75.3
19	46.9	47.3	46.9	47.2	4″			70.3	70.2
20	36.4	36.5	36.5	36.5	5″			76.9	76.9
21	79.4	79.2	79.4	79.2	6″			62.8	62.7
22	73.9	74.3	73.9	74.2	3′-*O*-Gal				
23	28.1	28.1	28.2	28.2	1‴	101.5	101.5	101.6	101.6
24	16.8	16.8	16.9	16.8	2‴	76.5	76.4	77.0	77.1
25	15.8	15.7	15.8	15.7	3‴	76.0	76.0	76.0	76.0
26	17.6	17.0	17.6	16.9	4‴	71.2	71.2	70.9	71.7
27	21.2	27.6	21.2	27.6	5‴	77.0	77.4	77.3	77.2
28	63.0	63.6	63.0	63.6	6‴	61.9	61.9	61.9	61.9
29	29.5	29.6	29.6	29.5	2‴-*O*-Rha				
30	20.0	20.1	20.0	21.1	1⁗	102.3	102.4	102.6	102.6
21-*O*-Tig					2⁗	72.5	72.5	72.5	72.4
1	168.1	168.1	168.1	168.0	3⁗	72.7	72.7	72.8	72.8
2	129.5	129.6	129.5	129.5	4⁗	73.9	74.3	74.0	74.0
3	136.7	136.6	136.7	136.5	5⁗	69.8	69.8	69.9	69.8
4	14.1	14.1	14.1	14.1		18.3	18.3	18.3	18.3
5	12.3	12.4	12.3	12.3					
22-*O*-Tig									
1	168.5	168.6	168.5	168.6					
2	129.1	129.6	129.3	129.3					
3	137.0	137.0	137.0	137.0					
4	14.0	14.1	13.9	14.0					
5	12.2	12.3	12.2	12.2					

Measured in pyridine-d_5.

Table 6. Effects of the Constituents from the Flower Buds of *C. oleifera* on Gastric Lesions Induced by EtOH in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	4	144.5±5.9	—
Quercetin 3-*O*-α-L-rhamnopyranoside (**6**)	50	3	95.1±16.6	34.2
Kaempferol 3-*O*-α-L-rhamnopyranoside (**7**)	50	3	78.3±15.7*	38.1
Control	—	9	117.4±15.6	—
Oleanolic acid (**8**)	50	7	95.4±22.8	18.7
Gallic acid	100	7	89.9±25.2	23.4

Ethanol (1.5 ml/rat) was orally administered to 24 h fasted rats. One hour later, rats were killed and the total length (mm) of gastric lesions was determined. Test sample was administered orally 1 h before administration of ethanol. Values represent the means±S.E.M. Significantly different from the control group, $*p<0.05$. Test sample was suspended in 5% acacia solution.

and leaves),[37] floratheasaponins (*Camellia sinensis*, flower buds),[7] and camelliosides (*Camellia japonica*, flower buds).[11] With regard to the relationships between the saponin structures and gastroprotective activities, the acyl groups at the 21- and 22-positions of the polyhydroxyoleanane-type triterpene part and the 3-*O*-glucuronide structure were found to be essential. Since the oligoglycosidic structures of yuchasaponins (**1**—**4**) are similar to those of gastroprotective saponins and yuchasaponins have two tigloyl groups at the 21- and 22-positions, yushasaponins seem to be the gastroprotective principle of the 1-BuOH fraction from the flower buds of *C. oleifera*.

Previously, we also reported the inhibitory effects of flavonoids on rat lens aldose reductase[38,39] and radical scavenging activities,[40,41] and some flavonol and their glycosides were found to show those activities. From the 1-BuOH- and EtOAc-soluble fractions, which showed potent inhibitory activity on aldose reductase and radical scavenging activities, quercetin, kaempferol, and their glycosides were isolated as the principal constituents, so the active principles of the flower buds of *C. oleifera* seems to be those flavonoids.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The flower buds of *C. oleifera* were cultivated in Fujian province of China and presented from Research Center of Kanou Shojuan Ltd., Ootsu, Japan *via* Research Center of Suntory Ltd., Osaka, Japan, at March, 2005. The botanical identification was undertaken by one of author (M. Y.). A voucher specimen of this plant is on file in our laboratory.

Isolation The flower buds of *C. oleifera* (4.0 kg, Fujian province, China) were cut and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (485 g), which was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (128 g) and aqueous phase. The aqueous phase was extracted with 1-BuOH to give 1-BuOH- (29 g) and H_2O- (251 g) soluble fractions.

The EtOAc-soluble fraction was subjected to ordinary-phase silica gel column chromatography [2.0 kg, hexane : EtOAc (10 : 1→5 : 1→1 : 1→1 : 2→1 : 5)→$CHCl_3$: MeOH : H_2O (15 : 3 : 1 (lower phase)→10 : 3 : 1 (lower phase)→6 : 4 : 1)→MeOH] to give quercetin 3-*O*-α-L-rhamnopyranoside (**6**, 19.0 g), kaempferol 3-*O*-α-L-rhamnopyranoside (**7**, 18.8 g), oleanolic acid (**8**, 4.3 g), quercetin (10.3 g) and six fractions [fr. 1 (20.0 g), 2 (1.45 g), 3 (6.58 g), 4 (1.82 g), 5 (2.89 g), and 6 (18.0 g)]. Fraction 2 (1.45 g) was separated by reversed-phase silica gel column chromatography [50 g, H_2O : MeOH (20 : 80→70 : 30→50 : 50→30 : 70)→MeOH] followed by HPLC [H_2O : MeOH (50 : 50)] to furnish 4-aminophenol (5.0 mg), 3-(4-hydroxyphenol) acrylic acid (29.7 mg), and kaempferol (20.0 mg). Fraction 4 (1.82 g) was purified by reversed-phase silica gel column chromatography [60 g, H_2O : MeOH (70 : 30→50 : 50→30 : 70)→MeOH] to provide gallic acid (458 mg). Fraction 5 (2.89 g) was purified by reversed-phase silica gel column chromatography [90 g, H_2O : MeOH (80 : 20→70 : 30→50 : 50)→MeOH] followed by HPLC [H_2O : MeOH (60 : 40)] to give (−)-epigallocatechin 3-*O*-β-D-glucopyranoside (2.0 mg) and quercetin 3-*O*-β-D-glucopyranoside (43.7 mg).

The 1-BuOH-soluble fraction (20.7 g) was subjected to ordinary-phase silica gel column chromatography [650 g, $CHCl_3$: MeOH : H_2O (20 : 3 : 1 (lower phase)→10 : 3 : 1 (lower phase)→6 : 4 : 1, v/v/v)→MeOH] to give nine fractions [fr.1 (242 mg), 2 (224 mg), 3 (397 mg), 4 (583 mg), 5 (1.08 g), 6 (971 mg), 7 (594 mg), 8 (8.5 g), 9 (3.8 g)]. Fraction 3 (397 mg), 4 (583 mg), 5 (1.08 g), and 7 (594 mg) were purified by HPLC [H_2O : MeOH (70 : 30)] to give **6** (172 mg), **7** (49 mg), gallic acid (409 mg), and quercetin (32.6 mg), respectively. Fraction 7 (270 g) was separated by reversed-phase silica gel column chromatography [900 g, H_2O : MeOH (80 : 20→70 : 30→60 : 40→50 : 50→40 : 60)→MeOH] to give saponin fraction (1.44 g), which was purified by ordinary-phase silica gel column chromatography [43 g, $CHCl_3$: MeOH : H_2O (20 : 3 : 1 (lower phase)→10 : 3 : 1 (lower phase)→6 : 4 : 1, v/v/v)→MeOH] followed by HPLC [1% aqueous AcOH : MeOH (28 : 72) and 1% aqueous AcOH : MeOH : MeCN (49 : 16 : 35)] to yield yuchasaponins A (**1**, 30.2 mg), B (**2**, 14.3 mg), C (**3**, 15.1 mg), and D (**4**, 24.5 mg) and jegosaponin B (**5**, 25.4 mg). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, MS, and ^{1}H- and ^{13}C-NMR) with authentic samples (**8**, quercetin, gallic acid) and reported values.[22—25]

Yuchasaponin A (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 234.9—235.7 °C; $[\alpha]_D^{20}$ −12.1° (c=0.3, MeOH); IR (KBr): ν_{max} 3475, 1719, 1655, 1078 cm^{-1}: high-resolution negative-ion FAB-MS: Calcd for $C_{64}H_{99}O_{28}$ $(M-H)^-$: 1315.6323. Found: 1315.6313; ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 1.02, 1.06, 1.13, 1.17, 1.34, 1.84 (3H each, all s, H_3-25, 26, 24, 29, 23, 30, 27), 3.24 (1H, dd, J=4.2, 11.1 Hz, H-3), 3.36, 3.74 (1H each, both d, J=10.3 Hz, H_2-28), 4.40 (1H, m, H-15), 4.89 (1H, d, J=7.2 Hz, H-1′), 5.53 (1H, m, H-12), 5.85 (1H, d, J=7.5 Hz, H-1″), 6.07 (1H, d, J=7.8 Hz, H-1‴), 6.20 (1H, br s, H-1⁗), 6.32 (1H, d, J=10.3 Hz, H-22), 6.70 (1H, J=10.3 Hz, H-21); ^{13}C-NMR: given in Table 5; positive-ion FAB-MS m/z: 1339 $(M+Na)^+$; negative-ion FAB-MS m/z: 1315 $(M-H)^-$, m/z 1169 $(M-C_6H_{11}O_4)^-$, m/z 1007 $(M-C_{12}H_{21}O_9)^-$.

Yuchasaponin B (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 236.5—237.0 °C; $[\alpha]_D^{18}$ −3.7° (c=1.0, MeOH); IR (KBr): ν_{max} 3475, 1718, 1655, 1074 cm^{-1}: high-resolution negative-ion FAB-MS: Calcd for $C_{64}H_{99}O_{27}$ $(M-H)^-$: 1299.6374. Found: 1299.6382; ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.82, 0.87, 1.07, 1.12, 1.16, 1.34, 1.84 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 3.24 (1H, dd, J=4.3, 11.6 Hz, H-3), 3.39, 3.65 (1H each, both d, J=10.7 Hz, H_2-28), 4.91 (1H, d, J=7.7 Hz, H-1′), 5.43 (1H, m, H-12), 5.87 (1H, d, J=7.7 Hz, H-1″), 6.08 (1H, d, J=7.9 Hz, H-1‴), 6.21 (1H, br s, H-1⁗), 6.27 (1H, d, J=10.4 Hz, H-22), 6.70 (1H, J=10.4 Hz, H-21); ^{13}C-NMR: given in Table 5; positive-ion FAB-MS m/z: 1323 $(M+Na)^+$; negative-ion FAB-MS m/z: 1299 $(M-H)^-$, m/z 1153 $(M-C_6H_{11}O_4)^-$, m/z 991 $(M-C_{12}H_{21}O_9)^-$, m/z 829 $(M-C_{18}H_{31}O_{14})^-$.

Yuchasaponin C (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 234.4—235.6 °C; $[\alpha]_D^{20}$ −21.3° (c=0.5, MeOH); IR (KBr): ν_{max} 3453, 2962, 1714, 1655, 1076 cm^{-1}: high-resolution negative-ion FAB-MS: Calcd for $C_{64}H_{99}O_{28}$ $(M-H)^-$: 1315.6323. Found: 1315.6318; ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.84, 1.02, 1.12, 1.16, 1.27, 1.34, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 3.27 (1H, dd, J=4.3, 11.7 Hz, H-3), 3.46, 3.74 (1H each, both d, J=10.7 Hz, H_2-28), 4.96 (1H, d, J=6.8 Hz, H-1′), 5.52 (1H, m, H-12), 5.71 (1H, d, J=7.6 Hz, H-1″), 6.02 (1H, d, J=7.7 Hz, H-1‴), 6.20 (1H, br s, H-1⁗), 6.28 (1H, d, J=10.2 Hz, H-22), 6.69 (1H, J=10.2 Hz, H-21); ^{13}C-NMR: given in Table 5; positive-ion FAB-MS m/z: 1339 $(M+Na)^+$; negative-ion FAB-MS m/z: 1315 $(M-H)^-$, m/z 1169 $(M-C_6H_{11}O_4)^-$, m/z 991 $(M-C_{12}H_{21}O_9)^-$.

Yuchasaponin D (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 232.9—233.6 °C; $[\alpha]_D^{22}$ −16.6° (c=0.6, MeOH); IR (KBr): ν_{max} 3456, 1716, 1655, 1076 cm^{-1}: high-resolution negative-ion FAB-MS: Calcd for $C_{64}H_{99}O_{28}$ $(M-H)^-$: 1299.6374 Found: 1299.6378; ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.82, 0.86, 1.11, 1.15, 1.30, 1.34, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 3.27 (1H, dd, J=4.2, 11.0 Hz, H-3), 3.38, 3.64 (1H each, both d, J=11.5 Hz, H_2-28), 4.97 (1H, d, J=7.6 Hz, H-1′), 5.42 (1H, m, H-12), 5.71 (1H, d, J=7.5 Hz, H-1″), 6.01 (1H, d, J=7.7 Hz, H-1‴), 6.19 (1H, br s, H-1⁗), 6.26 (1H, d, J=10.4 Hz, H-22), 6.68 (1H, J=10.4 Hz, H-21); ^{13}C-NMR: given in Table 5; positive-ion FAB-MS m/z: 1323 $(M+Na)^+$; negative-ion FAB-MS m/z: 1299 $(M-H)^-$, m/z 1153 $(M-C_6H_{11}O_4)^-$, m/z 991 $(M-C_{12}H_{21}O_9)^-$, m/z 829 $(M-C_{18}H_{31}O_{14})^-$.

Alkaline Hydrolysis of Yuchasaponins (1—4) A solution of each yuchasaponins [(**1**, 12.5 mg), **2**, **3**, **4** (8.0 mg each)] in 50% aqueous 1,4-dioxane (1.0 ml) was treated with 10% aqueous KOH (1.0 ml) and the whole was stirred at 37 °C for 1 h. The each reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a reaction product, which was separated by ordinary-phase silica gel column chromatography [600 mg, $CHCl_3$: MeOH : H_2O [10 : 3 : 1 (lower phase)→6 : 4 : 1] to give **1a** (8.0 mg from **1**), **2a**, **3a**, **4a** (4 mg each from **2**, **3**, **4**) and a tiglic acid fraction. The tiglic acid fraction (1 mg each) was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeCN–H_2O (50 : 50, v/v); detection: UV (254 nm); flow rate: 1.0 ml/min; column temperature: room temperature] to identify the *p*-nitrobenzyl ester of tiglic acid (t_R 30.6 min). **1a**: Colorless fine crystals; high-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{88}O_{26}$ $(M+Na)^+$: 1175.5453. Found: 1175.5462; positive-ion FAB-MS m/z: 1175 $(M+Na)^+$; negative-ion FAB-MS m/z: 1151 $(M-H)^-$, m/z 1005 $(M-C_6H_{11}O_4)^-$,

m/z 843 ($M-C_{12}H_{21}O_9$)$^-$. Desacyl-jegosaponin (**2a**) was identified by comparison of the physical data (IR, MS, ^{1}H-NMR) with an authentic sample.[34]

Acid Hydrolysis of Yuchasaponins (1a—4a) A solution of desacyl-derivatives (**1a**—**4a**, 2 mg each) in 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, 1.0 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. On removal of the solvent from the filtrate under reduced pressure, the residue was passed through a Sep-Pack C18 cartridge by elution with H_2O and the MeOH. The MeOH eluate was purified by normal-phase silica gel column chromatography [100 mg $CHCl_3$: MeOH : H_2O [10 : 3 : 1, lower phase] to give R_1-barrigenol (from **1a**, **3a**) and barringtogenol C (from **2a**, **4a**), which were identified with authentic samples by TLC, IR, and ^{1}H-NMR spectra. The H_2O eluate was concentrated and residue was treated with L-cysteine methyl ester hydrochloride (0.01 ml) in pyridine (0.02 ml) at 60 °C for 1 h. After this reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The reaction mixture was subjected to centrifugal separation and the supernatant was then subjected to GLC analysis [column: Supelco STB-1, 30 m×0.25 mm i.d.; column temperature: 230 °C; carrier gas: N_2] to identify the derivatives of D-glucuronic acid (t_R 23.9 min, from **1a**—**4a**), D-glucose (t_R 17.7 min, from **1a**, **2a**), D-galactose (t_R 21.1 min, from **1a**—**4a**), and L-rhamnose (t_R 12.4 min, from **1a**—**4a**).

Effect on Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats The acute gastric lesions were induced by oral administration of ethanol and indomethacin according to the method described previously.[19,36,42—45] Briefly, 99.5% ethanol and 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) were 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, the lengths of gastric lesions were measured as previously described, and the total length (mm) was expressed as a lesion index.

Statistics Values are expressed as mean±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 to present a statistically significant difference.

Effect on Rat Lens Aldose Reductase Aldose reductase activity was assayed by the method described in a previous paper.[38,39,46—49] The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135 mM phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μl of enzyme fraction, with 25 μl of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μl 0.5 M HCl. Then, 0.5 ml 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a fluorophotometer (Luminescence Spectrometer LS50B, Perkin Elmer, England) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

DPPH Radical Scavenging Activity The free radical scavenging activity was assessed using the DPPH radical.[50,51] An ethanol solution of DPPH (100 μM, 1.0 ml) was mixed with different concentrations of each test compound (0—200 μM, 0.5 ml) and 0.1 M acetate buffer (pH 5.5, 1.0 ml), and the absorbance change at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, SC_{50}) of 40 μM DPPH radical solution was determined graphically.

$^{\cdot}O_2^-$ Scavenging Activity The assay method for superoxide dismutase described by Ukeda *et al.*,[52] was used with a slight modification.[41] Briefly, a reaction mixture containing 100 μM xanthine, 100 μM EDTA, 25 μM WST-1, and *ca.* 1.9 mU/ml xanthine oxidase in 50 mM sodium carbonate buffer (pH 10.2) was incubated with or without each test sample for 20 min at 37 °C (total volume: 3.0 ml). After incubation, the solution was mixed with 0.1 ml of 2 M HCl to stop the reaction. The formazan formation was monitored at 450 nm. Measurements were performed in duplicate, and the concentration required for a 50% inhibition (IC_{50}) of the WST-1 formazan formation was determined graphically.

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