Brazilian Natural Medicines. III.[1] Structures of Triterpene Oligoglycosides and Lipase Inhibitors from Mate, Leaves of *Ilex paraguariensis*

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The methanolic extract from the leaves of *Ilex paraguariensis* (Aquifoliaceae) was found to show an inhibitory activity on porcine pancreatic lipase. From the methanolic extract, three new triterpene oligoglycosides, mateglycosides A, B, and C, were isolated together with 18 known compounds. The chemical structures of new oligoglycosides were elucidated on the basis of chemical and physicochemical evidence. Several constituents showed inhibitory activities on pancreatic lipase.

Key words mateglycoside; *Ilex paraguariensis*; lipase inhibitor; mate tea; Brazilian natural medicine; triterpene oligoglycoside

An Aquifolium plant, *Ilex* (*I.*) *paraguariensis* St. Hil., is distributed in South American countries: Brazil, Argentina, Paraguay, *etc.*, and is locally called as mate, erva-mate, or yerba-mate. The leaves of this plant are commonly consumed in South American countries for the preparation of several types of beverages, such as mate tea or Jesuit's tea. The leaves of *I. paragueriensis* are also known to have the therapeutic effects on arthritis, slow digestions, liver diseases, headache, rheumatism, and obesity in Brazilian traditional medicine. As chemical constituents of *I. paraguariensis*, various triterpene saponins[2] together with flavonoids, caffeoylquinic acids, and xanthines were isolated from the aerial parts. However, the biological activities of the saponin constituents have not yet characterized.

Previously, we have reported the isolation and structure elucidation of flavonoids, pseudoalkaloids, and steroidal saponins with antidiabetic activities from several Brazilian natural medicines[1,3—5] and also, we have examined the biological activities of triterpene saponins from natural medicines[6—11] and medicinal foodstuffs.[12—21] As a continuing study, the methanolic extract from the leaves of *I. paraguariensis* was found to exhibit an inhibitory effect against porcine pancreatic lipase. From the methanolic extract, we have isolated three new triterpene saponins named mateglycosides A (**1**), B (**2**), and C (**3**) together with 18 known constituents. This paper deals with the structure elucidation of new saponins (**1**—**3**) as well as the inhibitory effects of the principal constituents on pancreatic lipase.

Isolation from the Leaves of *I. paraguariensis* The methanolic extract (33.3% from the dried leaves) from the leaves of *Ilex paraguariensis* cultivated in Brazil, which showed the inhibitory effect on porcine pancreatic lipase, was partitioned into an EtOAc and water (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (11.2%) and an aqueous phase. The aqueous phase was further extracted with 1-BuOH to give 1-BuOH (16.7%)- and H_2O (6.4%)-soluble fractions. As shown in Table 1, the EtOAc- and 1-BuOH-soluble fractions were found to exhibit the inhibitory effects on pancreatic lipase, whereas the H_2O-soluble fraction showed weak activity. The active fractions were subjected to normal- and reversed-phase silica gel column chromatographies and finally HPLC to provide mateglycosides A (**1**, 0.20%), B (**2**, 0.02%), C (**3**, 0.001%), and D (**13**, 0.10%), which was reported as an acidic hydrolysis product of oleanolic acid tetraglycoside from *Aralia elata*,[22] together with ursolic acid[23] (**4**, 1.02%), 3β-hydroxyurs-11-en-13β(28)-olide[24] (**16**, 0.039%), matesaponins 1[25] (**5**, 0.09%), 2[26] (**6**, 0.01%), 3[26] (**7**, 0.034%), 4[26] (**8**, 0.034%), 5[27] (**9**, 0.009%), J3[28] (**10**, 0.06%), 3-*O*-β-

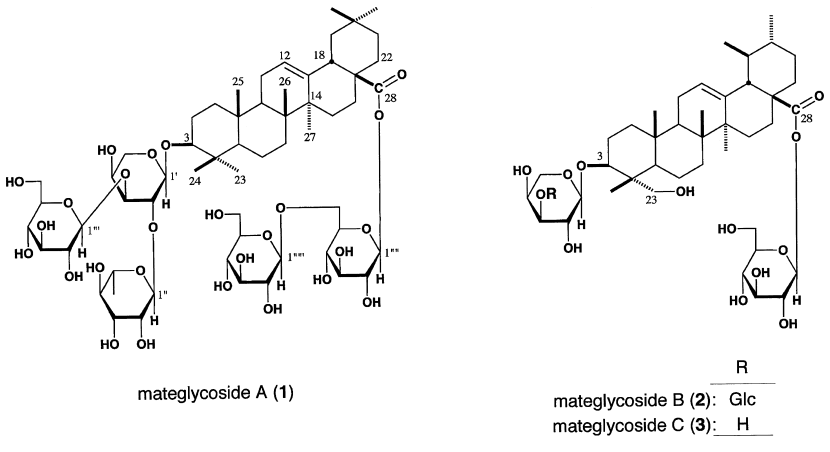

Chart 1. New Saponins (**1**—**3**) from the Leaves of *Ilex paraguariensis*

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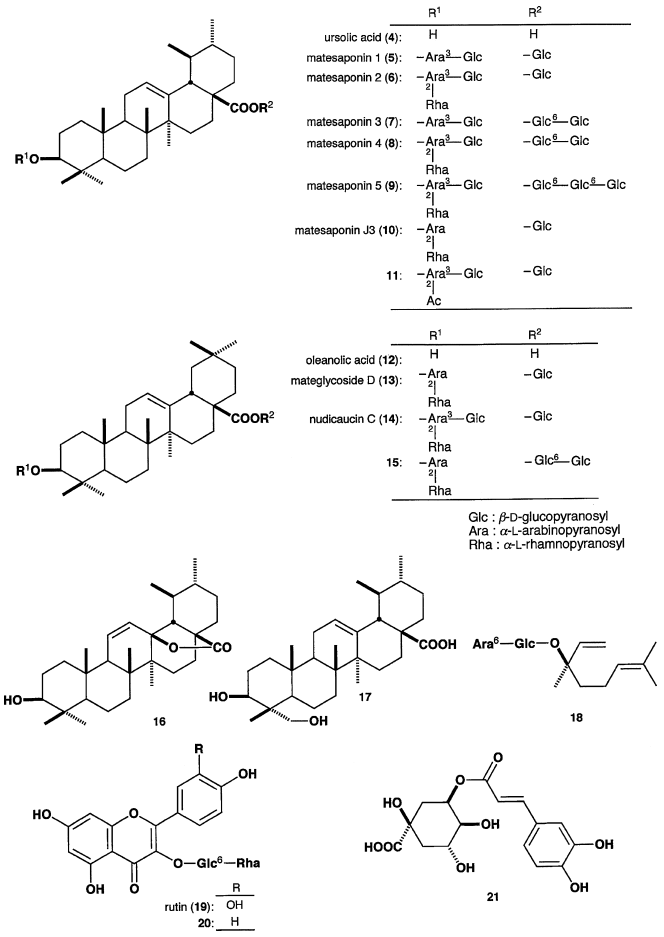

Chart 2. Known Constituents from the Leaves of *Ilex paraguariensis*

D-glucopyranosyl(1—3)-α-L-2-*O*-acetylarabinopyranosylurolic acid 28-*O*-β-D-glucopyranoside[29] (**11**, 0.047%), nudicaucin C[30] (**14**, 0.12%), 3-*O*-α-L-rhamnopyranosyl(1—2)-α-L-arabinopyranosyloleanolic acid 28-*O*-β-D-glucopyrnosyl(1—6)-β-D-glucopyranoside[31] (**15**, 0.29%), (*R*)-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside[32] (**18**, 0.090%), rutin[33] (**19**, 0.512%), kaempferol-3-*O*-rutinoside[34] (**20**, 0.178%), 5-*O*-caffeoyl-D-quinic acid[35] (**21**, 0.046%), caffeine[36] (0.55%), and theobromine[37] (0.15%).

The Structures of Mateglycosides A (1), B (2), and C (3) Mateglycoside A (**1**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{25}$ −5.6° in MeOH). The IR spectrum of **1** showed absorption bands at 1719 and 1650 cm^{-1} ascribable to carbonyl and olefin functions and broad bands at 3600 and 1075 cm^{-1} suggestive of an oligoglycoside structure. In the positive-ion FAB-MS spectrum of **1**, a quasimolecular ion peak was observed at *m/z* 1243 $(M+Na)^+$, whereas its negative-ion FAB-MS spectrum showed a quasimolecular ion peak at *m/z* 1219 $(M-H)^-$ together with fragment ion peak at *m/z* 895 $(M-C_{12}H_{21}O_{10})^-$, which was derived by cleavage of the ester glycoside linkage. The molecular formula of **1** was determined to be $C_{59}H_{96}O_{26}$ by high-resolution positive-ion FAB-MS analysis of the quasimolecular ion peak $(M+Na)^+$. Acid hydrolysis of **1** with 5% aqueous H_2SO_4 in 1,4-dioxane (1 : 1, v/v) liberated oleanolic acid (**12**) together with L-arabinose, D-glucose, and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[1,3,6] The ^1H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[38] indicated the presence of the following functions: an oleanolic acid part {seven methyls [δ 0.87, 0.88, 0.90, 1.09, 1.13, 1.21, 1.26 (all s, 25, 29, 30, 26, 24, 23, 27-H_3)], a methine bearing an oxygen function [δ 3.30 (dd, *J*= 3.5, 11.0 Hz, 3-H)], and an olefin [δ 5.42 (m, 12-H)]}, an α-L-arabinopyranosyl moiety [δ 4.86 (d, *J*=7.2 Hz, 1′-H)], three β-D-glucopyranosyl moieties [δ 5.05 (d, *J*=7.6 Hz, 1‴′-H), 5.11 (d, *J*=7.6 Hz, 1‴-H), and 6.27 (d, *J*=8.2 Hz, 1‴′-H)], and an α-L-rhamnopyranosyl moiety [δ 6.15 (br s, 1″-H)]. In the heteronuclear multiple bond connectivity (HMBC) experiment of **1**, long-range correlations were observed between the following protons and carbons: the 1′-proton and 3-carbon, the 1″-proton and 2′-carbon, the 1‴-proton and 3′-carbon, the 1‴′-proton and 28-carbon, and the 1‴′-proton and 6‴′-carbon. On the basis of those findings, the structure of mateglycoside A was determined to be 3-*O*-α-L-rhamnopyranosyl(1—2)[β-D-glucopyranosyl(1‴-3)]-α-L-arabinopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl(1—6)-β-D-glucopyranoside (**1**).

Table 1. Inhibitory Effects of the MeOH Extract and EtOAc-, 1-BuOH-, and H_2O-Soluble Fractions from the Leaves of *I. paraguariensis* against Porcine Pancreatic Lipase Activity

Conc. (μg/ml)	Inhibition (%)						
	0	50	100	200	400	800	1000
MeOH ext.	0.0	28	30	33	35	81	95
EtOAc-soluble fraction	0.0	42	55	69	80	89	88
1-BuOH-soluble fraction	0.0	31	37	39	94	99	99
H_2O-soluble fraction	0.0	21	32	43	48	53	58

Mateglycoside B (**2**) was also obtained as a white powder with positive optical rotation ($[\alpha]_D^{25}$ +18.7° in MeOH). The IR spectrum of **2** showed absorption bands at 3600, 1716, 1650, and 1070 cm^{-1} due to hydroxyl, carbonyl, and olefin functions. In the positive- and negative-ion FAB-MS spectra of **2**, pseudomolecular ion peaks were observed at *m/z* 951 $(M+Na)^+$ and *m/z* 927 $(M-H)^-$, respectively and high-resolution FAB-MS analysis of the pseudomolecular ion peak $(M+Na)^+$ revealed the molecular formula of **2** to be $C_{47}H_{76}O_{18}$. Acid hydrolysis of **2** with 5% aqueous H_2SO_4-1,4-dioxane provided 23-hydroxyursolic acid (**17**)[39] together with L-arabinose and D-glucose, which were identified by HPLC analysis using an optical rotation detector.[1,3,6] The ^1H-(pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[38] of **2** showed signals assignable to a 23-hydroxyursolic acid part, an α-L-arabinopyranosyl moiety [δ 4.98 (d, *J*=7.2 Hz, 1′-H)], and two β-D-glucopyranosyl moieties [δ 5.34 (d, *J*=7.6 Hz, 1″-H), 6.29 (d, *J*=7.9 Hz, 1‴-H)]. The HMBC experiment on **2** showed long-range correlations between the 1′-proton and 3-carbon, between the 1″-proton and 3′-carbon, and between the 1‴-proton and 28-carbon. Consequently, the structure of mateglycoside B was clarified to be 3-*O*-β-D-glucopyranosyl(1—3)-α-L-arabinopyranosyl 23-hydroxyurolic acid 28-*O*-β-D-glucopyranoside (**2**).

Mateglycoside C (**3**), which was isolated as a white powder with positive optical rotation ($[\alpha]_D^{29}$ +47.3° in MeOH), showed absorption bands due to hydroxyl, carbonyl, and olefin functions in the IR spectrum. The molecular formula, $C_{41}H_{66}O_{13}$, of **3** was determined from the positive-ion FAB-

Table 2. ^{13}C-NMR (125, 150 MHz) Data of Mateglycosides A (**1**), B (**2**) and C (**3**) (Measured in Pyridine-d_5)

C-	**1**	**2**	**3**	C-	**1**	**2**	**3**
1	39.0	39.3	39.1	3-*O*-Ara			
2	26.6	26.3	26.2	1′	104.6	106.6	106.7
3	88.2	81.8	81.9	2′	74.7	72.2	73.2
4	39.6	43.5	43.5	3′	82.0	84.2	74.8
5	56.0	47.5	47.6	4′	68.2	69.3	69.7
6	18.6	19.2	18.2	5′	64.8	67.1	67.0
7	32.5	33.2	33.2	2′-*O*-Rha			
8	39.9	39.1	40.2	1″	101.9		
9	48.0	48.1	48.1	2″	72.3		
10	37.0	36.8	36.9	3″	72.5		
11	23.4	24.7	24.7	4″	73.9		
12	123.0	126.7	126.2	5″	70.0		
13	144.1	138.4	138.4	6″	18.6		
14	42.1	42.5	42.5	3′-*O*-Glc			
15	28.3	28.7	28.5	1‴	104.7	106.4	
16	23.7	24.7	24.7	2‴	74.9	75.7	
17	47.0	48.4	48.4	3‴	78.3	78.4	
18	41.7	53.3	53.3	4‴	71.4	71.6	
19	46.2	40.2	39.1	5‴	78.5	78.4	
20	30.7	39.0	39.4	6‴	62.5	62.7	
21	33.1	30.8	30.8	28-*O*-Glc			
22	33.1	36.8	36.8	1⁗	95.6	95.7	95.7
23	28.1	64.2	64.5	2⁗	75.1	74.1	74.1
24	17.0	13.7	13.7	3⁗	78.2	78.9	79.2
25	15.7	16.4	16.4	4⁗	70.8	71.2	71.2
26	17.5	17.7	17.4	5⁗	77.9	78.8	78.9
27	26.0	23.7	23.8	6⁗	69.3	62.3	62.3
28	176.5	176.2	176.2	6⁗-*O*-Glc			
29	23.6	17.4	17.8	1′′′′′	105.3		
30	33.1	21.3	21.3	2′′′′′	75.1		
				3′′′′′	78.4		
				4′′′′′	71.3		
				5′′′′′	78.7		
				6′′′′′	62.5		

MS [m/z 789 $(M+Na)^+$] and by high-resolution MS measurement. The acid hydrolysis of **3** liberated **17**, L-arabinose, and D-glucose.[1,3,6] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[38] of **3** showed signals assignable to a 23-hydroxylursolic acid part, an α-L-arabinopyranosyl moiety [δ 4.99 (d, J=7.2 Hz, 1′-H)], and a β-D-glucopyranosyl moiety [δ 6.29 (d, J=8.1 Hz, 1″-H)]. In the HMBC experiment on **3**, long-range correlations were observed between the 1′-proton and the 3-carbon and between the 1″-proton and 28-carbon. This evidence led us to formulate the structure of mateglycoside C (**3**) as shown.

In the course of characterization studies on antiobesitic constituents from medicinal foodstuffs, we have reported the isolation and structure elucidation of several saponins and diterpenes with the inhibitory effect on pancreatic lipase from *Salvia officinalis*,[40] *Camellia sinensis*,[41] *Aesculus turbinata*,[8] and *Sapindus rarak*.[42,43] Pancreatic lipase is well known to play an important role in lipid digestion. Since mate tea is used for the treatment of obesity and also, the methanolic extract and its EtOAc- and 1-BuOH-soluble fractions from the leaves of *I. paraguariensis* showed an inhibitory activity on porcine pancreatic lipase, we examined the inhibitory effects of the principal constituents from the EtOAc- and 1-BuOH-soluble fractions. As shown in Table 3, several triterpene saponins (**5**, **14**, **15**) and monoterpene oligoglycosides (**18**) were found to exhibit the potent inhibitory activities and these compounds seems to be responsible for the antiobesitic activity of mate tea.

Table 3. Inhibitory Effects of Principal Constituents from *I. paraguariensis* against Porcine Pancreatic Lipase Activity

Conc. (μM)	Inhibition (%)					
	0	50	100	200	400	800
1	0.0	−2.7	−7.8	−2.5	15	46
4	0.0	13	10	16	22	17
5	0.0	47	94	95	96	96
7	0.0	2.1	−2.3	3.9	15	33
9	0.0	5.2	0.5	−0.9	−3.1	−7.8
14	0.0	36	78	94	96	96
15	0.0	8.0	77	94	97	98
18	0.0	39	83	102	101	99
19	0.0	15	24	27	36	39
20	0.0	10	12	23	38	49
21	0.0	37	39	39	53	47

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 leaves of *I. paraguariensis*, which were cultivated in Brazil, were purchased from Mitsuboshi Pharmaceutical Co., Ltd., Nara, Japan. The botanical identification was undertaken by research members of Mitsuboshi Pharmaceutical Co., Ltd. A voucher specimen of this plant is on file in our laboratory.

Isolation of Mateglycosides A (1), B (2), C (3), and D (4) The dried leaves (3.0 kg) of *I. paraguariensis* were finely cut and extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (1.0 kg). The methanolic extract (500 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (168.0 g) and aqueous phase, which was extracted with 1-BuOH to give 1-BuOH- (251 g) and H_2O- (96.5 g) soluble fractions.

The 1-BuOH-soluble fraction (150 g) was subjected to normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 3.0 kg), $CHCl_3$: MeOH : H_2O (10 : 3 : 1→7 : 3 : 1 lower phase→6 : 4 : 1, v/v/v)→MeOH] to give six fractions [fr.1 (14.7 g), 2 (26.9 g), 3 (30.8 g), 4 (41.3 g), 5 (34.5 g), 6 (5.5 g)]. Fraction 1 (14.0 g) was separated by reversed-phase silica gel column chromatography [420 g, H_2O–MeOH (90 : 10→70 : 30→50 : 50→30 : 70, v/v)→MeOH] to give theobromine (1.30 g), and caffeine (4.80 g). Fraction 2 (25.0 g) was subjected to reversed-phase silica gel column chromatography [750 g, H_2O : MeOH (90 : 10→70 : 30→50 : 50→30 : 70→20 : 80)→MeOH] to furnish seven fractions [fr. 2-1 (9.4 g), 2-2 (157 mg), 2-3 (1.2 g), 2-4 (9.0 g), 2-5 (1.7 g), 2-6 (1.5 g), 2-7 (238 mg)]. Fraction 2-2 (157 mg) was purified by HPLC [H_2O : MeOH (40 : 60)] to give (*R*)-linalyl 6-*O*-arabinopyranosyl-*β*-D-glucopyranoside (**18**, 74.8 mg). Fraction 2-4 (500 mg) was purified by HPLC [H_2O : MeOH (30 : 70)] to furnish matesaponin 1 (**5**, 31.4 mg). Fraction 2-7 (238 mg) was separated by HPLC [1% aqueous acetic acid : MeOH : MeCN (49 : 16 : 35)] to provide matesaponins J3 (**10**, 17.4 mg) and mateglycoside D (**13**, 30.3 mg), and 3-*O*-*β*-D-glucopyranosyl(1—3)-*α*-L-2-*O*-acetyl-arabinopyranosyl ursolic acid 28-*O*-*β*-D-glucopyranoside (**11**, 13.6 mg). Fraction 3 (30.0 g) was separated by reversed-phase silica gel column chromatography [900 g, H_2O : MeOH (90 : 10→70 : 30→50 : 50→30 : 70→20 : 80)→MeOH] to give rutin (**19**, 2.2 g), kaempferol 3-*O*-rutinoside (**20**, 1.5 g), and five fractions [fr. 3-1 (4.3 g), 3-2 (2.4 g), 3-3 (6.3 g), 3-4 (11.0 g), 3-5 (2.2 g)]. Fraction 3-3 (3.0 g) was subjected HPLC [H_2O : MeOH (30 : 70)] to give matesaponin 2 (**6**, 6.3 mg), nudicaucin C (**14**, 252 mg), and 3-*O*-*α*-L-rhamnopyranosyl(1—2)-*α*-L-arabinopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl(1—6)-*β*-D-glucopyranoside (**15**, 430 mg). Fraction 4 (40.0 g) was separated by reversed-phase silica gel column chromatography [1.2 kg, H_2O : MeOH (90 : 10→70 : 30→50 : 50→30 : 70→20 : 80)→MeOH] to give **19** (2.2 g) and six fractions [fr. 4-1 (26.4 g), 4-2 (1.0 g), 4-3 (2.7 g), 4-4 (2.9 g), 4-5 (0.79 g), 4-6 (3.9 g)]. Fraction 4-2 (500 mg) was separated by HPLC [H_2O : MeOH (40 : 60)] to give matesaponin 3 (**7**, 44.8 mg). Fraction 4-3 (2.4 g) was purified by HPLC [H_2O : MeOH (30 : 70)] followed by HPLC [H_2O : MeCN (70 : 30)] to give mateglycoside A (**1**, 513 mg) and matesaponin 5 (**9**, 72.4 mg). Fraction 4-4 (1.5 g) was purified by HPLC [H_2O : MeCN (70 : 30)] to furnish **1** (457 mg) and **9** (97.0 mg). Fraction 4-5 (0.79 g) was purified by HPLC [H_2O : MeOH (30 : 70)] followed by HPLC [H_2O : MeCN (70 : 30)] to give **1** (20.8 mg), matesaponin 4 (**8**, 29.7 mg), and **11** (65.7 mg). Fraction 5 (33.0 g) was subjected by reversed-phase silica gel column chromatography [1.2 kg, H_2O : MeOH (90 : 10→70 : 30→50 : 50→30 : 70)→MeOH] followed by HPLC [H_2O : MeOH (90 : 10)] to give 5-*O*-caffeoyl-D-quinic acid (**21**, 20.8 mg).

The EtOAc-soluble fraction (100 g) was subjected to normal-phase silica gel column chromatography [3.0 kg, hexane : EtOAc (5 : 1→1 : 1)→EtOAc→$CHCl_3$→$CHCl_3$: MeOH (5 : 1→1 : 1)→MeOH] to provide four fractions [fr. 1 (21.1 g), 2 (13.3 g), 3 (29.9 g), 4 (20.4 g)]. Fraction 3 (13.3 g) was separated by reversed-phase silica gel column chromatography [390 g, H_2O : MeOH (10 : 90)→MeOH] to give ursolic acid (**4**, 900 mg) and five fractions [fr. 3-1 (599 mg), 3-2 (10.2 g), 3-3 (312 mg), 3-4 (151 mg), 3-5 (993 mg)]. Fraction 3-2 (400 mg) was purified by HPLC [H_2O : MeOH (10 : 90)] to give 3-*β*-hydroxyurs-11-en-13*β*(28)-olide (**17**, 13.1 mg) and **4** (308 mg). Fraction 4 (20.3 g) was subjected to reversed-phase silica gel column chromatography [600 g, H_2O : MeOH (10 : 90)→MeOH] to give six fractions [fr. 4-1 (12.3 g), 4-2 (1.1 g), 4-3 (477 mg), 4-4 (663 mg), 4-5 (1.2 g), 4-6 (3.5 g)]. Fraction 4-2 (500 mg) was purified by HPLC [H_2O : MeOH (65 : 35)] to give mateglycoside C (**3**, 11.3 mg). Fraction 4-5 (250 mg) was purified by HPLC [H_2O : MeOH (65 : 35)] to furnish **5** (21.6 mg). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, MS, and ^{1}H- and ^{13}C-NMR) with reported values.[22—35] The obtained caffeine and theobromine were identified by comparison of the MS, ^{1}H- and ^{13}C-NMR data with those of commercial products.

Mateglycoside A (**1**): A white powder; $[\alpha]_D^{25}$ −5.6 (c=0.64, MeOH); high-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{96}O_{26}Na$ $(M+Na)^+$: 1243.6089. Found: 1243.6094; IR (KBr): ν_{max} 3600, 1719, 1650, 1075 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5) δ 0.87, 0.88, 0.90, 1.09, 1.13, 1.21, 1.26 (3H each, all s, H_3-25, 29, 30, 26, 23, 24, 27), 3.30 (1H, dd, J=3.5, 11.0 Hz, H-3), 4.86 (1H, d, J=7.2 Hz, H-1′), 5.05 (1H, d, J=7.6 Hz, H-1‴′), 5.11 (1H, d, J=7.6 Hz, H-1‴), 5.42 (m, H-12), 6.15 (1H, br s, H-1″), 6.27 (1H, d, J=8.2 Hz, H-1‴′); ^{13}C-NMR: given in Table 2; positive-ion FAB-MS: m/z 1243 $(M+Na)^+$; negative-ion FAB-MS: m/z 1219 $(M-H)^-$, m/z 895 $(M-C_{12}H_{21}O_{10})^-$, m/z 733 $(M-C_{18}H_{31}O_{15})^-$, m/z 587 $(M-C_{24}H_{41}O_{19})^-$, m/z 455 $(M-C_{29}H_{49}O_{23})^-$.

Mateglycoside B (**2**): A white powder; $[\alpha]_D^{25}$ +18.7 (c=0.13, MeOH); high-resolution positive-ion FAB-MS: Calcd for $C_{47}H_{76}O_{18}Na$ $(M+Na)^+$: 951.4933. Found: 951.4938; IR (KBr): ν_{max} 3600, 1716, 1650, 1070 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5) δ 0.89. 0.94 (both 3H, both d, J=5.9 Hz, H_3-30, 29), 0.95, 0.98, 1.15, 1.18 (3H each, all s, H_3-23, 25, 27, 26), 3.72 (1H, d, J=10.5 Hz, H-24), 4.26 (1H, m, H-24), 4.28 (1H, m, H-3), 4.98 (1H, d, J=7.2 Hz, H-1′), 5.34 (1H, d, J=7.6 Hz, H-1″), 6.29 (1H, d, J=7.9 Hz, H-1‴); ^{13}C-NMR: given in Table 2; positive-ion FAB-MS: m/z 951 $(M+Na)^+$; negative-ion FAB-MS: m/z 927 $(M-H)^-$, m/z 765 $(M-C_6H_{11}O_5)^-$, m/z 603 $(M-C_{12}H_{21}O_{10})^-$, m/z 471 $(M-C_{17}H_{29}O_{14})^-$.

Mateglycoside C (**3**): A white powder; $[\alpha]_D^{29}$ +47.3 (c=0.63, MeOH); high-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{66}O_{13}Na$ $(M+Na)^+$: 789.4412. Found: 789.4407; IR (KBr): ν_{max} 3507, 1653, 1072 cm^{-1}; ^{1}H-NMR (500 MHz, pyridine-d_5) δ 0.89. 0.93 (both 3H, both d, J=6.2 Hz, H_3-30, 29), 0.94, 0.99, 1.14, 1.19 (3H each, all s, H_3-23, 25, 27, 26), 3.73, 4.30 (both 1H, both d, J=10.7 Hz, H_2-24), 4.26 (1H, m, H-3), 4.99 (1H, d, J=7.2 Hz, H-1′), 6.29 (1H, d, J=8.1 Hz, H-1″); ^{13}C-NMR: given in Table 2; positive-ion FAB-MS: m/z 789 $(M+Na)^+$.

Acid Hydrolysis of Mateglycosides A (1), B (2), and C (3) A solution of **1** (33.1 mg), **2** (17.0 mg), and **3** (3.5 mg) in 5% aqueous H_2SO_4-1,4-dioxane (1 : 1, 1.0 ml) was heated under reflux for 2 h, respectively. 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. Then, the filtrate was extracted with EtOAc to give an EtOAc-soluble fraction and aqueous phase. The EtOAc-soluble fraction was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$→$CHCl_3$: MeOH (20 : 1)] to give oleanolic acid (**12**, 13.0 mg) from **1** and 23-hydroxyursolic acid (**17**, 4.9 mg and 1 mg) from **2** and **3**. Those triterpenes (**12**, **17**) were identified by comparison of their ^{1}H- and ^{13}C-NMR and MS data with those of authentic samples.

The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2–60–5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, MeCN–H_2O (80 : 20, v/v); flow rate 0.80 ml/min; column temperature, room temperature. Identification of L-arabinose (from **1**, **2**, and **3**) D-glucose (from **1**, **2**, and **3**), and L-rhamnose (from **1**), which were confirmed by comparison of its retention time and optical rotation with that of an authentic sample; t_R: 8.3 min (L-arabinose, positive optical rotation), 9.5 min (D-glucose, positive optical rotation), 6.5 min (L-rhamnose, negative optical rotation).

Effect on Pancreatic Lipase Activity A suspension of triolein (80 mg), phosphatidylcholine (10 mg), and sodium taurocholate (5 mg) in 9 ml of 0.1 M Tris–HCl buffer (pH=7.0) containing 0.1 M NaCl was sonicated for 10 min. This sonicated substrate suspension (0.1 ml) in a test tube was pre-incubated with 5 μl of test sample in DMSO and 95 μl of the tris–HCl buffer for 3 min at 37 °C. An aliquot of porcine pancreatic lipase (250 μg/ml, type II, Sigma Chemical Co.) (50 μl) or the tris–HCl buffer (50 μl) as a blank test was then added to start the reaction. After 30 min of incubation, the test tube was immediately immersed in boiling water for 2 min to stop the reaction, then cooled with water. Free fatty acid concentration was determined by a commercial kit (NEFA C-test Wako, Wako Pure Chemical Industries, Ltd.).

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References and Notes

1) Nakamura S., Hongo M., Sugimoto S., Matsuda H., Yoshikawa M., *Phytochemistry*, **69**, 1565—1572 (2008).
2) Schenkel E. P., Montanha J. A., Gosmann G., "Saponins Used in Food and Agriculture (Advances in Experimental Medicine and Biology vol. 405)," ed. by Waller G. R., Yamasaki K., Plenum Press, New York, London, 1996, pp. 47—56.
3) Yoshikawa M., Nakamura S., Ozaki K., Kumahara A., Morikawa T., Matsuda H., *J. Nat. Prod.*, **70**, 210—214 (2007).
4) Matsuda H., Nishida N., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 429—431 (2002).
5) Yoshikawa M., Shimada H., Nishida N., Li Y., Toguchida I., Matsuda H., *Chem. Pharm. Bull.*, **46**, 113—119 (1998).
6) Morikawa T., Matsuda H., Li N., Li X., Yoshikawa M., *Helv. Chim. Acta*, **90**, 2342—2348 (2007).
7) Morikawa T., Nakamura S., Kato Y., Muraoka O., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 293—298 (2007).
8) Hu J. N., Zhu X. M., Han L. K., Saito M., Sun Y. S., Yoshikawa M., Kimura Y., Zheng Y. N., *Chem. Pharm. Bull.*, **56**, 12—16 (2008).
9) Yoshikawa M., Morikawa T., Nakamura S., Li N., Li X., Matsuda H., *Chem. Pharm. Bull.*, **55**, 57—63 (2007).
10) Matsuda H., Nakamura S., Murakami T., Yoshikawa M., *Heterocycles*, **71**, 331—341 (2007).
11) Yoshikawa M., Morikawa T., Kobayashi H., Nakamura A., Matsuhira K., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 428—434 (2007).
12) Yoshikawa M., Xu F., Morikawa T., Pongpiriyadacha Y., Nakamura S., Asao Y., Kumahara A., Matsuda H., *Chem. Pharm. Bull.*, **55**, 308—316 (2007).
13) Nakamura S., Sugimoto S., Matsuda H., Yoshikawa M., *Heterocycles*, **71**, 577—588 (2007).
14) Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 571—576 (2007).
15) Yoshikawa M., Nakamura S., Kato Y., Matsuhira K., Matsuda H., *Chem. Pharm. Bull.*, **55**, 598—605 (2007).
16) Yoshikawa M., Morikawa T., Asao Y., Fujiwara E., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 606—612 (2007).
17) Yoshikawa M., Sugimoto S., Nakamura S., Sakumae H., Matsuda H., *Chem. Pharm. Bull.*, **55**, 1034—1038 (2007).
18) Nakamura S., Sugimoto S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1342—1348 (2007).
19) Yoshikawa M., Wang T., Sugimoto S., Nakamura S., Nagatomo A., Matsuda H., Harima S., *Yakugaku Zasshi*, **128**, 141—151 (2008).
20) Yoshikawa M., Li X., Nishida E., Nakamura S., Matsuda H., Muraoka O., Morikawa T., *Chem. Pharm. Bull.*, **56**, 559—568 (2008).
21) Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **56**, 1297—1303 (2008).
22) Saito S., Ebashi J., Sumita S., Furumoto T., Nagamura Y., Nishida K., Isiguro I., *Chem. Pharm. Bull.*, **41**, 1395—1401 (1993).
23) Taketa A. T. C., Breitmaier E., Schenkel E. P., *J. Braz. Chem. Soc.*, **15**, 205—211 (2004).
24) Pereira S. I., Freire C. S. R., Neto C. P., Silvestre A. J. D., Silva A. M. S., *Phytochem. Anal.*, **16**, 364—369 (2005).
25) Gosmann G., Schenkel E. P., Seligmann O., *J. Nat. Prod.*, **52**, 1367—1370 (1989).
26) Gosmann G., Guillaume D., Taketa A. T. C., Schenkel E. P., *J. Nat. Prod.*, **58**, 438—441 (1995).
27) Kraemer K. H., Taketa A. T. C., Schenkel E. P., Gosmann G., Guillaume D., *Phytochemistry*, **42**, 1119—1122 (1996).
28) Taketa A. T. C., Breitmaier E., Schenkel E. R., *J. Braz. Chem. Soc.*, **15**, 201—211 (2004).
29) Pezzuto D. A., Fabio D., Piacente S., Pizza C., Villegas W., *J. Agric. Food Chem.*, **50**, 255—261 (2002).
30) Nishimura K., Miyase T., Noguchi H., *J. Nat. Prod.*, **62**, 1128—1133 (1999).
31) Kong J., Li X. C., Wei B. Y., Yang C. R., *Phytochemistry*, **33**, 427—430 (1993).
32) Watanabe N., Nakajima R., Watanabe S., Moon J. H., Inagaki J., Sakata K., Yagi A., Ina K., *Phytochemistry*, **37**, 457—459 (1994).
33) Wenkert E., Gottlieb H. E., *Phytochemistry*, **16**, 1811—1816 (1977).
34) Kazuma K., Noda N., Suzuki M., *Phytochemistry*, **62**, 229—237 (2003).
35) Nakatani N., Kayano S., Kikuzaki H., Sumino K., Katagiri K., Mitani T., *J. Agric. Food Chem.*, **48**, 5512—5516 (2000).
36) Yanuka Y., Bergmann F., *Tetrahedron*, **42**, 5991—6002 (1986).
37) Fujii T., Saito T., Tamura K., *Chem. Pharm. Bull.*, **39**, 2855—2862 (1991).
38) The ^{1}H- and ^{13}C-NMR spectra of **1**—**3** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) experiments.
39) Inada A., Yamada M., Murata H., Kobayashi M., Toya H., Kato Y., Nakanishi T., *Chem. Pharm. Bull.*, **36**, 4269—4272 (1988).
40) Ninomiya K., Matsuda H., Shimoda H., Nishida N., Kasajima N., Yoshino T., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **14**, 1943—1946 (2004).
41) Yoshikawa M., Sugimoto S., Kato Y., Nakamura S., Wang T., Yamashita C., Matsuda H., *Chem. Biodiv.*, **50**, (2008) in press.
42) Morikawa T., Xie Y., Asao Y., Okamoto M., Yamashita C., Muraoka O., Matsuda H., Pongpiriyadacha Y., Yuan D., Yoshikawa M., *J. Nat. Prod.*, in press.
43) Asao Y., Morikawa T., Xie Y., Okamoto M., Hamao M., Matsuda H., Muraoka O., Yuan D., Yoshikawa M., *Chem. Pharm. Bull.*, **57**, 198—203 (2009).