A New Amide, Piperchabamide F, and Two New Phenylpropanoid Glycosides, Piperchabaosides A and B, from the Fruit of *Piper chaba*

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A new amide, piperchabamide F (1), and two new phenylpropanoid glycosides, piperchabaosides A (2) and B (3), were isolated from 80% aqueous acetone extract from fruit of *Piper chaba*. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence.

Key words *Piper chaba*; piperchabamide; piperchabaoside; Piperaceae; Thai natural medicine

During the course of our studies on Thai natural medicines,[1—13] we found that the 80% aqueous acetone extract from the fruit of *Piper chaba* HUNTER (syn. *P. retrofractum* VAHL., Piperaceae) was found to show protective effects on ethanol- or indomethacin-induced gastric lesions in rats,[1] inhibitory effects on the increase in serum aspartate aminotransaminase (sAST) and alanine aminotransaminase (sALT) levels induced by D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced liver injury in mice and on cell death induced by D-GalN/tumor necrosis factor-α (TNF-α) in primary cultured mouse hepatocytes,[6,12] and promoting effect on adipogenesis of 3T3-L1 cells.[13] As a continuing study on the constituents from the fruit of *P. chaba*, we additionally isolated a new amide constituent named piperchabamide F (**1**) and two new phenylpropanoid glycosides, piperchabaosides A (**2**) and B (**3**), together with three known sesquiterpenes (**4**—**6**) and a known phenylpropanoid glycoside (**7**). This paper deals with the isolation and stereostructure elucidation of three new compounds (**1**—**3**) from the fruit of *P. chaba*.

The 80% aqueous acetone extract from the fruit of *P. chaba* (19.7%, purchased in Thailand) was partitioned between EtOAc–H_2O (1 : 1, v/v) to give EtOAc-soluble fraction (9.7%) and an aqueous phase.[1] The aqueous phase was further extracted with *n*-BuOH to give *n*-BuOH-soluble fraction (2.1%) and H_2O-soluble fraction (7.0%). The EtOAc-soluble fraction was subjected to normal- and reversed-phase silica gel column chromatographies and finally HPLC to furnish **1** (0.0007%) together with three sesquiterpenes, 1-hydroxybisabola-2,10-dien-4-one[14] (**4**, 0.0006%), 1,4-dihydroxybisabola-2,10-diene[15] (**5**, 0.0007%), and 3,4-dihydroxybisabola-1,10-diene[15—17] (**6**, 0.0013%). From the *n*-BuOH-soluble fraction, **2** (0.012%) and **3** (0.0019%) were purified together with a phenylpropanoid glycoside, rosin[18] (**7**, 0.0007%) using normal- and reversed-phase silica gel chromatographies and finally HPLC.

Structure of Piperchabamide F (1) Piperchabamide F (**1**) was obtained as colorless oil with positive optical rotation ($[\alpha]_D^{27}$ +7.1° in $CHCl_3$). The IR spectrum of **1** showed absorption bands at 2924, 1626, 1561, 1509, 1491, and 1258 cm^{-1} ascribable to methylene, unsaturated amide group, and aromatic ring. The electron ionization (EI)-MS of **1** showed a molecular ion peak at *m/z* 343 (M^+), and the molecular formula was determined as $C_{21}H_{29}NO_3$ by high-resolution EI-MS measurement. The ^{1}H- and ^{13}C-NMR spectra of **1** ($CDCl_3$, Table 1) showed signals assignable to two methyls [δ 0.91 (3H, dd, J=6.6, 7.4 Hz, 4′-H_3), 0.92 (3H, d, J=6.6 Hz, 5′-H_3)], six methylenes [δ 1.16, 1.40 (1H each, both m, 3′-H_2), 1.49, 2.19 (4H each, both m, 5, 6, 4, 7-H_2), 3.13, 3.27 (1H each, both m, 1′-H_2)], a methine [δ 1.59 (1H, m, 2′-H)], a 3,4-methylenedioxyphenyl group [δ 5.92 (2H, br s, 12-H_2), 6.72 (2H, br s, 14, 15-H), 6.88 (1H, br s, 11-H)], two *trans*-olefinic proton pairs [δ 5.75 (1H, dd, J=1.2, 15.4 Hz, 2-H), 6.00 (1H, dt, J=16.5, 6.7 Hz, 8-H), 6.29 (1H, d, J=16.5 Hz, 9-H), 6.81 (1H, dt, J=15.4, 7.1 Hz, 3-H)], and a conjugated amide group [δ_C 165.8 (C-1)]. The planar structure of **1** was constructed on the basis of various NMR experiments.[19] Namely, the ^{1}H–^{1}H correlation spectroscopy (COSY) experiments on **1** indicated the presence of three partials written in bold lines, while in the heteronuclear multiple

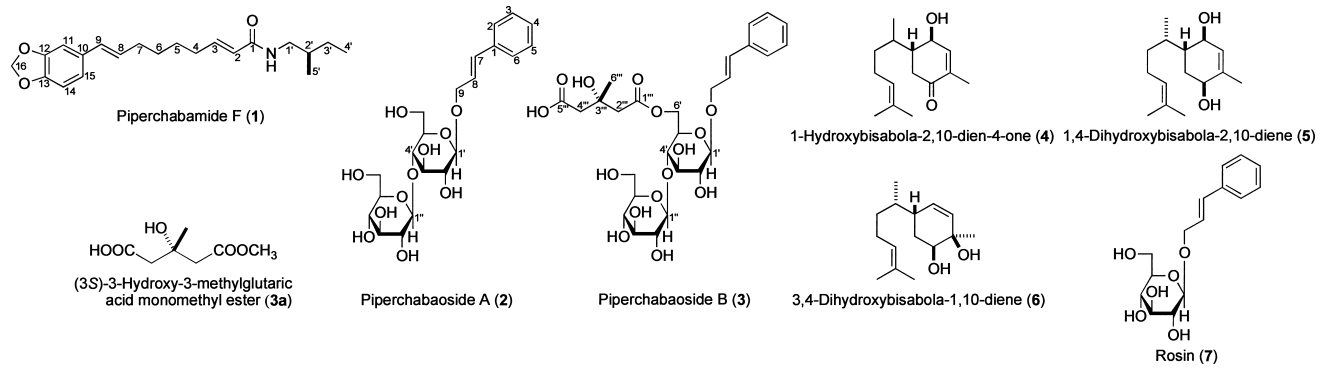

Chart 1

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bond connectivity (HMBC) experiments, long range correlations were observed between the following proton and carbon pairs: 2-H and 1-C; 8, 9-H and 10-C; 1′-H_2 and 1-C (Fig. 1). Finally, acid hydrolysis of **1** with 6 M HCl[12,20] liberated (*R*)-2-methylbutylamine,[21] which was identified by HPLC analysis using refractive index and optical rotation detectors. On the basis of above-mentioned evidence, the stereostructure of **1** was elucidated as shown.

Structures of Piperchabaosides A (2) and B (3) Piperchabaoside A (**2**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{23}$ −26.5° in MeOH). In the positive-ion fast atom bombardment (FAB)-MS of **2**, the quasimolecular ion peaks were observed at *m/z* 481 $(M+Na)^+$ and *m/z* 457 $(M-H)^-$, and the molecular formula $C_{21}H_{30}O_{11}$ was determined by high resolution FAB-MS measurement. The IR spectrum of **2** showed absorption bands at 3426, 1089, and 1030 cm^{-1} ascribable to hydroxyl and ether functions. Acid hydrolysis of **2** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis.[22—26] The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 2) spectra of **2** showed signals assignable to a methylene bearing an oxygen function [δ 4.43, 4.68 (1H each, both dd, *J*=5.8, 13.1 Hz, 9-H_2)], a *trans*-olefin pair [δ 6.45 (1H, dt, *J*=16.2, 5.8 Hz, 8-H), 6.76 (1H, d, *J*=16.2 Hz, 7-H)], and a monosubstituted benzene ring [δ 7.22 (1H, t, *J*=7.9 Hz, 4-H), 7.30 (2H, dd, *J*=7.3, 7.9 Hz, 3,5-H), 7.39 (2H, d, *J*=7.3 Hz, 2,6-H)] together with two β-D-glucopyranosyl parts [δ 4.88 (1H, d, *J*=7.6 Hz, 1′-H), 5.16 (1H, d, *J*=7.9 Hz, 1″-H)], which were superimposable on those of rosin (**7**), except for the signals due to an additional β-D-glucopyranosyl unit. Finally, the connectivities of the glycosyl linkages in **2** were elucidated on the basis of HMBC experiment, which showed long-range correlations between the following proton and carbon pairs as shown in Fig. 1: the *inner*-Glc-1-proton (1′-H) and the 9-carbon (δ_C 69.8), and the *terminal*-Glc-1-proton (1″-H) and the *inner*-Glc-4-carbon (δ_C 81.2, 4'-C). Consequently, piperchabaoside A was determined to be *trans*-cinnamyl alcohol *O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**2**).

Piperchabaoside B (**3**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −18.0°, MeOH). Its molecular formula $C_{27}H_{38}O_{15}$ was determined from the positive- and negative-ion FAB-MS and by high resolution FAB-MS measurements. The IR spectrum of **3** showed absorption bands at 3401, 1736, 1719, 1075, and 1040 cm^{-1} assignable to hydroxyl, ester carbonyl, carboxyl, and ether functions. Treatment of **3** with 1.0% sodium methoxide (NaOMe)–MeOH provided **2** together with (*S*)-3-hydroxy-3-methylglutaric acid monomethyl ester[27] (**3a**). The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 2) spectra of **3** were similar to those of **2**, except for the signals due to an acyl part [δ 1.74 (3H, s, 6‴-H_3), 3.13 (4H, m, 2‴,4‴-H_2)]. The connectivities of the acyl moiety and glycosyl linkages in **3** were elucidated on the basis of HMBC experiment, which showed long-range correlations were observed between following proton and carbon pairs as shown in Fig. 1: the *inner*-Glc-1-proton [δ 4.86 (1H, d, *J*=7.6 Hz, 1′-H)] and the 9-carbon (δ_C 69.9), the *terminal*-Glc-1-proton [δ 5.10 (1H, d, *J*=7.9 Hz, 1″-H)] and the *inner*-Glc-4-carbon (δ_C 81.6, 4′-C), and the *inner*-Glc-6-protons [δ 4.49 (1H, dd, *J*= 6.1, 12.8 Hz), 5.15 (1H, br d, *J*=*ca.* 13 Hz), 6′-H_2] and the acyl ester carbonyl carbon (δ_C 171.6, 1‴-C). Thus, the structure of piperchabaoside B was constructed as *trans*-cinnamyl alcohol *O*-β-D-glucopyranosyl-(1→4)-6-*O*-(*S*)-3-hydroxy-3-methylglutaroyl-β-D-glucopyranoside (**3**).

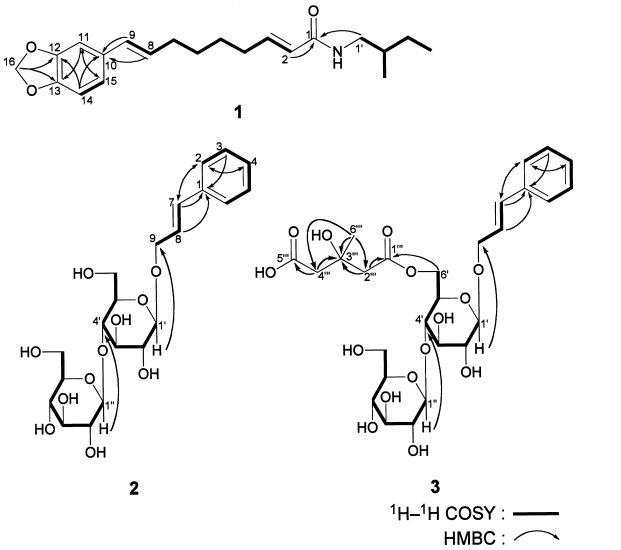

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**—**3**

Table 1. ^{1}H- and ^{13}C-NMR Data of Piperchabamide F (**1**)

Position	**1**	
	δ_H (*J* Hz)	δ_C
1		165.8
2	5.75 (dd, 1.2, 15.4)	123.5
3	6.81 (dt, 15.4, 7.1)	144.2
4	2.19 (2H, m)	31.8
5	1.49 (2H, m)	27.7[a]
6	1.49 (2H, m)	28.9[a]
7	2.19 (2H, m)	32.6
8	6.00 (dt, 16.5, 6.7)	128.6
9	6.29 (d, 16.5)	129.4
10		132.1
11	6.88 (br s)	105.2
12		147.7
13		146.3
14	6.72 (br s)	108.0
15	6.72 (br s)	120.0
16	5.92 (2H, br s)	100.8
1′	3.13, 3.27 (both m)	45.0
2′	1.59 (m)	34.9
3′	1.16, 1.40 (both m)	27.0
4′	0.91 (3H, dd, 6.6, 7.4)	17.2
5′	0.92 (3H, d, 6.6)	11.3

Measured in $CDCl_3$. *a*) May be interchangeable.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (*l*=5 cm); UV spectra, Shimazdu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H-NMR spectra, JEOL JNM-LA500 (500 MHz) and EX-270 (270 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-LA500 (125 MHz) and EX-270 (68 MHz) spectrometers with tetramethylsilane as an internal standard; EI-MS and high-resolution EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-10A refractive index, Shimadzu SPD-10A UV–VIS, and Shodex OR-2 optical rotation detectors. HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 250×4.6 mm

Table 2. ^{1}H- and ^{13}C-NMR Data of Piperchabaosides A (**2**) and B (**3**)

Position	**2**		**3**	
	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C
1		137.4		137.4
2,6	7.39 (2H, d, 7.3)	126.9	7.38 (2H, d, 7.9)	126.9
3,5	7.30 (2H, dd, 7.3, 7.9)	129.0	7.28 (2H, dd, 7.3, 7.9)	128.9
4	7.22 (t, 7.9)	127.9	7.21 (t, 7.3)	127.9
7	6.76 (d, 16.2)	132.2	6.77 (d, 16.2)	132.4
8	6.45 (dt, 16.2, 5.8)	126.8	6.44 (dt, 16.2, 6.1)	126.9
9	4.43, 4.68 (both dd, 5.8, 13.1)	69.8	4.45, 4.72 (both dd, 6.1, 12.8)	69.9
1′	4.88 (d, 7.6)	103.6	4.86 (d, 7.6)	103.5
2′	4.09 (m)	74.8	4.09 (m)	74.5
3′	4.24 (m)	76.8	4.21 (m)	76.6
4′	4.31 (m)	81.2	4.18 (m)	81.6
5′	3.87 (m)	76.5	4.03 (m)	73.5
6′	4.44, 4.52 (both m)	62.1	4.49 (dd, 6.1, 12.8) 5.15 (br d, *ca.* 13)	64.1
1″	5.16 (d, 7.9)	104.9	5.10 (d, 7.9)	105.2
2″	4.06 (m)	74.8	4.03 (m)	74.9
3″	3.99 (m)	78.4	4.18 (m)	78.4
4″	4.15 (dd, 8.3, 8.5)	71.6	4.09 (m)	71.9
5″	4.19 (m)	78.2	4.08 (m)	78.5
6″	4.29, 4.49 (both m)	62.5	4.24 (m) 4.54 (br d, *ca.* 12)	62.8
1‴				171.6
2‴			3.13 (2H, m)	46.8
3‴				70.1
4‴			3.13 (2H, m)	46.5
5‴				174.9
6‴			1.74 (3H, s)	28.3

Measured in pyridine-d_5.

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: normal-phase silica gel column chromatography (CC), silica gel 60N (Kanto Chemical Co., Ltd., 63—210 mesh, spherical, neutral); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); normal-phase TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with silica gel RP-18 F_{254S} (Merck, 0.25 mm); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm), detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material This item was described in a previous report.[1)]

Extraction and Isolation The dried fruit of *P. chaba* (4.0 kg) was finely cut and extracted four times with 80% (v/v) aqueous acetone at room temperature for 1 d. Evaporation of the solvent under reduced pressure provided an 80% aqueous acetone extract (788 g, 19.7% from the dried fruit). The 80% aqueous acetone extract (463 g) was partitioned in an EtOAc–H_2O (1 : 1, v/v) mixture. The aqueous phase was further extracted with *n*-BuOH. Removal of the solvent under reduced pressure yielded EtOAc-, *n*-BuOH-, and H_2O-soluble fractions [227 g (9.7%), 50 g (2.1%), and 164 g (7.0%)], respectively. Fractions 4-2 (0.21 g), 5-2 (0.62 g), and 6-2 (0.07 g) were obtained from the EtOAc-soluble fraction (118 g) as described previously.[1,12)] The fraction 4-2 (0.21 g) was subjected to preparative HPLC [CH_3CN–H_2O (50 : 50, v/v)] to give 1-hydroxybisabola-2,10-dien-4-one (**4**, 7 mg, 0.0006%) together with piperchabamide A (27 mg) and *N*-isobutyl-(2*E*,4*E*)-deca-2,4-dienamide (27 mg). The fraction 5-2 (0.62 g) was subjected to HPLC [CH_3CN–H_2O (70 : 30, v/v)] to give piperchabamide F (**1**, 9 mg, 0.0007%) together with piperchabamide D (20 mg), pipercide (=retrofractamide B, 100 mg), and guineensine (292 mg). The fraction 6-2 (0.07 g) was further purified by HPLC [CH_3CN–H_2O (45 : 55, v/v)] to give 1,4-dihydroxybisabola-2,10-diene (**5**, 9 mg, 0.0007%) and 3,4-dihydroxybisabola-1,10-diene (**6**, 16 mg, 0.0013%).

The *n*-BuOH-soluble fraction (40.5 g) was subjected to normal-phase silica gel CC [1.5 kg, $CHCl_3$–MeOH–H_2O (30 : 3 : 1→15 : 3 : 1→10 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give six fractions [Fr. 1 (3.3 g), Fr. 2 (2.4 g), Fr. 3 (4.3 g), Fr. 4 (1.7 g), Fr. 5 (0.8 g), Fr. 6 (25.7 g)]. The fraction 2 (2.4 g) was subjected to reversed-phase silica gel CC [120 g, MeOH–H_2O (20 : 80→40 : 60→80 : 20, v/v)→MeOH] to give three fractions [Fr. 2-1 (2.05 g), 2-2 (0.20 g), 2-3 (0.07 g)]. The fraction 2-2 (0.20 g) was further purified by HPLC [MeOH–H_2O (20 : 80, v/v)] to give rosin (**7**, 13 mg, 0.0007%). The fraction 4 (1.7 g) was subjected to reversed-phase silica gel CC [50 g, MeOH–H_2O (20 : 80→40 : 60, v/v)→MeOH] to give three fractions [Fr. 4-1 (0.67 g), 4-2 (0.57 g), 4-3 (0.17 g)]. The fraction 4-2 (0.57 g) was further purified by HPLC [MeOH–H_2O (10 : 90, v/v)] to give piperchabaosides A (**2**, 54 mg, 0.0028%) and B (**3**, 36 mg, 0.0019%). The fraction 5 (0.8 g) was purified by HPLC [MeOH–H_2O (10 : 90, v/v)] to give **2** (177 mg, 0.0092%).

Piperchabamide F (**1**): Colorless oil, $[\alpha]_D^{25}$ +7.1° (c=0.27, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{21}H_{29}NO_3$ (M^+) 343.2147; Found 343.2152. UV [λ_{max} (log ε), EtOH]: 260 (4.1) nm. IR (KBr, cm^{-1}): 2924, 1626, 1561, 1509, 1491, 1258, 1043, 965, 924. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: given in Table 1. ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS (%): *m*/*z* 343 (M^+, 41), 135 (100).

Piperchabaoside A (**2**): A white powder, $[\alpha]_D^{23}$ −26.5° (c=2.40, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{30}O_{11}Na$ $(M+Na)^+$ 481.1686; Found 481.1678. UV [λ_{max} (log ε), MeOH]: 251 (4.5) nm. IR (KBr, cm^{-1}): 3426, 1089, 1030. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m*/*z*: 481 $(M+Na)^+$. Negative-ion FAB-MS *m*/*z*: 457 $(M-H)^-$.

Piperchabaoside B (**3**): A white powder, $[\alpha]_D^{26}$ −18.0° (c=3.21, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{27}H_{38}O_{15}Na$ $(M+Na)^+$ 625.2108; Found 625.2115. UV [λ_{max} (log ε), MeOH]: 251 (4.3) nm. IR (KBr, cm^{-1}): 3401, 1736, 1719, 1075, 1040. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS *m*/*z*: 625 $(M+Na)^+$. Negative-ion FAB-MS *m*/*z*: 601 $(M-H)^-$.

Acid Hydrolysis of Piperchabamide F (1) A solution of **1** (5 mg) in 6 M HCl (0.5 ml) was heated at 110 °C for over night in a sealed tube. After cooling, the reaction mixture was basified (pH 10) with aq. NaOH and then the mixture was extracted with *n*-hexane. The *n*-hexane phase was evaporated *in vacuo* gave a residue, which was subjected to HPLC analysis [column: Cosmosil 5C_{18}-MS-II, 250×4.6 mm i.d.; mobile phase: MeOH–H_2O (50 : 50, v/v); detection: RI and OR; flow rate: 1.0 ml/min] to identify (*R*)-2-

methylbutylamine [t_R 9.1 min (positive)].

Acid Hydrolysis of Piperchabaoside A (2) A solution of **2** (3.0 mg) in 1 M HCl (1.0 ml) was heated at 80 °C for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and then the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure, the residue was separated by Sep-Pak C18 cartridge column ($H_2O \rightarrow MeOH$). The H_2O-eluted fraction was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 250×4.6 mm i.d. (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate 0.8 ml/min]. Identification of D-glucose (**i**) present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R: (i) 13.9 min (positive optical rotation).

Deacylation of Piperchabaoside B (3) A solution of **3** (20.2 mg) in 1.0% sodium methoxide (NaOMe)–MeOH (3.0 ml) was stirred at room temperature for 3 h. The 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 gave a residue, which was purified by HPLC [Cosmosil 5C_{18}-MS-II, MeOH–H_2O (40 : 60, v/v)] to furnish **2** (12.0 mg) and (*S*)-3-hydroxy-3-methylglutaric acid monomethyl ester[27] (**3a**, 1.6 mg).

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