HETEROCYCLES, Vol. 68, No. 11, 2006, pp. 2335 - 2342. © The Japan Institute of Heterocyclic Chemistry
Received, 7th August, 2006, Accepted, 11th September, 2006, Published online, 12th September, 2006. COM-06-10858

MEDICINAL FOODSTUFFS. XXXII.[1] NOVEL SESQUITERPENE GLYCOSIDE SULFATE, FUKINOSIDE A, WITH ANTIALLERGIC ACTIVITY FROM JAPANESE BUTTERBUR (*PETASITES JAPONICUS*)

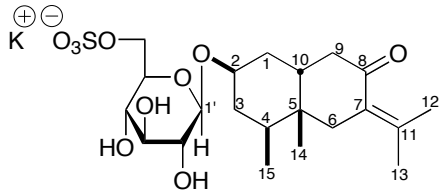
Masayuki Yoshikawa,*,[a)] Toshio Morikawa,[a,b)] Junji Tanaka,[c)]
and Hiroshi Shimoda[c)]

a) Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan
b) Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan
c) Research and Development Division, Oryza Oil & Fat Chemical Company, Ltd., 1 Numata Kitagata-cho, Ichinomiya, Aichi 493-8001, Japan

Abstract — Novel sesquiterpene glycoside sulfate, fukinoside A, was isolated as the potassium salt from the aerial parts of *Petasites japonicus*. The absolute sterostructure of fukinoside A was elucidated on the basis of chemical and physicochemical evidence. In addition, fukinoside A was found to inhibit release of β-hexosaminidase, as a marker of antigen-induced degranulation, in RBL-2H3 cells.

The Compositae plant *Petasites* (*P.*) *japonicus* MAXIM. (Japanese butterbur in English, Fuki in Japanese) has been cultivated as a vegetable in Japan. The fresh stems of *P. japonicus* have been used as a food garnish in Japanese-style dishes. On the other hand, the rhizomes of this plant have been used for the treatment of tonsillitis, contusions, and poisonous-snake bite in China.[2] In previous studies, several sesquiterpenes, triterpenes, anthraquinones, and phenolic compounds were isolated from the rhizomes of *P. japonicus*.[3–14] In the course of our characterization studies on the bioactive constituents in medicinal foodstuffs,[1,15–19] we have reported that the 70% aqueous ethanol extract from the dried aerial parts of *P. japonicus* was found to show an anti-allergic effect.[20] As a continuing study on this herbal medicine, we have isolated a novel eremophilane-type sesquiterpene glycoside sulfate named fukinoside A as the potassium salt (**1**). In addition, **1** was found to show inhibitory effect on the release of β-hexosaminidase in RBL-2H3 cells. This paper deals with the absolute stereostructure elucidation of **1** as well as the inhibitory effect of **1** on the release of β-hexosaminidase in RBL-2H3 cells.

The dried aerial parts of *P. japonicus* (cultivated in Aichi prefecture, Japan) were extracted with 70% aqueous ethanol (EtOH) at 70 ºC for 2 h to give an aqueous EtOH extract (12.4% from this herbal medicine). The aqueous ethanolic extract was partitioned in an ethyl acetate (EtOAc)/water mixture to



fukinoside A potassium (**1**)

give an EtOAc-soluble fraction (1.0%) and an aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH and H_2O-soluble fractions (0.7, 9.4%, respectively). In our previous report, 2β-hydroxyfukinone (**2**), (+)-fukinone, fukinolic acid, chlorogenic acid, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid methyl ester, caffeic acid, dotorioside II, and mussaendoside R were isolated from this extract.[20] Continuing the isolation study for this herbal medicine, the *n*-BuOH-soluble fraction was subjected to silica gel and ODS column chromatography and finally HPLC to furnish fukinoside A potassium (**1**, 0.0005%).

Absolute Stereostructure of 1

Fukinoside A was isolated as the potassium salt with positive optical rotation ($[\alpha]_D^{26}$ +12.4°, in MeOH). In the positive-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 539 $(M+Na)^+$ and *m/z* 555 $(M+K)^+$. On the other hand, quasimolecular ion peaks were observed at *m/z* 477 $(M-K)^-$ and *m/z* 515 $(M-H)^-$ in the negative-ion FAB-MS. High-resolution MS analysis of quasimolecular ion peaks in the positive-ion FAB-MS revealed the molecular formula of **1** to be $C_{21}H_{33}KO_{10}S$, so that the presence of a potassium sulfate function in **1** was confirmed.[21] The IR spectrum of **1** showed absorption bands at 3454, 1684, 1256, 1065, and 1042 cm^{-1} ascribable to hydroxyl, α,β-unsaturated carbonyl, sulfate, and ether functions, while its UV spectrum indicated the presence of enone chromophore with absorption maximum at 251 (log ε 3.79) nm. Solvolysis[22,23] of **1** with pyridine–1,4-dioxane (4:1, v/v) gave **1a** as shown in Figure 2. Acid hydrolysis of **1a** with 1 M hydrochloric acid (HCl) liberated 2β-hydroxyfukinone (**2**)[24] as an aglycone, whose absolute configuration was left uncharacterized, together with D-glucose, which was identified by HPLC analysis using an optical rotation detector.[18,19] The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[25] showed signals assignable to four methyls [δ 0.91 (3H, d, J = 6.7 Hz, 15-H_3), 1.00 (3H, s, 14-H_3), 1.83 (3H, s, 13-H_3),1.92 (3H, d, J = 1.8 Hz, 12-H_3)], four methylenes [δ 1.35, 1.90 (1H each, both m, 3β-H and 3α-H), 1.78 (2H, m, 1-H_2), 2.07, 2.73 (1H each, both d, J = 15.0 Hz, 6β-H and 6α-H), 2.31 (1H, dd, J = 5.5, 16.5 Hz, 9α-H), 2.59 (1H, dd, J = 12.8, 16.5 Hz, 9β-H)], two methines and a methine bearing an oxygen function [δ 1.78 (1H, m, 4-H), 2.02 (1H, m, 10-H), 3.97 (1H, m, 2-H)], and four quaternary carbons (5, 7, 8, and 11-C) together with a hexose moiety [δ 4.15 (1H, dd, J = 5.5, 11.0 Hz), 4.29 (1H, dd, J = 1.8, 11.0 Hz), 6'-H_2], 4.40 (1H, d, J = 7.6 Hz, 1'-H)].

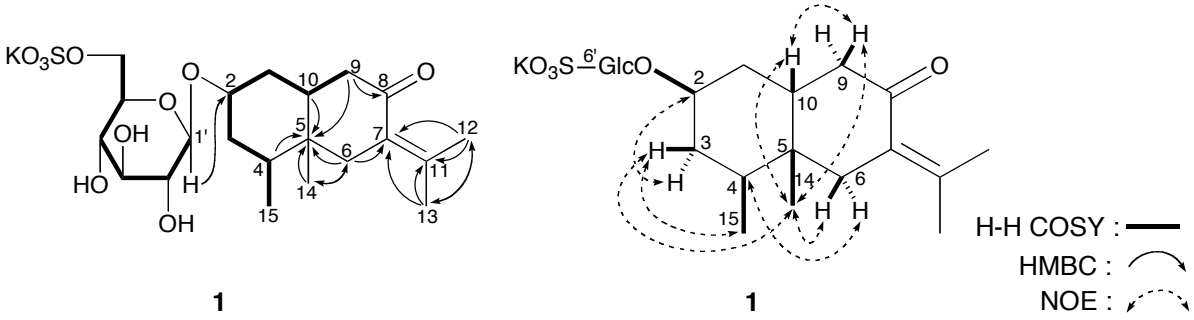


Figure 1. H–H COSY, HMBC, and NOE correlations of **1**

The proton and carbon signals due to the 6'-position in the ^{1}H-NMR and ^{13}C-NMR spectra of **1** were observed at lower fields compared to those of **1a** {δ [3.63 (1H, dd, J = 5.8, 11.9 Hz), 3.85 (1H, dd, J = 1.8, 11.9 Hz), 6'-H_2]; δ_C 62.9 (6'-C)}, so that the position of the potassium sulfate function in **1** was clarified to be the 6'-position. The eremophil-7(11)-en-8-one type sesquiterpene structure of **1** was constructed on the basis of ^{1}H—^{1}H correlation spectroscopy (^{1}H—^{1}H COSY) and heteronuclear multiple bond correlation (HMBC) experiments (Figure 1). Thus, the ^{1}H—^{1}H COSY experiment on **1** indicated the presence of partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons (1'-H and 2-C; 4-H, 6-H_2, 9-H_2, 10-H, 14-H_3 and 5-C; 14-H_3 and 6-C; 6-H_2, 12-H_3, 13-H_3 and 7-C; 9-H_2 and 8-C, 12-H_3, 13-H_3 and 11-C; 6-H_2 and 14-C). Furthermore, the stereostructure of **1** was characterized on the basis of the nuclear Overhauser enhancement spectroscopy (NOESY) experiment, in which NOE correlations were observed between the following proton pairs of **1** (2-H and 3α-H; 3β-H and 14-H_3, 15-H_3; 4-H and 6α-H; 6β-H and 14-H_3; 9β-H and 10-H, 14-H_3; 10-H and 14-H_3).

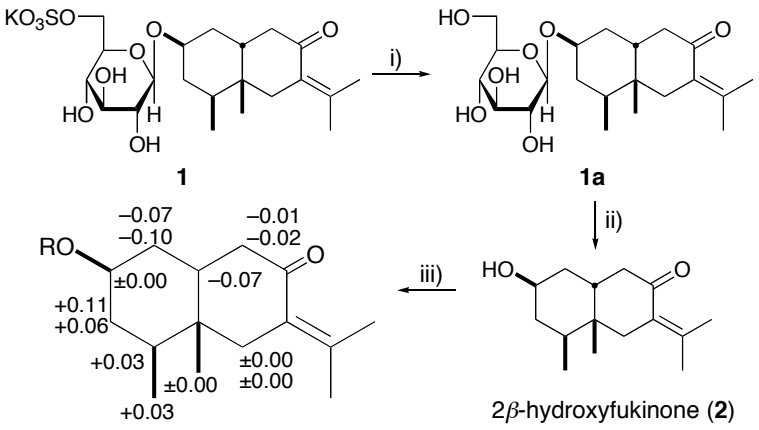


Figure 2.

Table 1. ^{13}C-NMR Data for **1**, **1a**, and **2**

	1[a]	**1a**[a]	**2**[b]	**2**[c]
C-1	35.0	35.1	36.0	37.0
C-2	75.0	74.2	66.4	65.4
C-3	37.1	37.1	39.8	41.0
C-4	31.9	31.8	31.0	31.2
C-5	37.6	37.7	36.4	36.7
C-6	41.2	41.3	40.3	40.6
C-7	131.8	131.9	130.5	131.4
C-8	207.7	207.8	205.0	204.2
C-9	45.3	45.4	44.4	45.0
C-10	43.9	44.0	42.5	43.0
C-11	142.0	142.1	140.9	139.2
C-12	22.7	22.7	22.6	22.5
C-13	21.9	21.8	21.7	21.4
C-14	21.1	21.2	20.9	21.0
C-15	16.7	16.8	16.2	16.5
C-1'	102.5	102.2		
C-2'	74.9	75.2		
C-3'	77.7	77.9		
C-4'	71.4	71.8		
C-5'	75.7	78.1		
C-6'	68.2	62.9		

Measured in [a]CD_3OD, [b]$CDCl_3$, and [c]pryidine-d_5 (125 MHz).

Finally, the absolute configurations of **1** and **2** were characterized by the application of the modified Mosher's method.[26] Namely, treatment of **2** with (*R*)- or (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) yielded the MTPA esters (**2a**, **2b**), respectively, whose ^{1}H-NMR data showed an acylation shift at the 2-position in **2**. As shown in Figure 2, the signals due to protons attached to the 3, 4, and 15-positions in the 2-(*S*)-MTPA ester (**2b**) were observed at lower fields compared with those of the 2-(*R*)-MTPA ester (**2a**) [$\Delta\delta$: positive], while the signals due to protons on the 1, 9, and 10-positions in **2b** were observed at higher fields compared with those of **2a** [$\Delta\delta$: negative].

Consequently, the absolute configuration at the 2-position of **2** was determined as *R* configuration and the absolute stereostructures of **1** and **2** were elucidated to be as shown.

Inhibitory Effect of 1 on the Release of β-Hexosaminidase in RBL-2H3 Cells

Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker *in vitro* experiments on immediate allergic reactions. β-Hexosaminidase is also stored in the secretory granules of mast cells and is released concomitantly with histamine when mast cells are immunologically activated.[27,28] Therefore it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. As a part of our characterization studies on the bioactive components of natural medicines, we previously reported several inhibitors of the release of β-hexosaminidase such as diarylheptanoids,[19,29,30] sesquiterpenes,[31] diterpenes,[32] flavonoids,[33] anthraquinones,[34] stilbenes,[35] phenanthrenes,[35] phenylpropanoids,[36] and alkaloids.[37,38] In our previous report, several constituents from *P. japonicus* showed inhibitory activities on the release of β-hexosaminidase in RBL-2H3 cells.[20] Compound (**1**) (IC_{50} = 16.6 μM) was also inhibited on the release of β-hexosaminidase and this activity was stronger than that of antiallergic compounds, tranilast[36] (IC_{50} = 282 μM) and ketotifen fumarate[36] (IC_{50} = 158 μM).

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, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV-VIS detectors; HPLC column, GL Science Inertsil ODS-3 (250 × 4.6 mm i.d.) and (250 × 10 mm i.d.) columns were used for analytical and preparative purposes, respectively.

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 60F_{254} (Merck, 0.25 mm) (normal-phase) and 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) (reversed-phase). Detection was done by spraying with 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4, followed by heating.

Extraction and Isolation

The dried aerial parts of *P. japonicus* (cultivated in Aichi prefecture, Japan) were extracted with 70% aqueous EtOH at 70 ºC for 2 h to give an aqueous EtOH extract (12.4% from this herbal medicine). The aqueous EtOH extract was partitioned in an EtOAc/water mixture to give an EtOAc-soluble fraction (1.0%) and aqueous layer. The aqueous layer was extracted with *n*-BuOH to give *n*-BuOH and H_2O-soluble fractions (0.7, 9.4%, respectively), which were described previously.[20] The *n*-BuOH-soluble

fraction (17.0 g) was to reversed-phase ODS column chromatography [300 g, MeOH–H_2O (15:85 → 70:30) → MeOH] to afford 16 fractions [fr. 1 (3.47 g), fr. 2 (1.85 g), fr. 3 (0.90 g),fr. 4 (0.95 g), fr. 5 (0.41 g), fr. 6 (0.25 g), fr. 7 (0.39 g), fr. 8 (0.48 g), fr. 9 (0.33 g), fr. 10 (0.40 g), fr. 11 (0.27 g), fr. 12 (0.26 g), fr. 13 (0.30 g), fr. 14 (0.29 g), fr. 15 (0.06 g), and fr. 16 (0.26 g)]. Fraction 4 (0.95 g) was further purified by HLPC [Inertsil ODS-3 (GL Science), MeOH–H_2O (30:70, v/v)] to give **1** (12 mg, 0.0005%).

Fukinoside A potassium (**1**): A white powder, $[\alpha]_D^{26}$ +12.4° (*c*=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{33}KO_{10}SNa$ $(M+Na)^+$: 539.1330. Found: 539.1326; Calcd for $C_{21}H_{33}KO_{10}SK$ $(M+K)^+$: 555.1069. Found: 555.1063. UV [nm (log ε), MeOH]: 251 (3.79). IR (KBr): 3454, 1684, 1256, 1065, 1042 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 0.91 (3H, d, *J* = 6.7 Hz, 15-H_3), 1.00 (3H, s, 14-H_3), 1.35 (1H, m, 3β-H), 1.78 (3H, m, 1-H_2 and 4-H), 1.83 (3H, s, 13-H_3), 1.90 (1H, m, 3α-H), 1.92 (3H, d, *J* = 1.8 Hz, 12-H_3), 2.02 (1H, m, 10-H), 2.07, 2.73 (1H each, both d, *J* = 15.0 Hz, 6β-H and 6α-H), 2.31 (1H, dd, *J* = 5.5, 16.5 Hz, 9α-H), 2.59 (1H, dd, *J* = 12.8, 16.5 Hz, 9β-H), 3.16 (1H, dd, *J* = 7.6, 8.8 Hz, 2'-H), 3.35 (1H, dd, *J* = 7.9, 8.6 Hz, 4'-H), 3.37 (1H, dd, *J* = 8.6, 8.8 Hz, 3'-H), 3.48 (1H, m, 5'-H), 3.97 (1H, m, 2-H), [4.15 (1H, dd, *J* = 5.5, 11.0 Hz), 4.29 (1H, dd, *J* = 1.8, 11.0 Hz), 6'-H_2], 4.40 (1H, d, *J* = 7.6 Hz, 1'-H). ^{13}C-NMR (CD_3OD, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 539 $(M+Na)^+$, 555 $(M+K)^+$. Negative-ion FAB-MS: *m/z* 477 $(M–K)^–$, 515 $(M–H)^–$.

Solvolysis of 1

A solution of **1** (10.0 mg) in pyridine–1,4-dioxane (4:1, v/v, 3.0 mL) was heated at 80 °C for 1 h. After removal of the solvent under reduced pressure, the residue was subjected to HPLC [MeOH–H_2O (50:50, v/v)] to furnish **1a** (6.5 mg, 84%).

1a: A white powder, $[\alpha]_D^{17}$ –4.0° (*c*=0.23, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{34}O_7Na$ $(M+Na)^+$: 421.2202. Found: 421.2208. IR (KBr): 3415, 1684, 1076 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz) δ: 0.91 (3H, d, *J* = 6.7 Hz, 15-H_3), 1.01 (3H, s, 14-H_3), 1.33 (1H, m, 3β-H), 1.72, 1.80 (1H each, both m, 1-H_2), 1.80 (1H, m, 4-H), 1.83 (3H, s, 13-H_3), 1.90 (3H, s, 12-H_3), 1.93 (1H, m, 3α-H), 2.02 (1H, m, 10-H), 2.06, 2.76 (1H each, both d, *J* = 14.9 Hz, 6β-H and 6α-H), 2.27 (1H, dd, *J* = 5.5, 16.5 Hz, 9α-H), 2.61 (1H, dd, *J* = 12.5, 16.5 Hz, 9β-H), 3.12 (1H, dd, *J* = 8.0, 9.1 Hz, 2'-H), 3.24 (1H, m, 5'-H), 3.32 (1H, m, 4'-H), 3.35 (1H, m, 3'-H), [3.63 (1H, dd, *J* = 5.8, 11.9 Hz), 3.85 (1H, dd, *J* = 1.8, 11.9 Hz), 6'-H_2], 4.02 (1H, m, 2-H), 4.38 (1H, d, *J* = 8.0 Hz, 1'-H). ^{13}C-NMR (CD_3OD, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 421 $(M+Na)^+$.

Acid Hydrolysis of 1a

A solution of **1a** (6.0 mg) in 1 M HCl (0.5 mL) was heated under reflux for 3 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. Then, the filtrate was extracted with EtOAc. 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, CH_3CN–H_2O (75:25, v/v); flow rate 0.8 mL/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of an authentic sample. t_R: 12.3 min (positive optical rotation). The EtOAc layer was evaporated *in vacuo* gave the residue, which was purified by HPLC [MeOH–H_2O (85:15, v/v)] to give 2β-hydroxyfukinone (**2**, 3.0 mg, 84%).

2β-Hydroxyfukinone (**2**): ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.89 (3H, d, J = 6.4 Hz, 15-H_3), 0.99 (3H, s, 14-H_3), 1.31 (1H, m, 3β-H), 1.68 (2H, m, 1-H_2), 1.78 (1H, m, 3α-H), 1.81 (1H, m, 4-H), 1.81 (3H, s, 13-H_3), 1.94 (3H, d, J = 1.8 Hz, 12-H_3), 2.03 (1H, m, 10-H), 2.07, 2.67 (1H each, both d, J = 15.9 Hz, 6β-H and 6α-H), 2.31 (1H, dd, J = 5.5, 16.5 Hz, 9α-H), 2.49 (1H, dd, J = 12.8, 16.5 Hz, 9β-H), 3.89 (1H, m, 2-H). ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.81 (3H, d, J = 6.7 Hz, 15-H_3), 0.91 (3H, s, 14-H_3), 1.56 (1H, br dd, J = *ca.* 11, 13 Hz, 3β-H), 1.71 (3H, d, J = 0.9 Hz, 13-H_3), 1.75 (1H, m, 4-H), 1.82, 1.93 (1H each, both m, 1-H_2), 1.91 (1H, m, 3α-H), 1.91 (1H, m, 10-H), 1.97 (1H, d-like, 6β-H), 2.06 (3H, d, J = 1.9 Hz, 12-H_3), 2.61 (1H, d, J = 15.6 Hz, 6α-H), 2.38 (1H, dd, J = 5.2, 16.2 Hz, 9α-H), 2.65 (1H, dd, J = 12.5, 16.2 Hz, 9β-H), 4.09 (1H, m, 2-H). ^{13}C-NMR ($CDCl_3$ and pyridine-d_5, 125 MHz) δc: given in Table 1.

Preparation of the (*R*)-MTPA Ester (2a) and (*S*)-MTPA Ester (2b) from 2

A solution of **2** (2.0 mg) in CH_2Cl_2 (0.5 mL) was treated with (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid [(*R*)-MTPA, 9.9 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 8.1 mg) and 4-dimethylaminopyridine (4-DMAP, 3.1 mg), and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole 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 separated by ordinary-phase silica-gel column chromatography [*n*-hexane–EtOAc (10:1, v/v)] to give **2a** (1.9 mg, 49%). Using a similar procedure, (*S*)-MTPA esters [**2b** (2.3 mg, 60%)] was obtained from **2** (2.0 mg), using (*S*)-MTPA (9.9 mg), EDC·HCl (8.1 mg), and 4-DMAP (3.1 mg).

2a: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.87 (3H, d, J = 6.8 Hz, 15-H_3), 0.98 (3H, s, 14-H_3), 1.40, 1.88 (1H each, both m, 3-H_2), 1.74, 1.88 (1H each, both m, 1-H_2), 1.81 (3H, s, 13-H_3), 1.88 (1H, m, 4-H), 1.95 (3H, d, J = 1.6 Hz, 12-H_3), [2.07 (1H, d-like), 2.69 (1H, d, J = 15.5 Hz), 6-H_2], 2.10 (1H, m, 10-H), [2.38 (1H, dd, J = 4.8, 16.1 Hz), 2.56 (1H, dd, J = 12.2, 16.1 Hz), 9-H_2], 3.55 (3H, s, –OCH_3), 5.23 (1H, m, 2-H), [7.41 (3H, m), 7.51 (2H, m), Ph-H].

2b: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.90 (3H, d, J = 6.7 Hz, 15-H_3), 0.98 (3H, s, 14-H_3), 1.51, 1.94 (1H each, both m, 3-H_2), 1.67, 1.78 (1H each, both m, 1-H_2), 1.81 (3H, s, 13-H_3), 1.91 (1H, m, 4-H), 1.95 (3H, d, J = 1.8 Hz, 12-H_3), [2.07 (1H, d-like), 2.69 (1H, d, J = 15.3 Hz), 6-H_2], 2.03 (1H, m, 10-H), [2.37 (1H, dd, J = 5.8, 16.5 Hz), 2.54 (1H, dd, J = 12.5, 16.5 Hz), 9-H_2], 3.55 (3H, s, –OCH_3), 5.23 (1H, m, 2-H), [7.41 (3H, m), 7.51 (2H, m), Ph-H].

Bioassay

Inhibitory effect on the release of *β*-hexosaminidase in RBL-2H3 cells was assayed by the method described in a previous paper.[20]

ACKNOWLEDGMENTS

This research was supported by the 21st COE program, Academic Frontier Project, and Grand-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the Food Technology Development Office, Food Industry Policy Division, General Food Policy Bureau, Ministry of Agriculture, Forestry and Fisheries in Japan.

REFERENCES AND NOTES

1 Part XXXI: T. Morikawa, J. Tao, K. Ueda, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2003, **51**, 62.
2 Shanghai Scientific and Technologic Publishers Ed, 'Dictionary of Chinese Traditional Medicines', Shogakkan, Tokyo, 1985, 2386.
3 Y. Yaoita, K. Nagata, N. Suzuki, and M. Kikuchi, *Chem. Pharm. Bull.*, 1992, **40**, 3277.
4 Y. Yaoita and M. Kikuchi, *Chem. Pharm. Bull.*, 1994, **42**, 1944.
5 Y. Yaoita and M. Kikuchi, *Pyhtochemistry*, 1994, **37**, 1765.
6 Y. Yaiota and M. Kikuchi, *Pyhtochemistry*, 1994, **37**, 1773.
7 Y. Yaoita and M. Kikuchi, *Chem. Pharm. Bull.*, 1995, **43**, 1738.
8 Y. Yaoita and M. Kikuchi, *Nat. Med.*, 1996, **50**, 49.
9 Y. Yaoita and M. Kikuchi, *Phytochemistry*, 1996, **42**, 751.
10 Y. Yaoita and M. Kikuchi, *Chem. Pharm. Bull.*, 1996, **44**, 1731.
11 Y. Yaoita and M. Kikuchi, *Nat. Med.*, 1997, **51**, 372.
12 M. Tori, M. Kawahara, and M. Sono, *Tetrahedron Lett.*, 1997, **38**, 1965.
13 M. Tori, M. Kawahara, and M. Sono, *Phytochemistry*, 1998, **47**, 401.
14 M. Tori, M. Kume, K. Nakashima, M. Sono, and M. Tanaka, *Heterocycles*, 2005, **65**, 887.
15 H. Matsuda, T. Morikawa, H. Ueda, and M. Yoshikawa, *Heterocycles*, 2001, **55**, 1499.
16 H. Matsuda, T. Morikawa, H. Ueda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2001, **49**, 1368.
17 H. Matsuda, T. Morikawa, I. Toguchida, K. Ninomiya, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2001, **49**, 1558.
18 T. Morikawa, H. Matsuda, K. Ninomiya, and M. Yoshikawa, *Biol. Pharm. Bull.*, 2002, **25**, 627.
19 T. Morikawa, J. Tao, I. Toguchida, H. Matsuda, and M. Yoshikawa, *J. Nat. Prod.*, 2003, **66**, 86.
20 H. Shimoda, J. Tanaka, E. Yamada, T. Morikawa, N. Kasajima, and M. Yoshikawa, *J. Agric. Food Chem.*, 2006, **54**, 2915.
21 K. Xiao, L. Xuan, Y. Xu, and D. Bai, *J. Nat. Prod.*, 2000, **63**, 1373.
22 I. Kitagawa, M. Kobayashi, and T. Sugawara, *Chem. Pharm. Bull.*, 1978, **26**, 1852.
23 I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, 1978, **26**, 1864.

24 K. Naya, M. Kawai, M. Naito, and T. Kasai, *Chem. Lett.*, 1972, 241.

25 The ^{1}H- and ^{13}C-NMR spectra of **1** and **1a** 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 connectivity (HMBC) experiments.

26 I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.

27 L. B. Schwartz, R. A. Lewis, D. Seldin, and K. F. Austen, *J. Immunol.*, 1981, **126**, 1290.

28 D. L. Marquardt and S. I. Wasserman, *J. Immunol*, 1983, **131**, 934.

29 H. Matsuda, T. Morikawa, J. Tao, K. Ueda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2002, **50**, 208.

30 H. Matsuda, S. Tewtrakul, T. Morikawa, A. Nakamura, and M. Yoshikawa, *Bioorg. Med. Chem.*, 2004, **12**, 5891.

31 T. Morikawa, H. Matsuda, I. Toguchida, K. Ueda, and M. Yoshikawa, *J. Nat. Prod.*, 2002, **65**, 1468.

32 T. Morikawa, H. Matsuda, Y. Sakamoto, K. Ueda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2002, **50**, 1045.

33 H. Matsuda, T. Morikawa, K. Ueda, H. Managi, and M. Yoshikawa, *Bioorg. Med. Chem.*, 2002, **10**, 3123.

34 J. Tao, T. Morikawa, S. Ando, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2003, **51**, 654.

35 H. Matsuda, T. Morikawa, H. Xie, and M. Yoshikawa, *Planta Med.*, 2004, **70**, 847.

36 H. Matsuda, T. Morikawa, H. Managi, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3197.

37 B. Sun, T. Morikawa, H. Matsuda, S. Tewtrakul, L. J. Wu, S. Harima, and M. Yoshikawa, *J. Nat. Prod.*, 2004, **67**, 1464.

38 T. Morikawa, B. Sun, H. Matsuda, L. J. Wu, S. Harima, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2004, **52**, 1194.