Table 1. ^{13}C- and ^{1}H-NMR Chemical Shifts for Sugar Moieties of **1** and **1-b** (Pyridine-d_5)

	1	**1-b**			**1**	**1-b**
	δ_C	δ_C	δ_H[a)]		δ_C	δ_C
Glc				C-1	37.2	37.1
1	102.4	102.4	4.88 d (7.3)	2	29.9	29.8
2	73.2	73.2	4.41 t (7.8)	3	77.4	77.3
3	75.6	75.5	4.08—4.14	4	34.8	34.8
4	79.9	79.9	4.60—4.62	5	44.7	44.6
5	75.3	75.3	4.0—4.05	6	28.9	28.9
6	60.6	60.6	4.7 dd (16.1, 10.1)	7	32.1	32.4
			4.21—4.24	8	35.2	35.2
Glc'				9	54.4	54.3
1	105.1	105.2	5.2 d (7.8)	10	35.8	35.7
2	81.3	81.4	4.45 t (8.7)	11	21.2	21.2
3	86.7	86.6	4.18 t (8.7)	12	40.0	40.1
4	70.4	70.4	3.83 t (9.2)	13	41.0	40.7
5	77.7	77.7	3.87—3.90	14	56.3	56.4
6	62.9	63.0	4.01—4.05	15	32.4	32.1
			4.5—4.55	16	81.3	81.2
Glc''				17	64.3	62.8
1	104.8	104.8	5.58 d (7.4)	18	16.5	16.6
2	76.2	76.2	4.11 t (9.2)	19	12.3	12.3
3	78.6	78.6	4.03—4.10	20	40.5	42.4
4	71.0	70.9	4.21—4.24	21	16.3	14.9
5	78.7	78.7	3.90—3.93	22	112.6	109.7
6	62.4	62.4	4.6 dd (11.9, 2.7)	23	31.0	26.2
			4.36 dd (11.9, 5.9)	24	28.2	26.3
Xyl				25	34.4	27.5
1	104.9	104.9	5.24 d (7.8)	26	74.9	65
2	75.0	75.0	3.96 td (8.2, 3.7)	27	17.5	16.3
3	77.6	77.6	4.07—4.13	OCH_3	47.3	
4	70.7	70.7	4.05—4.13			
5	67.3	67.3	3.67 t (10.6)			
			4.21—4.24			
Glc'''						
1	105.0					
2	75.2					
3	78.6					
4	71.6					
5	78.5					
6	62.8					

All spectra recorded at 800 MHz. *a)* *J* values (in Hz) parentheses.

lated **1** showed no fragment 619, which was derived from a terminal diglucose heptaacetate [Glc(OAc)$_4$-Glc(OAc)$_3$-]. Based on this information, the glycosyl linkage position of **1-b** was determined by 1D-HOHAHA technique and 2D-NMR (800 MHz) (Fig. 1). The orientation of a methoxyl group at C-22 of **1** was determined to be α because the nuclear Overhauser effect (NOE) correlation between a methoxyl group and H-16 was observed. Thus the chemical structure of **1** was established as shown in Fig. 2. To our knowledge, this compound has not been reported previously.

The IC_{50} values of the leishmanicidal activities of **1** against *L. major*, *L. guyanensis*, and *L. panamensis* were 0.3, 5.5, and 8.0 μg/ml, respectively.

In conclusion, a new compound, **1**, was isolated together with three known compounds. **1** was found to show leishmanicidal activity against *L. major*, *L. guyanensis*, and *L. panamensis*, and was especially potent against *L. major*. Compound **1** possesses a methoxyl group at C-22, which was thought to be inserted during extraction with methanol.

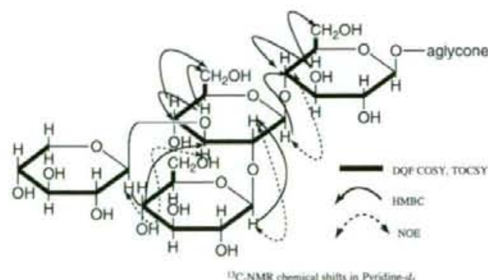

Fig. 1. DQF COSY, TOCSY, HMBC and NOESY Correlations for the Sugar Moiety of **1-b**

Experimental

Plant Material *Brunfelsia grandiflora* material was collected in the Peruvian Amazon region and identified by Dr. Elsa Rengifo (The Peruvian Research Institute of the Amazon, Iquitos, Peru). A voucher specimen (No. P07-01) is on file at the Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation (1–2 Hachimandai, Tsukuba, Ibaraki 305–0843, Japan).

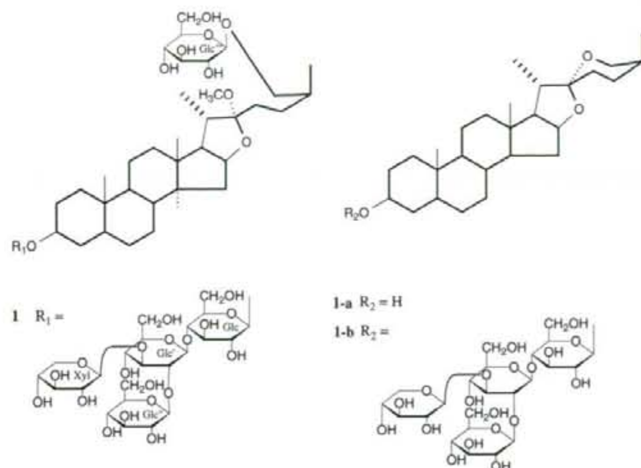

Fig. 2. Chemical Structures of **1**, **1-a** and **1-b**

General Experimental Procedures Melting points were determined by Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-370 automatic polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL Alpha 500 spectrometer (500 MHz) and JEOL ECA 800 (800 MHz). IR spectra were recorded on a JASCO FTIR-5300 spectrometer. GC-MS and LC-MS were performed with a SHIMADZU QP 5050A and an Applied Biosystems QSTAR XL with an APCI, and ESI ion source apparatus, respectively. FAB-MS was measured by JEOL HX110 spectrometer. HPLC was run on Shimadzu LC-10VP system with JASCO OR-2090 Plus chiral detector.

Extraction and Isolation The methanolic extract of *B. grandiflora* leaves was prepared in Peru by Dr. Victor Zorrilla (Institute of Tropical Medicine, Lima, Peru). The concentrated extract (367 g) was dissolved in methanol and the solution was passed through a column of activated charcoal (70 g). Methanol (10 l), 30% chloroform/methanol (10 l), and chloroform were then each used as an eluent to give 3 Fractions (FR. A—B). Each fraction was concentrated *in vacuo* to give a syrup. The fraction eluted with methanol (FR. A) was subjected to column chromatography on silica gel (developing solvent: gradient with chloroform–methanol system) to afford 20 Fractions (FR. 1—20). Fractions eluted with 30—40% methanol/chloroform (FR. 7—9) were combined and evaporated under reduced pressure, and the residue was crystallized from methanol to give ursolic acid (670 mg). Fractions eluted with 60—70% methanol/chloroform (FR. 12—16) were combined and concentrated then partitioned between *n*-buthanol and water. The *n*-buthanol layer was concentrated *in vacuo* and the residue was chromatographed on Sephadex LH-20 with 80% methanol/water as an eluent to give 83 fractions (FR. 1′—83′). FR. 67′—83′ were combined and concentrated to yield chlorogenic acid (565 mg). FR. 13′—33′ were combined and rechromatographed on silica gel with a mixture of chloroform–methanol–water (100 : 20 : 1) as an eluent to afford 294 fractions (FR. 1″—294″). FR. 148″—177″ were combined and the concentrated residue was subjected to HPLC (CapcelPak C-18, Shiseido, developing solvent: methanol/water (=3 : 1—4 : 1)) to afford cirensenoside P (12 mg). FR. 208″—242″ were combined and crystallized with methanol to yield **1** (1.08 g) as colorless fine needles. FR. B (eluted with 30% methanol/chloroform) was subjected to column chromatography on silica gel with a mixture of methanol and chloroform as an eluent then crystallized with chloroform–methanol to afford scopoletin (145 mg). **1**: colorless fine needles. mp 209—205 °C $[\alpha]_D^{23}$ −44.6° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.59 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3), 1.02 (3H, d, J=6.9 Hz, 27-CH_3), 1.14 (3H, d, J=6.9 Hz, 21-CH_3), 3.23 (3H, s, 22-OCH_3), 4.83 (1H, d, J=7.8 Hz, 26-Glc-1), 4.87 (1H, d, J=7.9 Hz, Glc-1), 5.17 (1H, d, J=7.3 Hz, Glc′-1), 5.22 (1H, d, J=7.8 Hz, Xyl-1), 5.55 (1H, d, J=7.4 Hz, Glc″-1). IR (KBr) cm^{-1}: 3407, 2927, 1654, 1451, 1378, 1073. HR-FAB-MS (positive mode) m/z: 1229.6195 $[M+H]^+$ (Calcd for $C_{57}H_{97}O_{28}$: 1229.6166). FAB-MS (positive mode) m/z: 1251 $[M+Na]^+$. APCI-TOF-MS (negative mode): m/z 1227 $[M-H]^-$, 1095 $[M-Xyl]^-$, 1065 $[M-Glc]^-$, 933 $[M-Glc-Xyl]^-$, 771 $[M-2Glc-Xyl]^-$, 609 $[M-3Glc-Xyl]^-$.

Acid Hydrolysis of 1 Compound **1** (32 mg) was dissolved in 3% hydrochloric acid (12 ml) and the mixture was refluxed for 4 h. After cooling, the mixture was poured into ice water, and the precipitate was filtered to give neotigogenin (10 mg).[5] The filtrate was evaporated *in vacuo*, then a half residue was analyzed by HPLC with chiral detector [column: high-performance carbohydrate column (Waters), solvent system: 80% acetonitrile/water, flow rate: 1.0 ml/min, Peak A: 5.0 min(+), Peak B: 4.5 min(+), D-glucose standard: 5.0 min(+), D-xylose standard: 4.5 min(+)]. A trimethylsilylating reagent (TMS-HT, Tokyo Kasei Kogyo Co., Ltd.) was added to another residue and the supernatant solution was analyzed by GC-MS [column: ZB-1701 capillary column (I.D. 0.25 mm×30 m) (Zebron), column temperature program: initial temperature 100 °C (0 to 5 min), rising from 100 to 245 °C (5 to 65 min)]. In GC-MS analysis, 4 peaks (17.5, 18.5, 20.7, 22.3 min) were detected. They were identified to be a trimethylsilylated glucose (TMSGlc) and a trimethylsilylated xylose (TMSXyl) by comparison of retention times and MS fragment patterns with standard samples (TMSGlc: 20.7, 22.3 min, TMSXyl: 17.5, 18.4 min).

Enzymatic Hydrolysis of 1 Compound **1** (30 mg) was suspended in citrate buffer solution (pH 4.25) (10 ml), β-glucosidase (from almond, Oriental Yeast Co., Ltd.) (30 mg) was added, and the mixture was stirred at 30 °C for 3 d. The mixture was poured into water, extracted with *n*-buthanol, and evaporated to afford **1-b** (10 mg). **1-b**: colorless amorphous powder. $[\alpha]_D$ −37.7° (c=0.19, MeOH). HR-FAB-MS (positive mode) m/z: 1035.5347 $[M+H]^+$ (Calcd for $C_{50}H_{83}O_{22}$ 1035.5376). FAB-MS (positive mode) m/z: 1058 $[M+Na]^+$. ESI-TOF-MS (negative mode) m/z: 1033 $[M-H]^-$, 901 $[M-Xyl]^-$, 871 $[M-Glc]^-$, 739 $[M-Glc-Xyl]^-$, 577 $[M-2Glc-Xyl]^-$, 415 $[M-3Glc-Xyl]^-$.

Permethylation, Acid Hydrolysis, Reduction and Acetylation of 1[8] A solution of sodium hydride (60% oil suspended) (100 mg) in dimethyl sulfoxide (dried with molecular sieves 4A) (7.5 ml) was heated at 65 °C with stirring for 60 min, and a solution of **1** (50 mg) in dimethyl sulfoxide (2.5 ml) was then added and the mixture was stirred for 1 h. Methyl iodide (0.75 ml) was added and the mixture was stirred at room temperature for 12 h. After the reaction, the mixture was poured into water and extracted twice with chloroform, and the organic layer was washed with water and evaporated *in vacuo*. The residue was subjected to preparative thin-layer chromatography (TLC) (development solvent system; chloroform/methanol (=20 : 1)) to give a permethylated compound (**1-c**). **1-c**: ^{1}H-NMR ($CDCl_3$) δ: 3.35, 3.37, 3.40, 3.44, 3.44, 3.48, 3.51, 3.52, 3.53, 3.56, 3.56, 3.58, 3.60, 3.61, 3.62, 3.62, 3.62 (each 3H, s), 4.19 (1H, d, J=7.3 Hz), 4.30 (1H, d, J=7.8 Hz), 4.70 (1H, d, J=7.3 Hz), 4.93 (1H, d, J=7.3 Hz), 4.99 (1H, d, J=7.8 Hz). **1-c** was dissolved in 0.5 N-sulfuric acid in 90% acetic acid (10 ml), and the solution was stirred at room temperature for 30 min then at 77 °C for 5 h. After being cooled, the reaction mixture was neutralized with silver carbonate and filtered. The filtrate was freeze-dried to give a residue. The residue was dissolved in water (5 ml) and sodium borohydride was added until halting foaming. The solution was stirred at room temperature for 2 h and then a few drops of acetic acid were added to stop the reaction. Methanol was added

and the solution was concentrated under reduced pressure to afford a residue. Acetic anhydride (2 ml) and pyridine (2 ml) were added to the residue and the mixture was heated at 70 °C for 2 h. After being cooled, water was added to the reaction mixture and the solution was stirred at room temperature for 30 min, extracted with chloroform, and evaporated *in vacuo* without heating to give a mixture of partially methylated alditol acetates. The mixture was analyzed by GC-MS. GC-MS analysis was performed with a DB-225 capillary column (I.D. 0.25 mm×30 m) (Agilent Technologies Co., Ltd.) at a column temperature of 170 °C. The relative retention times and mass fragmentation patterns of detected peaks were compared with the values in the literature.[6] The following peaks were detected and their mass fragmentation patterns were identical to those reported previously: 4,6-dimethylglucitol acetate (retention time; 3.29 min), 2,3,6-trimethylglucitol acetate (2.09 min), 2,3,4,6-tetramethylglucitol acetate (1.0 min), and 2,3,4-trimethylxylitol acetate (0.61 min).

Leishmanicidal Activity Assay Cultivation of *Leishmania* promastigotes and leishmanicidal activity assay were carried out as we previously described.[9] Amphotericin B was used as a positive control (IC_{50} 0.04 μg/ml). The moderate leishmanicidal activities for ursolic acid,[10] chlorogenic acid,[11] and scopoletin[12] have been reported in the literature. Activity for cirensenoside P was not carried out due to amount shortage.

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Leishmanicidal Active Constituents from Nepalese Medicinal Plant Tulsi (*Ocimum sanctum* L.)

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In the course of screening leishmanicidal active compounds from Asian and South American medicinal plants, a Nepalese medicinal plant, Tulsi (*Ocimum sanctum* L.), showed strong activity. We therefore studied the isolation and structural elucidation of the active constituents from *O. sanctum* L. From the ethyl acetate soluble fraction of the plant, seven new novel neolignan derivatives were isolated along with 16 known compounds. The structures of the new compounds (1—7) were elucidated as 6-allyl-3′,8-dimethoxy-flavan-3,4′-diol (1), 6-allyl-3-(4-allyl-2-methoxyphenoxy)-3′,8-dimethoxyflavan-4′-ol (2), 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (3), 1,2-bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane (4), 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy)propane (5), 1-allyl-4-(5-allyl-2-hydroxy-3-methoxyphenoxy)-3-(4-allyl-2-methoxyphenoxy)-5-methoxybenzene (6), and 3-(5-allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)-prop-1-ene (7) by means of ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectral data. Some of these compounds showed leishmanicidal activity.

Key words *Ocimum sanctum*; Labiatae; leishmanicidal activity; neolignan; eugenol oligomer

Leishmaniases are a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania* that survive and multiply in macrophages in the mammalian host and are transmitted by female flying insects of genera *Phleobotomus* and *Lutzomyia*. Currently, 12 million people in developing countries are affected by the disease annually.[1,2] In many cases, the medicines employed for treatment are toxic, only slightly effective, given by injection, and compromised by the development of resistance.[3] Since safe, effective, and affordable drugs for leishmaniases are needed in developing countries, there have been many studies on leishmanicidal constituents from plant sources,[4—6] but there are as yet no safe, effective, and reasonably priced drugs. In our project, sesquiterpene lactone[7] and steroid saponines[8] were isolated as leishmanicidal constituents. We have been testing tropical medicinal plants for developing lead compounds for leishmanicidal medicines. Of these testing samples, the Nepalese traditional crude drug, Tulsi (*Ocimum sanctum* L.), was identified as a potent leishmanicidal sample. Seven new neolignan derivatives were isolated from the sources, derived by polymerization of eugenol. This paper deals with the isolation and structural elucidation of new novel neolignan derivatives and the leishmanicidal activity of compounds isolated from *O. sanctum* L.

Results and Discussion

Methanol (MeOH) extract of *O. sanctum* L. was partitioned with ethylacetate (AcOEt) and *n*-buthanol (*n*-BuOH) to give AcOEt, *n*-BuOH, and aqueous fractions. Active AcOEt fraction was separated by successive chromatographies, as described in the Experimental section, to give seven new compounds (**1—7**) and 16 known compounds (**8—23**) (Fig. 1). The structures of the known compounds were determined by data from MS and NMR spectra as follows: eugenol (**8**), citrusin C (**9**),[9] ferulaldehyde (**10**), bieugenol (**11**),[10] dehydrodieugenol B (**12**),[11] oleanolic acid (**13**), ulsoric acid (**14**), stigmasterol (**15**),[12] β-sitosterol-3-O-β-D-glucopyranoside (**16**), caryophyllene oxide (**17**),[13] apigenin (**18**),[14] luteolin (**19**),[15] crysoeriol (**20**),[16] 4′,5-dihydroxy-7,8-dimethoxyflavone (**21**),[17] 4′,5-dihydroxy-3′,7,8-trimethoxyflavone (**22**),[18] and vanillin (**23**).

Compound **1** was obtained as a colorless amorphous powder. HR-ESI-MS of **1** showed a pseudomolecular ion at m/z 365.1371 $[M+Na]^+$ $C_{20}H_{22}O_5Na$, which accorded to the molecular formula $C_{20}H_{22}O_5$. The IR spectrum of **1** showed absorptions at 3433, 2361, 1495, 1272 cm^{-1}. The UV spectrum of **1** showed absorption at 238 (ε 10600) and 279 (ε 7100) nm. The ^{1}H-NMR spectrum of **1** showed the presence of two methoxy groups [δ_H 3.83 (3H, s), 3.88 (3H, s)], one allyl group [δ_H 3.31 (2H, br d, J=6.8 Hz), 5.96 (1H, ddt, J=6.6, 10.3, 17.1 Hz), 5.07 (1H, br d, J=10.3 Hz), 5.10 (1H, br d, J=17.1 Hz)], 3,4-dioxygenated phenyl group [δ_H 6.95 (1H, d, J=1.7 Hz), 6.91 (1H, d, J=8.1 Hz), 6.93 (1H, dd, J=8.1, 1.7 Hz)], two *m*-coupled protons [δ_H 6.58 (1H, d, J=1.5 Hz), 6.54 (1H, d, J=1.5 Hz)], two oxygen-bearing methine groups [δ_H 4.76 (1H, d, J=7.8 Hz), 4.11 (1H, dt, J=5.8, 8.1 Hz)], and a metylene group [δ_H 2.89 (1H, dd, J=8.8, 16.1 Hz), 3.06 (1H, dd, J=5.4, 16.1 Hz)]. The ^{13}C-NMR data of **1** showed the presence of two secondary oxygen-bearing methine carbons (δ_C 82.0, 68.1), two methylene carbons (δ_C 32.7, 39.8), and 14 sp^2 carbons (Table 2). This indicated that **1** has a flavan-3-ol skeleton having two methoxy groups, an allyl group, and a hydroxyl group. The positions of functional groups were determined by heteronuclear multiple bond connectivity (HMBC) experiments of **1**, as shown in Fig. 2. H-7′ (δ_H 4.76, d, J=7.8 Hz) showed a correlation to C-4, 2′, 6′, 8′, and 9′ (δ_C 141.8, 109.5, 120.4, 68.1, 32.7); H-8′ (δ_H 4.11, dt, J=5.1, 8.1 Hz) to C-5 and 1′ (δ_C 120.7,

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Table 1. ^{1}H-NMR Data of Compounds **1**—**7** (in $CDCl_3$, 500 MHz)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
H-2	6.58 (1H, d, 1.5)	6.59 (1H, d, 2.0)	6.65 (1H, br s)	7.05 (1H, d, 1.7)	7.17 (1H, d, 1.7)	6.75 (1H, d, 1.7)	6.57 (1H, d, 1.5)
5				6.85 (1H, d, 8.1)	6.82 (1H, d, 8.1)	6.80 (1H, d, 8.1)	
6	6.54 (1H, d, 1.5)	6.49 (1H, d, 2.0)	6.76 (1H, br s)	6.91 (1H, dd, 8.1, 1.7)	7.01 (1H, dd, 8.1, 1.7)	6.67 (1H, dd, 8.1, 1.7)	6.61 (1H, br s)
7	3.31 (2H, br d, 6.8)	3.30 (2H, br d, 5.1)	3.33 (2H, br d, 5.4)	4.62 (1H, d, 4.9)	5.54 (1H, d, 4.6)	3.35 (2H, d, 6.6)	3.30 (2H, d, 6.6)
8	5.96 (1H, ddd, 17.1, 10.3, 6.6)	5.89—5.96 (1H, overlap)	5.95 (1H, overlap)	4.53 (1H, dt, 5.1, 4.9)	4.79 (1H, q, 4.9)	5.84—5.98 (1H, overap)	5.94 (1H, ddt, 16.8, 10.0, 6.6)
9	5.07 (1H, br d, 10.3) 5.10 (1H, br d, 17.1)	5.03—5.10 (2H, overlap)	5.07 (2H, overlap)	3.91 (1H, dd, 10.3, 4.9) 4.26 (1H, dd, 10.3, 5.1)	3.96 (1H, dd, 10.3, 5.4) 4.46 (1H, dd, 10.3, 4.4)	4.98—5.10 (2H, overlap)	5.04 (1H, br d, 10.0) 5.07 (1H, br d, 17.6)
2′	6.95 (1H, d, 1.7)	6.89 (1H, d, 1.7)	6.98 (1H, d, 2.2)	6.67 (1H, d, 1.7)	6.64 (1H, overlap)	6.51 (1H br s)	6.55 (1H, d, 2.7)
5′	6.91 (1H, d, 8.1)	6.83 (1H, d, 8.1)	6.86 (1H, d, 8.8)	6.71 (1H, d, 8.1)	6.75 (1H, d, 7.8)		6.81 (1H, d, 8.8)
6′	6.93 (1h, dd, 8.1, 1.7)	6.91 (1H, dd, 8.1, 1.7)	6.97 (1H, dd, 8.8, 2.2)	6.61 (1H, dd, 8.1, 1.7)	6.64 (1H, overlap)	6.30 (1H, d, 1.7)	6.49 (1H, dd, 8.8, 2.7)
7′	4.76 (2H, d, 7.8)	5.23 (1H, d, 6.4)	5.66 (2H, br d, 6.6)	3.30 (2H, br d, 3.9)	3.29 (2H, br d, 6.1)	3.26 (2H, d, 6.6)	6.44 (1H, d, 12.0)
8′	4.11 (1H, dt, 5.8, 8.1)	4.65 (1H, q, 6.4)	3.93 (1H, m)	5.92 (1H, ddt, 16.9, 10.3, 1.8)	5.90 (1H, overlap)	5.84—5.98 (1H, overlap)	5.46 (1H, dt, 12.0, 7.3)
9′	2.88 (1H, dd, 16.1, 8.8) 3.06 (1H, dd, 16.1, 5.8)	3.04 (2H, d, 5.4)	4.16 (1H, t, 8.6) 4.27 (1H, dd, 9.5, 5.9)	5.05 (2H overlap)	5.00—5.08 (2H, overlap)	4.98—5.10 (2H, overlap)	3.33 (2H, d, 7.3)
2″		6.66 (1H, d, 1.7)	6.71 (1H, d, 2.0)	6.65 (1H, d, 2.0)	6.64 (1H, overlap)	6.40 (1H, d, 1.5)	
5″		6.63 (1H, d, 8.1)	6.80 (1H, d, 8.3)	6.87 (1H, d, 8.1)	7.00 (1H, d, 7.8)		
6″		6.58 (1H, dd, 8.1, 1.7)	6.68 (1H, dd, 8.3, 2.0)	6.63 (1H, dd, 8.1, 2.0)	6.64 (1H, overlap)	6.51 (1H, br s)	
7″		3.29 (2H, br d, 6.1)	3.32 (2H, br d, 6.1)	3.30 (2H, br d, 4.9)	3.30 (2H, br d, 5.1)	3.20 (2H, overlap)	
8″		5.89—5.96 (1H, overlap)	5.95 (1H, overlap)	5.93 (1H, ddt, 16.9, 10.3, 6.6)	5.90 (1H, overlap)	5.84—5.98 (1H, overlap)	
9″		5.03—5.15 (2H, overlap)	5.05—5.11 (2H, overlap)	5.05 (2H, overlap)	5.00—5.08 (2H, overlap)	4.98—5.10 (2H, overlap)	
2‴					6.64 (1H, overlap)		
5‴					6.74 (1H, d, 8.1)		
6‴					6.52 (1H, dd, 8.1, 1.7)		
7‴					3.25 (2H, br d, 6.6)		
8‴					5.90 (1H, overlap)		
9‴					5.00—5.08 (2H, overlap)		
3-OMe	3.83 (3H, s)	3.89 (3H, s)	3.89 (3H, s)	3.79 (3H, s)	3.78 (3H, s)	3.74 (3H, s)	3.86 (3H, s)
3′-OMe	3.88 (3H, s)	3.77 (3H, s)	3.84 (3H, s)	3.80 (3H, s)	3.76 (3H, s)	3.82 (3H, s)	3.85 (3H, s)
3″-OMe		3.75 (3H, s)	3.82 (3H, s)	3.76 (3H, s)	3.74 (3H, s)	3.84 (3H, s)	
3‴-OMe					3.73 (3H. s)		
7-OMe				3.30 (3H, s)			

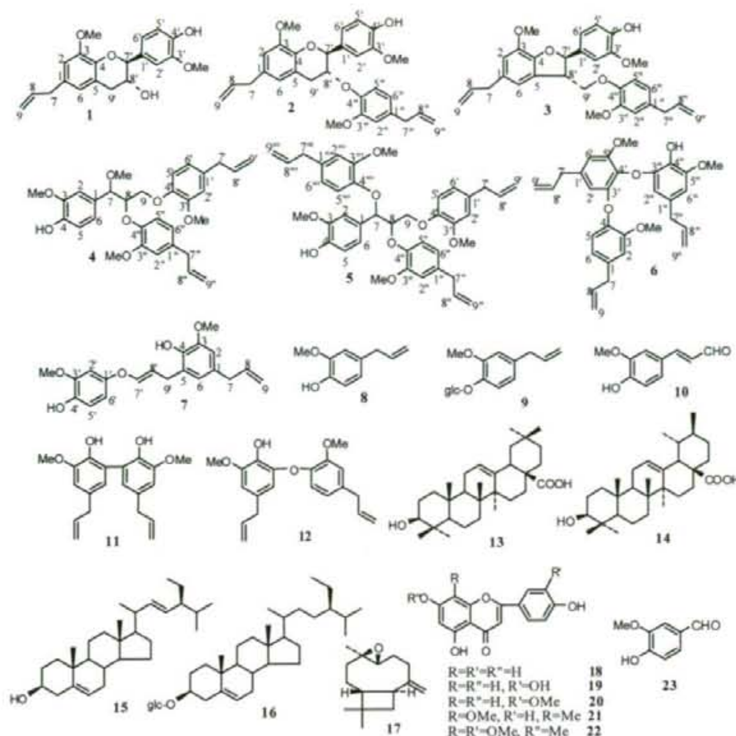

Fig. 1. Structures of Constituents Isolated from *O. sanctum*

129.7); H-6 (δ_H 6.54, d, J=1.5 Hz) to C-2, 4, 7, and 9′ (δ_C 110.2, 141.8, 39.8, 32.7); OMe at C-3 (δ_H 3.83, s) to C-3 (δ_C 148.1); OMe at C-3′ (δ_H 3.88, s) to C-3′ (δ_C 146.8); H-5′ (δ_H 6.91, d, J=8.1 Hz) to C-1′ and 3′ (δ_C 129.7, 146.8); H-2′ (δ_H 6.95, J=1.7 Hz) and H-6′ (δ_H 6.93, dd, J=8.1, 1.7 Hz) to C-4′ and 7′ (δ_C 146.0, 82.0), and so on, as shown in Fig. 2. These data indicated that **1** was 6-allyl-3′,8-dimethoxy-flavan-3,4′-diol, and the compound was named tulsinol A. The relative configuration between C-7′ and 8′ resulted in *trans* from the coupling constant of H-7′ with H-8′ (J=7.8 Hz).[19] The absolute configuration of **1** was not studied because of low optical rotation ($[\alpha]_D$ +1.5°) and no Cotton effect in circular dichroism (CD) spectrum of **1**.

Compound **2** was obtained as a pale yellow viscous oil. HR-ESI-MS of **2** showed a pseudomolecular ion at *m/z* 511.2113 $[M+Na]^+$ $C_{30}H_{32}O_6Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. The IR spectrum of **2** showed absorptions at 3444, 2935, 1594, and 1269 cm^{-1}. The UV spectrum of **2** showed absorption at 234 (ε 13300) and 279 (ε 6100) nm. The ^{1}H-NMR spectrum of **2** showed a similar signal pattern to that of **1** along with a further eugenol unit [δ_H 3.75 (3H, s), 3.29 (2H, br d, J=6.1 Hz), 5.03—5.10 (2H, m), 5.89—5.96 (1H, m), 6.58 (1H, dd, J=8.1, 1.7 Hz), 6.63 (1H, d, J=8.1 Hz), and 6.66 (1H, d, J=1.7 Hz)]. The ^{13}C-NMR spectrum of **2** also showed the signals of **1** and an eugenol unit (δ_C 39.8, 55.9, 112.9, 115.7, 119.7, 120.6, 135.1, 137.7, 144.9, 151.1). These data indicated that **2** was a derivative of **1** with one more eugenol unit. The position of the extra eugenol unit in **2** was determined from the HMBC experiment. H-8′ (δ_H 4.65, 1H, q, 6.4 Hz) showed a correlation to C-4″, C-1′, and C-5 (δ_C 144.9, 130.8, 120.4). The relative configuration at C-7′ and 8′ was determined to be *trans* from the coupling constant (J=6.4 Hz) between H-7′ and H-8′. Optical rotation of **2** gave a low value ($[\alpha]_D$ +1.5°) and the CD spectrum of **2** showed no Cotton effect, indicating the possibility of low optical purity in **2**; therefore, the absolute configuration of **2** was not studied. The structure was determined to be 6-allyl-3-(4-allyl-2-methoxyphenoxy)-3′,8-dimethoxyflavan-4′-ol, and the compound was named tulsinol B.

Compound **3** was obtained as a pale yellow viscous oil. HR-ESI-MS of **3** showed a pseudomolecular ion at *m/z* 511.2119 $[M+Na]^+$ $C_{30}H_{32}O_6Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. $[\alpha]_D$ +4.3 (c=0.03, MeOH). The IR spectrum of **3** showed absorptions at 3427, 2936, 1604, and 1267 cm^{-1}. The UV spectrum of **3** showed absorption at 215 (ε 10300), 229 (ε 9100), and 281 (ε 3500) nm. The ^{1}H-NMR spectrum of **3** showed the presence of two allyl groups [δ_H3.32 (2H, d, J=6.1 Hz), 3.33 (2H, d, J=5.4 Hz), 5.92—5.98 (2H, over lap), 5.05—5.11 (4H, overlap)], two 3,4-dioxygenated phenyl groups [δ_H 6.75 (1H, d, J=7.8 Hz), 7.00 (1H, d, J=7.8 Hz), 6.62—6.66 (4H, overlap)], two *m*-coupled H [δ_H 6.65 (1H, br s), 6.76 (1H, br s)], a methine group [δ_H 3.93 (1H, m)], an oxygen-bearing methine group [δ_H 5.65 (1H, d, J=6.6 Hz)], and an oxygen-bearing methylene group [δ_H 4.16 (1H, t, J=8.6 Hz), 4.27 (1H, dd, J=9.5, 5.9 Hz)]. The ^{13}C-NMR spectrum of **3** showed 30 carbon signals, which showed that **3** was a trimer of eugenol. The

Table 2. ^{13}C-NMR Data of Compounds **1**—**7** (in $CDCl_3$, 125 MHz)

Position	1	2	3	4	5	6	7
C-1	132.6	132.2	133.8	130.4	130.0	136.7	131.1
2	110.2	110.3	112.6	110.2	110.0	113.2	121.6
3	148.1	148.0	144.1	146.5	146.4	150.8	146.2
4	141.8	141.6	146.5	145.2	145.1	143.1	141.6
5	120.7	120.4	127.7	113.8	113.9	120.4	126.1
6	121.3	121.2	116.9	120.6	120.0	120.8	109.0
7	39.8	39.8	40.1	82.6	80.6	39.9	40.0
8	137.6	137.4	137.8	82.7	82.7	137.3	137.8
9	115.6	115.5	115.7	68.1	68.4	115.9	115.4
1′	129.7	130.8	133.3	134.2	133.1	130.4	151.0
2′	109.5	109.6	108.6	112.7	112.6	106.8	101.3
3′	146.8	146.2	146.4	149.6	150.7	147.6	146.8
4′	146.0	145.3	145.3	146.8	146.8	133.0	141.1
5′	114.5	114.1	114.1	114.3	114.1	146.1	114.2
6′	120.4	119.9	119.2	120.5	120.3	110.7	108.5
7′	82.0	79.2	88.3	39.8	39.7	40.0	143.8
8′	68.1	76.2	51.4	137.6	137.6	136.8	110.2
9′	32.7	30.0	71.7	115.5	115.1	115.4	27.3
1″		135.1	133.4	133.3	134.3	137.4	
2″		119.7	112.7	112.5	112.6	106.8	
3″		151.1	149.8	150.6	150.7	153.2	
4″		144.9	146.5	146.6	146.8	134.4	
5″		112.9	114.3	118.2	118.9	151.1	
6″		120.6	120.5	120.5	120.5	106.9	
7″		39.8	39.8	39.8	39.8	40.1	
8″		137.7	137.5	137.6	137.6	137.7	
9″		115.7	115.6	115.6	115.5	116.1	
1‴					133.4		
2‴					112.7		
3‴					150.0		
4‴					146.1		
5‴					116.5		
6‴					120.6		
7‴					39.9		
8‴					137.7		
9‴					115.5		
3-OMe	55.9	55.8	56.0	55.7	55.7	55.9	55.9
3′-OMe	55.9	55.8	55.8	55.8	55.8	56.2	56.0
3″-OMe		55.9	55.9	55.9	55.8	56.2	
3‴-OMe					55.9		
7-OMe				57.2			

HMBC spectral data showed the following correlations; H-7′ (δ_H 5.66, d, J=6.6 Hz) to C-4, C-5, C-2′, C-6′, and C-9′ (δ_C 146.5, 127.7, 108.6, 119.2, 71.7); H-8′ (δ_H 3.93, 1H, m) to C4, C-6, and C-1′ (δ_C 146.5, 116.9, 133.3); H-9′ (δ_H 4.16, 1H, t, J=8.6 Hz, 4.27, 1H, dd, J=9.5, 5.9 Hz) to C-5, C-4″, and C-7′ (δ_C 127.7, 146.5, 88.3) and so on, as shown in Fig. 2. These data showed that **3** has a dihydrobenzfurane skeleton. Thus the structure of **3** was determined to be 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran. Stereochemistry at C-7′ and 8′ was determined as *trans* from observation of the NOE between H-7′ and CH_2-9′, and the compound was named tulsinol C.

Compound **4** was obtained as a pale yellow viscous oil. HR-ESI-MS of **4** showed a pseudomolecular ion at m/z 543.2339 $[M+Na]^+$ $C_{31}H_{36}O_7Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. The IR spectrum of **4** showed absorptions at 3418, 2936, 1670, 1595, and 1270 cm^{-1}. The ^{1}H-NMR spectrum of **3** showed the presence of three aromatic methoxy groups [δ_H 3.76 (3H, s), 3.79 (3H, s), and 3.80 (3H, s)], an aliphatic methoxy group (δ_H 3.30, 3H, s), two allyl moieties [δ_H 3.30 (4H, br d, J=6.9 Hz), 5.92 (1H, ddt, J=16.9, 10.3, 6.8 Hz), 5.93 (1H, ddt, J=16.9, 10.3, 6.6 Hz), 5.05 (4H, over lap)], three 3,4-dioxygenated phenyl groups [δ_H 7.05 (1H, d, J=1.7 Hz), 6.67 (1H, d, J=1.7 Hz), 6.65 (1H, d, J=2.0 Hz), 6.85 (1H, d, J=8.1 Hz), 6.71 (1H, d, J=8.1 Hz), 6.87 (1H, d, J=8.1 Hz), 5.05 (3H, over lap)], two oxygen-bearing methine groups [δ_H 4.62 (1H, d, J=4.9 Hz), 4.53 (1H, dt, J=5.1, 4.9 Hz)], and an oxygen-bearing methylene group [δ_H 3.91 (1H, dd, J=10.3, 4.9 Hz), 4.26 (1H, dd, J=10.3, 5.1 Hz)]. The ^{13}C-NMR spectrum of **4** showed four methoxy carbons (δ_C 55.7, 55.8, 55.9, 57.2), two oxygen-bearing methine carbons (δ_C 82.6, 82.7), an oxygen-bearing methylene carbon (δ_C 68.1), 18 aromatic carbons, and two allyl groups (δ_C 115.5, 115.6, 137.6×2, 39.8×2). These data indicate that **4** is a trimer of eugenol units, as shown in Fig. 1. The structure and position of functional groups in **4** were determined by HMBC experiment as follows. The alcoholic methoxy H (δ_H 3.30, 3H, s) showed a correlation to C-7 (δ_C 82.6); H-7 (δ_H 4.62, 1H, d, J=4.9 Hz) to C-1, 6, and 9 (δ_C 110.2, 120.6, 68.1); H-8 (δ_H 4.53, 1H, q, J=4.9 Hz) to C-1 and 4″ (δ_C 130.4, 146.6); H-9 (δ_H 3.91, 1H, dt, J=4.9, 10.3, 4.26, 1H, dd, J=5.1, 10.3 Hz) to C-4′ and 7 (δ_C 146.8, 82.6) and so on, as shown in Fig. 2. Thus the structure of **4** was de-

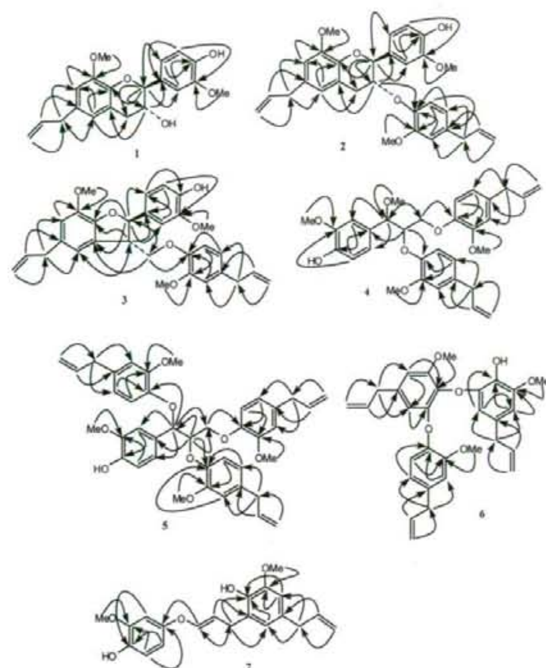

Fig. 2. Key HMBC Correlations of **1**—**7**

termined to be 1,2-bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane, and the compound was named tulsinol D. Stereochemistry at C-7 and 8 was not determined.

Compound **5** was obtained as a pale yellow viscous oil. HR-ESI-MS of **5** showed a pseudomolecular ion at *m/z* 675.2963 $[M+Na]^+$ $C_{40}H_{44}O_8Na$, which accorded to the molecular formula $C_{40}H_{44}O_8$. The ^{1}H-NMR spectrum of **5** showed the presence of three eugenol units [δ_H 3.73 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 3.25 (2H, br d, J=6.6 Hz), 3.29 (2H, d, J=6.1 Hz), 3.30 (2H, d, J=5.1 Hz), 5.85—5.97 (3H, overlap), 5.00—5.08 (6H, overlap), 6.74 (1H, d, J=8.1 Hz), 6.52 (1H, dd, J=8.1, 1.7 Hz), 6.75 (1H, d, J=8.1 Hz), 7.00 (1H, d, J=7.8 Hz), 6.62—6.66 (5H, overlap)], a phenolic methoxy group [δ_H 3.78 (3H, s)], 3,4-dioxyphenyl group [δ_H 6.82 (1H, d, J=8.1 Hz), 7.01 (1H, dd, J=8.1, 1.7 Hz), 7.17 (1H, d, J=1.7 Hz)], two oxygen-bearing methine groups [δ_H 5.54 (1H, d, J=4.6 Hz), 4.79 (1H, q, J=4.9 Hz)] and an oxygen-bearing methylene group [δ_H 3.96 (1H, dd, J=10.3, 5.4 Hz), 4.46 (1H, dd, J=10.3, 4.4 Hz)]. These data showed the replacement of the methyl group in **4** to the eugenol moiety in **5**. The ^{13}C-NMR data (in Table 2) also supported the structure of **5**, in which one more eugenol moiety was substituted at C-7. The structure of **5** was confirmed by the HMBC spectrum. The HMBC of **5** showed correlation of H-7 (δ_H 5.54, 1H, d, J=4.6 Hz) to C-2, 6, 9, and 4‴ (δ_C 110.0, 120.0, 68.4, 146.1); H-8 (δ_H 4.79, 1H, q, J=4.9 Hz) to C-1, 7, 9, and 4″ (δ_C 130.0, 80.6, 68.4, 146.8); H-9 (δ_H 3.96, 1H, dd, J=10.3, 4.5 Hz, 4.46, 1H, dd, J=10.3, 4.4 Hz) to C-4′ and 7 (δ_C 146.8, 80.6), and so on, as shown in Fig. 2. Thus the structure of **5** was determined to be 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy)propane, and the compound named tulsinol E. Stereochemistry was not determined.

Compound **6** was obtained as a pale yellow viscous oil. HR-ESI-MS of **6** showed a pseudomolecular ion at *m/z* 489.2300 $[M+H]^+$ $C_{30}H_{33}O_6$ in HR-ESI-MS, which accorded to the molecular formula $C_{30}H_{33}O_6$. The ^{1}H-NMR spectrum of **6** showed the presence of three allyl moieties, three methoxy groups [δ_H 3.74 (3H, s), 3.82 (3H, s), 3.84 (3H, s)], five *m*-coupled protons [δ_H 6.30 (1H, d, J=1.7 Hz), 6.40 (1H, d, J=1.5 Hz), 6.51 (2H, br s), 6.75 (1H, d, J=1.7 Hz)], an *o*-coupled proton [δ_H 6.80 (1H, d, J=8.1 Hz)], and an *o*, *m*-coupled proton [δ_H 6.67 (1H, dd, J=8.1, 1,7 Hz)]. The ^{13}C-NMR spectrum of **6** showed the presence of three methoxy groups (δ_C 55.9, 56.2×2), three allyl moieties (δ_C 115.4, 115.9, 116.1, 136.8, 137.3, 137.7, 39.9, 40.9, 40.1), and 18 aromatic carbons (δ_C 106.8×2, 106.9, 110.7, 113.2, 120.4, 120.8, 130.4, 133.0, 134.4, 136.7, 137.4, 143.1, 146.1, 147.6, 150.8, 151.1, 153.2). These data indicate that **6** is an eugenol trimer. The three eugenol units of **6** were confirmed as two 1,2-dioxy-3-methoxy-5-allylbenzene and a 1-methoxy-2-oxy-5-allylbenzene structures from HMBC experiments, as shown in Fig. 2. Compound **6** should be derived by the phenol oxidation reaction between C-5 and oxygen at C-4 of eugenol. Thus, the structure of **6** was inevitably determined to be 1-allyl-4-(5-allyl-2-hydroxy-3-methoxyphenoxy)-3-(4-allyl-2-methoxyphnoxy)-5-methoxybenzene, and the compound was named tulsinol F.

Compound **7** was obtained as a colorless viscous oil. HR-ESI-MS of **7** showed a pseudomolecular ion at *m/z* 365.1401 $[M+Na]^+$ $C_{20}H_{22}O_5Na$ in HR-ESI-MS, which accorded to the molecular formula $C_{20}H_{22}O_5$. The ^{1}H-NMR spectrum of **7** showed the presence of an allyl group [δ_H 5.04 (1H, br d, J=10.0 Hz), 5.07 (1H, br d, J=16.8 Hz), 5.94 (1H, ddt, J=16.8, 10.0, 6.6 Hz), 3.30 (2H, d, J=6.6 Hz)], 1,2-dioxyphenoxy moiety [δ_H 6.49 (1H, dd, J=8.8, 2.7 Hz), 6.55 (1H, d, J=2.7 Hz), 6.81 (1H, d, J=8.8 Hz)], two *m*-coupled H [δ_H 6.57 (1H, d, J=1.5 Hz), 6.61 (1H, d, J=1.5 Hz)], two methoxy groups [δ_H 3.85 (3H, s), 3.86 (3H, s)], and a propene moiety [δ_H 3.33 (2H, d, J=7.3 Hz), 5.46 (1H, dt, J=12.0, 7.3 Hz), 6.44 (1H, d, J=12.0 Hz)]. The connecting pattern of these moieties was determined from the HMBC experiment of **7**. H-7 (δ_H 3.30, 2H, d, J=6.6 Hz) showed correlations to C-2, 6, and 9 (δ_C 121.6, 109.0, 115.4); H-2 (δ_H 6.57, 1H, d, J=1.5 Hz) to C-3, 4, 6, and 7 (δ_C 146.2, 141.6, 109.0, 40.0), H-9′ (δ_H 3.33, 2H, d, J=7.3 Hz)) to C-4, 6, and 7′ (δ_C 141.6, 109.0, 143.8); H-8′ (δ_H 5.46, 1H, dt, J=12.0, 7.3 Hz) to C-5, 7′, and 9′ (δ_C 126.1, 143.8, 27.3); H-7′ (δ_H 6.44, 1H, d, J=12.0 Hz) to C-1′ and 9′ (δ_C 151.0, 27.3), and so on, as shown in Fig. 2. In the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiment, H-7′ showed a correlation with H-9′. Thus the C-7′,8′ double bond was an *E* configuration, but the coupling constant (J=12.0 Hz) between H-7′ and H-8′ was small for ordinal *E* configuration. This could be explained from substitution of the electro-negative atom oxygen at C-7′. From these data, the structure of **7** was determined to be 3-(5-allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)-propene, and the compound was named tulsinol G.

Compounds **1** and **2** have very interesting structures. They should be synthesized from eugenol through oligomerization, thus they belong to the neolignan group in biosynthetic

Table 3. Leishmanicidal Activity of Isolated Compounds against *L. major*

Compound	IC_{50} (μg/ml)
1	>25
2	43.9
3	9.1
5	47.1
6	23.8
7	89.7
8	>25
10	0.9
11	13.6
12	16.9
13	17.1
14	2.2
15	>25
17	>25
18	358.7
19	73.9
21	>25
22	>25
Amphotericin B	0.04

standpoint, but they have a flavan-3-ol skeleton and belong to the flavone group in structural standpoint.

Some isolated compounds were tested for leishmanicidal activity against promastigotes of *Leishmania major* and the results are shown in Table 3. Known compounds, ferulaldehyde and ulsoric acid, showed strong leishmanicidal activity (IC_{50} 0.9 and 2.2 μg/ml, respectively). Of the new compounds, **3** showed strong activity (IC_{50} 9.1 μg/ml). Eugenol dimers **11** and **12** also showed activity (IC_{50} 13.6 and 16.9 μg/ml, respectively). Some of the new compounds showed medium activity, as shown in Table 3.

Experimental

UV and IR spectra were obtained by U-2001 (Hitachi) and FT-IR spectroscopy (Perkin Elmer). Optical rotations were measured by JASCO P-1010 polarimeter at room temperature. NMR spectra were recorded on a Unity INOVA 500 spectrometer (Varian Inc., Palo Alto, CA, U.S.A.). HR-ESI-TOF-MS spectra were obtained on a Micromass Q-Tof micro mass spectrometer (Waters Corp., Milford, MA, U.S.A.). Preparative and analytical HPLC was carried out on reverse-phase columns (Mighty sil RP-18 and 8, Kantho Chemical Co., Ltd.) with the CH_3CN–H_2O solvent system. Silica gel 60N (Kantho Chemical Co., Ltd.) was used for column chromatography. Analytical and preparative TLC were carried out on precoated Kieselgel $60F_{254}$ (Merck) and spots were visualized by spraying the plates with 50% H_2SO_4 solution, followed by heating.

Plant Material The crude drug, Tulsi, was purchased at a market in Kathmandu, Nepal, in August 2003. The botanical identification was made by Dr. A. Takano of Showa Pharmaceutical University. A voucher specimen is deposited in the laboratory of Natural Product Chemistry of the Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Shobara, Hiroshima, Japan.

Isolation of Constituents Dried leaves of Tulsi (*Ocimum sanctum* L.) (1 kg) were extracted with methanol (MeOH) under reflux to give MeOH extract (106 g). The MeOH extract was partitioned between ethyl acetate (AcOEt) and water to give an AcOEt layer and aqueous layer. The aqueous layer was partitioned with *n*-butanol (*n*-BuOH) to give a *n*-BuOH layer. The AcOEt layer and *n*-BuOH layer were evaporated to give AcOEt extract (37.4 g) and *n*-BuOH extract (20.3 g). Both extracts showed leishmanicidal activity, but the AcOEt extract showed many constituents on TLC analysis. Thus the AcOEt extract was chromatographed on a silica gel column using a gradient chloroform ($CHCl_3$)–MeOH solvent system to give 11 fractions, Fr. 1—Fr. 11. Fr. 2 (6.19 g) was purified by successive column chromatography and HPLC using an ODS column to give compounds **8** (1.8 g), **10** (3 mg), **12** (118 mg) and **17** (43 mg). Fr. 3 (4.96 g) was also purified by similar procedure to give compounds **2** (51 mg), **3** (7 mg), **5** (3 mg), **6** (5 mg), and **7** (5 mg); Fr. 4 (1.62 g) gave compounds **11** (71 mg), **15** (69 mg), and **23** (47 mg); Fr. 5 (2.93 g) gave compounds **4** (6 mg) and **10** (21 mg); Fr. 6 (4.05 g) gave compounds **1** (16 mg) and **22** (42 mg); Fr. 7 (2.93 g) gave compound, **13** (173 mg) and **14** (262 mg); Fr. 8 (2.82 g) gave compound **21** (15 mg); Fr. 9 (4.27 g) gave compounds **18** (72 mg) and **20** (9 mg); and Fr. 10 (1.98 g) gave compounds **9** (10 mg), **16** (104 mg), and **19** (19 mg).

Compound **1**: Colorless amorphous powder. $[\alpha]_D$ +1.5° (c=0.04, MeOH), HR-ESI-MS m/z: 365.1371 $[M+Na]^+$ (Calcd for $C_{20}H_{22}O_5Na$, 365.1365), IR ν_{max} cm^{-1} (KBr): 3433, 2361, 2343, 1495, 1272, 1223. UV λ_{max} nm (ε): 238 (10600), 279 (7100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **2**: Pale yellow viscous oil. $[\alpha]_D$ +1.5°(c=0.02, MeOH), HR-ESI-MS m/z: 511.2113 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$, 511.2097), IR ν_{max} cm^{-1} (KBr): 3444, 2935, 1594, 1511, 1495, 1269, 1222. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **3**: Pale yellow viscous oil. HR-ESI-MS m/z: 511.2119 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$; 511.2097). $[\alpha]$ +4.3° (c=0.03, MeOH). IR ν_{max} cm^{-1}: 3427, 2936, 1604, 1514, 1267, 1140, 1031. UV λ_{max} nm (ε): 215 (10300), 229 (9100), 281 (3500), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **4**: Pale yellow viscous oil. ESI-MS m/z: 543.2339 $[M+Na]^+$ (Calcd for $C_{31}H_{36}O_7Na$; 543.2359). IR ν_{max} cm^{-1}: 3418, 2936, 1670, 1595, 1512, 1270, 1139, 1032. ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **5**: Pale yellow viscous oil. $[\alpha]_D$ +8.0° (c=0.01, MeOH), HR-EI-MS m/z: 511.2113 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$, 511.2097), IR ν_{max} cm^{-1} (KBr): 3409, 2936, 1595, 1510, 1268, 1222, 1138. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **6**: Pale yellow viscous oil. HR-ESI-MS m/z: 489.2300 $[M+H]^+$ (Calcd for $C_{30}H_{33}O_6$; 489.2277). IR ν_{max} cm^{-1} (KBr): 3422, 2940, 1673, 1591, 1508, 1211, 1131, 1090. UV λ_{max} nm (ε): 222 (11300), 275 (2400), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **7**: Colorless viscous oil. HR-ESI-MS m/z: 365.1401 $[M+Na]^+$ (Calcd for $C_{20}H_{22}O_5Na$; 365.1365). UV λ_{max} nm (ε): 217 (7200), 233 (6800), 284 (2300), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Leishmanicidal Activity Test The leishmanicidal activities of isolated compounds were tested by an improved MTT method as follows. Basically, the reported method[20] was employed except that Tetra Color One {the mixture of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-disulfophenyl)-2*H*-tetrazorium, monosodium salt] and 1-methoxy PMS (1-methoxy-5-methylphenazinium methosukfate) (SeikagakuKogyou Co., Ltd.)} were used instead of MTT. Cultured promastigotes were centrifuged at 600 ***g*** at 4 °C for 5 min. The parasites were resuspended in each culture and diluted to a density of 1×10^5/ml. *L. major* promastigotes were seeded at 0.5×10^4/50 μl in medium/well in a 96-well microplate, and then a further 50 μl medium/well with different concentrations of test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well. Each concentration was tested in triplicate. As positive controls, amphotericin B and pentamidine were investigated. The microplate was incubated at 27 °C in 5% CO_2 for 72 h. Tetra Color One was added to each well and the plates were incubated at 27 °C for 6 h. Optical density at 630 nm was measured using a microplate reader (Molecular Devices Co., Ltd.). Leishmanicidal activity was expressed as the 50% inhibitory concentration (IC_{50}).

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Flavonol Caffeoylglycosides as α-Glucosidase Inhibitors from *Spiraea cantoniensis* Flower

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In the screening experiments for rat intestinal α-glucosidase inhibitors in 218 plants cultivated in the Japanese temperate region, potent maltase-inhibiting activity was found in the extract of flowers of *Spiraea cantoniensis*. The enzyme assay guided fractionation of the extract led to the isolation of three flavonol caffeoylglycosides, quercetin 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**1**), kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**2**), and kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside (**3**), as rat intestinal maltase inhibitors. This is the first report on the α-glucosidase-inhibitory activity of those flavonol caffeoylglycosides. Comparison in the activity of the isolates indicated the importance of caffeoyl substructures in the molecule for the α-glucosidase-inhibiting activity. The relatively high contents of the active isolates in the plant suggest that *S. cantoniensis* could be physiologically useful for treatment of diabetes.

KEYWORDS: *Spiraea cantoniensis*; α-glucosidase inhibitor; flavonol acylglycoside; caffeoyl group

INTRODUCTION

Diabetes mellitus is one of the most serious chronic diseases that is developing along with an increase in both obesity and aging in the general population (*1*). One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive organs (*2*). In recent years, many efforts have addressed the search for effective α-glucosidase inhibitors from natural sources in order to develop a physiological functional food or lead compounds for use against diabetes (*3*). In the course of our search for rat intestinal α-glucosidase-inhibiting principles from plants, we have isolated and identified several active compounds from a variety of plants grown not only in Japan (*4–6*) but also in Thailand (*7, 8*), China (*9, 10*), and Nepal (*11*). In this paper, we present results of a screen of temperate plants in Japan for α-glucosidase inhibition. In the screening experiments for rat intestinal maltase and/or sucrase inhibitors in 218 plants cultivated in Tsukuba, Japan, potent maltase-inhibiting activity was found in extracts of flowers of *Spiraea cantoniensis* (Rosaceae), an ornamental deciduous shrub. There have been only a few reports on the chemical constituents of this plant (*12–14*), but no medicinal usage is known, although some rosaceous plants are known to contain anthocyanins and soluble tannins showing antiglucosidase and antiamylase activities (*15*). Hence, the promising screening result prompted us to isolate and elucidate the structure of active compounds from this plant species.

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MATERIALS AND METHODS

Materials. Two hundred and eighteen species of Japanese temperate plants were cultivated and collected in the experiment field in Tsukuba, Japan. All voucher specimens are deposited in Tsukuba Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Tsukuba, Japan. All chemicals used were of reagent grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan) unless otherwise stated. All solvents were distilled before use.

General Procedure. NMR spectra were recorded on a Bruker AMX500 instrument (^{1}H, 500 MHz; ^{13}C, 125 MHz). Chemical shifts were determined relative to residual signals of methanol-d_4 as a solvent (δ_H 3.3 ppm, δ_C 49.0 ppm). Field desorption (FD) and fast atom bombardment (FAB) mass spectra were determined by a JEOL SX102A instrument. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Melting points were measured on a hot stage and are uncorrected.

Intestinal α-Glucosidase Inhibitory Activity Determination. The maltase- and sucrase-inhibitory activities designating an inhibition of maltose- and sucrose-hydrolyzing activities, respectively, in rat intestinal glucosidase complexes were measured as described previously (*10*). The crude enzyme solution prepared from rat intestinal acetone powder (Sigma-Aldrich Japan Co., Tokyo, Japan) was used as the small intestinal α-glucosidases, maltase and sucrase, showing specific activities of 0.70 and 0.34 U/mL, respectively. The reaction mixture consisted

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Table 1. NMR Assignments of **1**−**3** (Methanol-d_4)

	δ_C, ppm			δ_H, ppm (mult, J in Hz)		
	1	2	3	1	2	3
2	158.4	159.2	159.4			
3	135.6	135.6	135.2			
4	179.1	179.6	179.4			
5	162.6	162.9	162.9			
6	99.7	100.1	100.1	6.26(d, 2.1)	6.14(d, 1.7)	6.14(d, 2.0)
7	165.2	166.2	166.0			
8	94.7	94.9	94.9	6.51(d, 2.1)	6.32(d, 1.7)	6.31(d, 2.0)
9	157.9	158.5	158.4			
10	105.3	105.5	105.6			
1′	122.7	122.6	122.7			
2′	117.4	132.3	132.2	7.87(d, 2.2)	8.05(d, 9.0)	7.98(d, 8.9)
3′	145.3	116.2	116.1		6.84(d, 9.0)	6.81(d, 8.9)
4′	149.3	161.6	161.5			
5′	115.7	116.2	116.1	6.92(d, 8.5)	6.84(d, 9.0)	6.81(d, 8.9)
6′	123.2	132.3	132.2	7.70(dd, 8.5, 2.2)	8.05(d, 9.0)	7.98(d, 8.9)
1″	105.3	105.2	104.1	5.22(d, 7.9)	5.07(d, 7.9)	5.20(d, 7.4)
2″	72.5	72.9	75.8	3.83(dd, 9.6, 7.9)	3.80(dd, 9.9, 7.9)	3.47(m)
3″	74.6	74.9	78.0	3.66(dd, 9.6, 3.3)	3.56(dd, 9.9, 3.3)	3.47(m)
4″	69.5	70.2	71.7	3.90(dd, 3.3, 1.2)	3.80(dd, 3.3, 1.5)	3.34(m)
5″	74.2	74.8	75.7	3.81(ddd, 7.1, 4.9, 1.2)	3.73(ddd, 8.0, 4.4, 1.5)	3.44(m)
6″	63.8	64.3	64.3	4.21(dd, 11.9, 7.1) 4.26(dd, 11.9, 4.9)	4.12(dd, 11.3, 8.0) 4.32(dd, 11.3, 4.4)	4.18(dd, 11.9, 6.7) 4.28(dd, 11.9, 2.2)
1‴	127.5	127.7	127.7			
2‴	115.1	115.2	115.2	7.10(d, 2.0)	6.92(d, 1.6)	6.95(d, 1.7)
3‴	146.2	147.0	147.0			
4‴	148.7	149.5	149.5			
5‴	116.3	116.5	116.5	6.85(d, 8.1)	6.76(d, 8.1)	6.76(d, 8.4)
6‴	122.7	123.0	123.1	6.88(dd, 8.1, 2.0)	6.76(dd, 8.1, 1.6)	6.79(dd, 8.4, 1.7)
7‴	145.9	146.7	146.7	7.37(d, 16.0)	7.32(d, 16.0)	7.34(d, 15.8)
8‴	115.1	114.6	114.7	6.08(d, 16.0)	6.00(d, 16.0)	6.03(d, 15.8)
9‴	167.2	168.8	168.9			

of crude enzyme solution (0.05 mL of maltase and 0.2 mL of sucrase), substrate (maltose, 3.5 mM, 0.35 mL; sucrose, 56 mM, 0.2 mL) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample in 50% aqueous dimethyl sulfoxide (0.1 mL). After incubation for 15 min at 37 °C, the reaction was stopped by adding 0.75 mL of 2 M Tris-HCl buffer (pH 7.0). The reaction mixture was passed through a short column of basic alumina (ICN Alumina B, grade I, ICN Biomedical GmbH, Eschwege, Germany) to remove phenolic compounds, which might interfere with enzymatic glucose quantification in the following step. The amount of liberated glucose was measured by the glucose oxidase method, using a commercial test kit (Glucose B-test Wako, Wako Pure Chem. Co., Osaka, Japan).

Screening Experiment. The screening experiments for rat intestinal sucrase and maltase inhibition were carried out with extracts of 509 plant parts from 218 species. Each dried plant part was extracted with 50% aqueous methanol. The extracts were evaporated, redissolved in 50% aqueous dimethyl sulfoxide, and subjected as the test sample to the assay for rat intestinal α-glucosidase inhibitory activity at the final concentration of the extractable constituents obtained from 50 mg of plant material in 1 mL of solution.

Isolation of Quercetin 3-*O*-(6-*O*-Caffeoyl)-*β*-galactoside (1), Kaempferol 3-*O*-(6-*O*-Caffeoyl)-*β*-galactoside (2), and Kaempferol 3-*O*-(6-*O*-Caffeoyl)-*β*-glucoside (3) from *S. cantoniensis* Flower. Dried flowers (100 g) of *S. cantoniensis* were extracted with 50% aqueous methanol and the extracts were concentrated and partitioned between ethyl acetate and water. The inhibitory activity assay was carried out at the concentration of each fraction obtained from 0.1 g of plant material in 1 mL of reaction solution throughout the fractionation. The ethyl acetate fraction showed a strong inhibitory activity of 62% against maltase, whereas the sucrase-inhibitory activity was relatively low (36%). In contrast, the aqueous phase showed inconspicuous activities for both maltase (37%) and sucrase (26%). Hence, further fractionation was performed for isolating maltase inhibitors from the ethyl acetate fraction. This active fraction (6 g of dry weight) was charged onto a silica gel column and eluted with a chloroform−methanol gradient. The maltase-inhibitory activity was eluted in the chloroform−methanol (6:1) eluate (2.1 g of dry weight, 50% inhibition). The eluate was further purified by preparative HPLC (column, Inertsil PREP-ODS, 20 × 250 mm, GL-Science Co.; mobile phase, 27.5% MeCN in water; flow rate, 5.0 mL/min; detection, UV 254 nm). Eight principal peaks were detected in the region of t_R = 15−50 min. Among them, a peak eluted at t_R = 19.4 min, showing the highest activity of 38%, was rechromatographed under the same condition except for using 23% MeCN in water as the mobile phase to give **1** (33 mg, 0.03%, t_R = 39.2 min). A peak cluster that eluted at t_R = 22.5−25.4 min in the first HPLC, showing the second highest activity of 31%, was rechromatographed (column, Inertsil PREP-ODS, 20 × 250 mm × 2 (serial connection); mobile phase, 20% MeCN in water; flow rate, 7.0 mL/min (0−30 min) and 5.0 mL/min (30−120 min); detection, UV 254 nm) to give **2** (26 mg, 0.03%, t_R = 89.0 min) and **3** (29 mg, 0.03%, t_R = 101.8 min). **1**: yellow powder; mp 190−195 °C; $[\alpha]_D^{23}$ −11° (*c* 0.22, MeOH); FD-MS *m/z* 626 ($[M]^+$), 464 (M − 162), 302 (M − 162 × 2); FAB-HR-MS (negative) *m/z* 625.1185 ($[M - H]^-$, calcd for $C_{30}H_{25}O_{15}$, 625.1193). **2**: yellow powder; mp 210−212 °C; $[\alpha]_D^{23}$ −15° (*c* 0.22, MeOH); FD-MS *m/z* 611 ($[M + H]^+$), 448 (M − 162), 286 (M − 162 × 2); FD-HR-MS *m/z* 611.1423 (calcd for $C_{30}H_{27}O_{14}$, 611.1400). **3**: yellow powder; mp 205−208 °C; $[\alpha]_D^{23}$ −41° (*c* 0.24, MeOH); FD-MS *m/z* 611 ($[M + H]^+$), 448 (M − 162), 286 (M − 162 × 2); FD-HR-MS *m/z* 611.1397 (calcd for $C_{30}H_{27}O_{14}$, 611.1400). For ^1H and ^{13}C NMR data, see **Table 1**.

RESULTS AND DISCUSSION

In the screening experiment, 73 and 40 samples showed more than 50% enzyme−inhibitory activity for maltase and sucrase, respectively, out of 509 samples from 218 plant species. Among them, notable inhibitory activity (>90%) against rat intestinal maltase was observed in *Cicuta virosa* (leaf, 97%), *Akebia trifoliate* (berry, 97%), *Punica granatum* (seed, 95%; fruit skin, 100%), *Quercus myrsinaefolia* (seed, 90%), *Wisteria floribunda* (leaf, 95%), *S. cantoniensis* (flower, 91%), *Paeonia suffruticosa* (flower, 96%; leaf, 98%), *Paeonia lactiflora* (fruit, 97%; leaf, 94%), *Rheum unduratum* (root, 92%), and *Lythrum salicaria*

(leaf, 90%), and activity against sucrase was seen for *Akebia trifoliate* (berry, 96%), *Trichosanthes rostrata* (stem, 91%), *Chaenomeles sinensis* (leaf, 93%; stem, 99%), *Elaegnus umbellata* var. *rotundifolia* (fruit, 97%), and *Lythrum salicaria* (leaf, 93%). Among these promising species, we first chose extracts of *S. cantoniensis* flower for identifying active principles, since only a limited number of studies (*12–14*) have been carried out concerning chemical constituents of this species.

Dried flowers of *S. cantoniensis* were extracted with 50% aqueous methanol. After evaporation, the crude extracts were partitioned between ethyl acetate and water. The maltase-inhibiting activity was found principally in the ethyl acetate-soluble part. The active part was chromatographed on silica gel followed by HPLC purification to yield three major compounds, **1–3**.

Compound **1** showed a molecular ion at *m/z* 626 in FD-MS and the molecular formula was determined as $C_{30}H_{26}O_{15}$ from the high resolution FAB-MS analysis. The characteristic mass spectral fragments at *m/z* 464 (M − 162) and 302 (M − 162 × 2), resulting from successive loss of the 162 mass unit, suggested **1** to be a quercetin caffeoylglycoside. The ^{1}H NMR spectrum strongly supported this indication. The aromatic proton region (δ 6.0–8.0) contained 10 protons assignable to H-6, -8, -2′, -5′, and -6′ of quercetin and H-2‴, -5‴, -6‴, -7‴, and -8‴ of caffeic acid. These assignments were supported by the COSY cross peaks of two 1,2,4-trisubstituted benzenes and an isolated *trans*-olefin. The proton signals of a sugar unit appeared at δ 3.6–5.6 and could be fully correlated by COSY. The small coupling constants of 3.3 Hz between H-3″ (δ 3.66) and H-4″ (δ 3.90) and 1.2 Hz between H-4″ and H-5″ (δ 3.81) strongly supported the presence of galactose. In the HMBC spectrum, interunit cross peaks between the anomeric proton of the sugar (H-1″, δ 5.22) and C-3 (δ 135.6) of the aglycon and between the nonequivalent methylene protons (H-6″, δ 4.21 and 4.26) and the ester carbonyl (C-9‴, δ 167.2) were observed, which showed that the galactose was connected to the C-3 of quercetin and that caffeic acid was esterified with 6-OH of the galactose. Thus, **1** was concluded to be quercetin 3-*O*-(6-*O*-caffeoyl)-β-D-galactoside.

Compound **2** showed a pseudomolecular ion ($[M + H]^+$) at *m/z* 611 in FD-MS and the high-resolution analysis indicated the molecular formula of $C_{30}H_{26}O_{14}$. The mass spectral fragments at *m/z* 448 (M − 162) and 286 (M − 162 × 2) again indicated the presence of a caffeoylglycoside unit like **1**. The difference in molecular formula of **2** and **1** was one oxygen. Hence, **2** was suggested to be the kaempferol analog of **1**. The NMR spectrum of the aromatic region contained a pair of two-proton doublets (J = 9.0 Hz) at δ 6.84 and 8.05, being characteristic of a 4-hydroxyphenyl group in place of one of the 3,4-dihydroxyphenyls in **1**. The sugar unit was determined to be galactose in the similar manner as **1**, that is, J (H-3″/H-4″) = 3.3 Hz and J (H-4″/H-5″) = 1.5 Hz. The HMBC correlations confirmed the connectivity of each unit to be the same as that of **1**. The structure of **2** was determined to be kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside.

Compound **3** gave a similar spectral pattern to **2**, except for the sugar protons and carbons in the NMR spectra. Overlapping of H-2″, -3″, and -5″ at δ 3.44–3.47 and higher field resonance of H-4″ indicated the presence of glucose in **3** in place of galactose in **2** (*16, 17*). In addition, relatively higher chemical shifts of the sugar carbons also support the glucoside structure (*17, 18*). The structure of **3** was thus determined to be kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside.

Table 2. Inhibitory Activity of **1–3** and Related Compounds against Rat Intestinal Glucosidases

compd	IC_{50} (mM)	enzyme activity	ref
1	0.085	maltase	this work
2	0.35	maltase	this work
3	0.47	maltase	this work
4	(19%)[a]	maltase	(*29*)
5	(3%)[a]	maltase	(*29*)
6	(28%)[a]	maltase	(*29*)
7	(8%)[a]	maltase	(*29*)
8	0.029	sucrase	(*31*)
9	0.038	sucrase	(*31*)
10	1.89	maltase (immobilized)	(*3*)
11	1.91	maltase (immobilized)	(*3*)
12	18.9	maltase (immobilized)	(*3*)
13	0.024	maltase (immobilized)	(*3*)

[a] Percent inhibition at 0.5 mM.

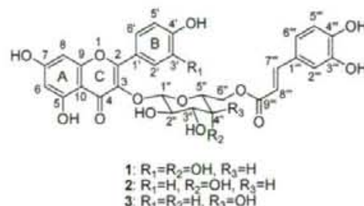

Figure 1. Structures of **1–3**.

Quercetin 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**1**) has been identified in several plants including *Hydrocotyle sibthorpioides* (*19*), *Scorzonera columnae* (*20*), *Polygonum viscosum* (*21*), *Blechnum novae-zelandiae* (*22*), *Monochaetum multiflorum* (*23*), and *Vaccinium corymbosum* (*24*), whereas kaempferol 3-*O*-(6-*O*-caffeoyl)-β-galactoside (**2**) was only isolated from *Conyza filaginoides* (*25*) and kaempferol 3-*O*-(6-*O*-caffeoyl)-β-glucoside from *Pteridium aquilinum* (*26*) and *Rubus ulmifolius* (*27*). However, this is the first report on the α-glucosidase-inhibitory activity of those compounds (**Table 2**). The similar extraction and fractionation of dried leaves of *S. cantoniensis* resulted in an isolation of **1** in 0.06% yield.

Among the isolates, the quercetin derivative **1** showed a higher activity (IC_{50} = 0.085 mM) than the kaempferol derivatives **2** (IC_{50} = 0.35 mM) and **3** (IC_{50} = 0.47 mM). The inhibition mode of **1** against rat intestinal maltase was determined to be mixed-inhibition type, as has been seen in other flavonoids (*29*) and the K_i value was calculated to be 110 μM. The moderate inhibitory activities of flavonols and their glycosides against mammalian intestinal glucosidases have been reported (*3, 30*). However, the maltase inhibitory activity (IC_{50} > 1 mM) of both quercetin and its glucoside was apparently lower than that of the caffeoylglycoside **1**. The substitution of the sugar moiety in flavonol glycosides by a phenolic acid, in particular, caffeic acid, could thus enhance their glucosidase inhibitory activity. In addition, the quercetin analogues having the caffeoyl substructure of C-2, -3, -4, and -1′–6′ in the B/C-rings of the flavonoid skeleton showed relatively higher glucosidase inhibitory activity than the corresponding kaempferol derivatives (*28, 29*). In the present case, the most potent compound, **1**, contains two caffeoyl substructures as the acyl substituent on the sugar and the B/C-rings of the aglycon, whereas **2** and **3** carry only one caffeoyl moiety. As shown in **Figures 1** and **2** and **Table 2**, the comparative study on the inhibitory activity of simple flavones and flavonols against rat intestinal glucosidase showed that luteolin (**4**) and quercetin (**6**) were more potent than apigenin (**5**) and kaempferol (**7**),

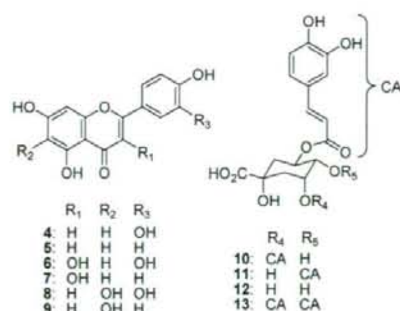

Figure 2. α-Glucosidase inhibitors having caffeoyl moieties.

respectively (*29*). This tendency was also seen in our structure−activity relationship study for baicalein (5,6,7-trihydroxyflavone) derivatives, in which 6-hydroxyluteolin (**8**) showed a higher activity than 6-hydroxyapigenin (**9**) (*31*). In any case, compounds possessing a caffeoyl substructure in the B/C ring of the flavone skeleton, **4**, **6**, and **8**, were more active than their *p*-coumaroyl counterparts, **5**, **7**, and **9**, respectively. In addition, in a series of caffeoylquinic acids, dicaffeoylquinic acids (**10**, **11**) showed a higher activity than monocaffeoyolquinic acid (chlorogenic acid, **12**), and a tricaffeoyl analogue (**13**) was much higher than those dicaffeoyl acids (*3*). These results strongly support the importance of a caffeoyl substructure in the molecule for exerting an effective glucosidase inhibitory activity. In contrast, the difference in sugar part, galactose and glucose, did not significantly affect the inhibitory activity.

In conclusion, the enzyme-assay guided fractionation of the extract from the dried flowers of *S. cantoniensis* led to the isolation of three flavonol caffeoylglycosides, **1**−**3**, as rat intestinal maltase inhibitors. The relatively high contents (0.03% each) of **1**−**3** in the plant compared to those in other plants (<0.002% *19, 21, 23–25*) suggest that *S. cantoniensis* could be physiologically useful for treatment of diabetes, although in vivo experiments are needed.

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