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Isolation of α-glusosidase inhibitors from hyssop (*Hyssopus officinalis*)

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Abstract

α-Glucosidase inhibitory activity was found in aqueous methanol extracts of dried hyssop (*Hyssopus officinalis*) leaves. Active principles against α-glucosidase, prepared from rat small intestine acetone powders, were isolated and characterized. The structures of these isolated compounds were determined to be (7*S*, 8*S*)-syringoylglycerol-9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside and (7*S*, 8*S*)-syringoylglycerol 9-*O*-β-D-glucopyranoside by analysis of physical and spectroscopic data (FDMS, ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC experiments) together with chemical syntheses.

Keywords: *Hyssopus officinalis*; Labiatae; Hyssop; α-Glucosidase inhibitor; Diabetes; Syringoylglycerol

1. Introduction

Diabetes is a lifestyle-related disease known to trigger many complications. Recently, the rapid increase in the already large number of patients has become a major health issue. The α-glucosidase complex in the small intestine is involved in sugar absorption. Thus, inhibitors of α-glucosidase would limit the absorption of dietary carbohydrates and in turn suppress postprandial hyperglycemia. Therefore, α-glucosidase inhibitors are promising drug candidates in the treatment and prevention of diabetes. Nojirimycin (Inouye et al., 1968; Reese and Parrish, 1968) and acarbose (Truscheit et al., 1981) are known to be powerful α-glucosidase inhibitors derived from microorganisms. However, the potency of these inhibitors precludes their use in humans without careful medical consultation. In the search for alternative α-glucosidase inhibitors, we speculated that analysis of the secondary metabolites synthesized by plants would be a promising line of inquiry, since plants are known to have many nutritional and health benefits. We surveyed a number of herbs and edible wild plants for α-glucosidase inhibitors, and reported the inhibitor from balsam pear (*Momordica charantia*) and *Grifola frondosa* (Matsuura et al., 2002). We report here the isolation and characterization of α-glucosidase inhibitors from hyssop.

2. Results and discussion

Extracts were obtained in MeOH–H_2O (7:3) of 13 varieties of herb; yarrow (*Achillea millefolium*), basil (*Ocimum basilicum*), spearmint (*Mentha spicata*), sage (*Saliva officinalis*), rocket (*Eruca vesicaria*), parsley (*Petroselinum crispum*), watercress (*Nasturtium officinale*), lemon balm (*Melissa officinalis*), chervil (*Anthriscus cerefolium*), oregano (*Origanum vulgare*), hyssop (*Hyssopus officinalis*), chamomile (*Matricaria chamomilla*), and tansy (*Tanacetum vulgare*). Each extract was divided into EtOAc and H_2O soluble fractions. The H_2O and EtOAc soluble layers were examined for their inhibitory activities. Both layers of oregano, tansy, and

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hyssop extract showed inhibitory activities (data not shown). Air-dried leaves of hyssop (1 kg) were soaked in MeOH–H_2O (7:3). The extract was partitioned with EtOAc and H_2O. The H_2O and EtOAc soluble layers were purified by a series of various chromatography techniques to obtain (7*S*, 8*S*)-syringoylglycerol 9-*O*-β-D-glucopyranoside (**1**, 565 mg) and (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**2**, 90 mg), respectively (Fig. 1). Compound **1** was obtained as a colorless residue with ions at *m*/*z* 405 $[M-H]^-$ (negative, matrix:TEA) and 429 $[M+Na]^+$ (positive, matrix:glycerol) by FAB-MS spectrometry. The molecular formula of heptaacetyl derivative (**3**), which was derived from **1**, was determined to be $C_{31}H_{40}O_{18}$ by HR-FD–MS spectrometry. Thus, the molecular formula of **1** was determined to be $C_{17}H_{26}O_{11}$. Spectroscopic data including the specific rotation, $[\alpha]_D^{23}$ −17.7° (*c* = 0.5, MeOH), agreed well with previously reported data (Kijima et al., 1997), and therefore, the planar structure of **1** was determined to be as in Fig. 1. The analysis of ^1H NMR, ^{13}C NMR, DEPT, HMQC, and HMBC spectral data gave the total ^1H and ^{13}C assignments for **1,** as in Tables 1 and 2, respectively.

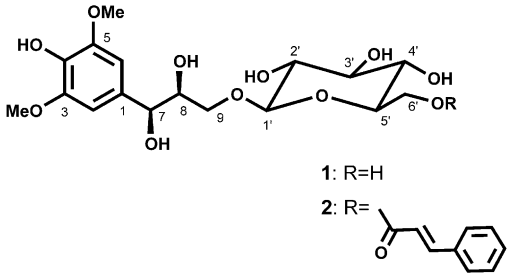


Fig. 1. α-Glucosidase inhibitors isolated from hyssop (*H. officinalis*).

In order to ascertain the absolute configurations of C-7 and 8, compound **1** was converted according to Scheme 1 to afford (7*S*, 8*S*)-4-*O*-methylsyringoylglycerol (**5**), with a specific rotation of $[\alpha]_D^{23}$ −21.1° (*c* = 0.59, MeOH). Independently, (7*R*, 8*R*)-4-*O*-methylsyringoylglycerol (**8**) was synthesized according to Scheme 2 using 3, 4, 5-trimethoxycinnamic acid (**6**) as a starting material. Its chirality of (7*R*, 8*R*) was introduced by a reaction of the catalytic asymmetric dihydration developed by Kolb et al. (1994) using the reagent of AD-mix-β, and the total assignment of ^1H for **8** was performed by a ^1H–^1H COSY experiment. The ^1H NMR, IR, and EI-MS spectra of (7*R*, 8*R*)-4-*O*-methylsyringoylglycerol (**8**) agreed well with those of **5**, but the direction of its specific rotation, $[\alpha]_D^{23}$ −17.0° (*c* = 0.50, MeOH), was opposite. Therefore, the absolute configurations of C-7 and -8 of **1** were determined to be (7*S*) and (8*S*), respectively. Although the expected $[\alpha]_D^{23}$ of **8** should be close to −21.1°, the observed value was due to incomplete enantio-selectivity of catalytic asymmetric dihydroxylation conducted by our group.

Table 1
^1H NMR (270 MHz, CD_3OD) assignments of **1** and **2**

	1	**2**
H-2	6.62 (1H, *s*)	6.62 (1H, *s*)
H-6	6.62 (1H. s)	6.62 (1H, *s*)
H-7	4.54 (1H, *d*, *J* = 6.2 Hz)	4.59 (1H, *d*, *J* = 6.5 Hz)
H-8	3.73 (1H, *m*)	
H-9a	3.63 (1H, m)	3.73–3.20 (3H, *m*)
H-9b	3.47 (1H, *dd*, *J* = 10.0, 3.5 Hz)	
H-1′	4.15 (1H, *d*, *J* = 7.6 Hz)	4.20 (1H, *d*, *J* = 7.6 Hz)
H-2′	3.30–3.10 (4H, *m*)	3.73–3.20 (4H, *m*)
H-3′		
H-4′		
H-5′		
H-6′a	3.73 (1H, *m*)	4.46 (1H, *dd*, *J* = 13.0, 2.1 Hz)
H-6′b	3.55 (1H, *dd*, *J* = 11.6, 5.4 Hz)	4.23 (1H, *dd*, J 13.0, 6.2 Hz)
		6.43 (1H, *d*, *J* = 16.1 Hz)
H3″		7.58 (1H, *d*, *J* = 16.1 Hz)
H5″		7.49 (1H, *m*)
H6″		7.33 (1H, *m*)
H-7″		7.33 (1H, *m*)
H-8″		7.33 (1H, *m*)
H-9″		7.49 (1H, *m*)
Ph–OCH_3	3.77 (6H, *s*)	3.73 (6H, *s*)

Table 2
^{13}C NMR (67.5 MHz, CD_3OD) assignments of **1** and **2**

	1	**2**
C-1	133.6	133.5
C-2	105.3	105.1
C-3	148.9	148.8
C-4	135.9	135.7
C-5	148.9	148.8
C-6	105.3	105.1
C-7	75.5	75.3
C-8	76.0	75.9
C-9	72.0	72.1
C-1′	104.6	104.9
C-2′	71.7	71.8
C-3′	75.2	75.1
C-4′	77.9	75.2
C-5′	78.0	77.7
C-6′	62.8	64.9
C-1″		168.2
C-2″		118.4
C-3″		146.4
C-4″		135.5
C-5″		129.2
C-6″		129.9
C-7″		131.4
C-8″		129.9
C-9″		129.2
Ph–O$\underline{C}H_3$	56.9	56.7

Compound **2** was obtained as a colorless residue and gave a molecular ion at m/z 536 $[M]^+$ by FD–MS spectrometry. Compound **2** was treated with a solution of pyridine and acetic anhydride to afford **9**, whose molecular formula was determined to be $C_{38}H_{44}O_{18}$ (m/z 788.2508 $[M]^+$) by HR-FD–MS spectrometry. Thus, the molecular formula of **2** was determined to be $C_{26}H_{32}O_{12}$. Compounds **1** and **2** showed similar features in their ^1H and ^{13}C NMR spectra (Tables 1 and 2), and the acetyl-derivatives also exhibited similar tendencies in ^1H and ^{13}C NMR spectra. The ^{13}C NMR resonance of δ 168.2 of **2** suggested that an ester moiety existed in the structure. As shown in Scheme 3, alkaline hydrolysis of **2** afforded **1** and **10**. Compound **10** was identified as cinnamic acid since spectral data of EI-MS, ^1H and ^{13}C NMR of **10** agreed closely with those of an authentic specimen of cinnamic acid (data not shown). This result revealed that compound **2** was composed of **1** and cinnamic acid in its ester form, and the partial structures were connected by HMBC correlation to construct the structure of **2** (Fig. 2). The analysis of ^1H NMR, ^{13}C NMR, DEPT, HMQC, and HMBC spectral data gave the total ^1H and ^{13}C assignments for **2**, as in Tables 1 and 2, respectively.

The inhibitory activities of **1** and **2** were compared with 1-deoxynojirimycin (**11**) (Yoshikuni, 1988) (Fig. 3). Compounds **1** and **2** exhibited 53 and 54% inhibitory activity at the concentration of 3×10^{-3} M, but 1-deoxynojirimycin (**11**) exhibited 58% inhibitory activity at 3×10^{-7} M. Compared with the activity of 1-deoxynojirimycin (**11**), those of **1** and **2** were very low. However, the activity of 1-deoxynojirimycin (**11**) is too potent to administer without medical supervision. On the other hand, hyssop has been consumed as a food material for a long time, thus it has a proven safety record. If the plant is regularly incorporated into the diet, it could compensate for the low α-glucosidase inhibitory activity.

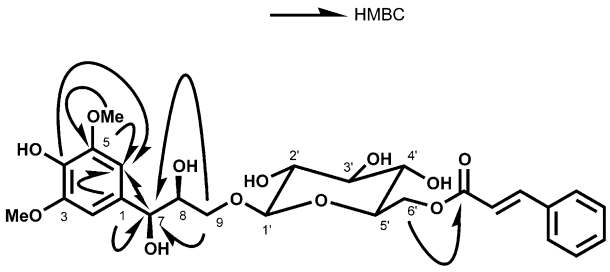
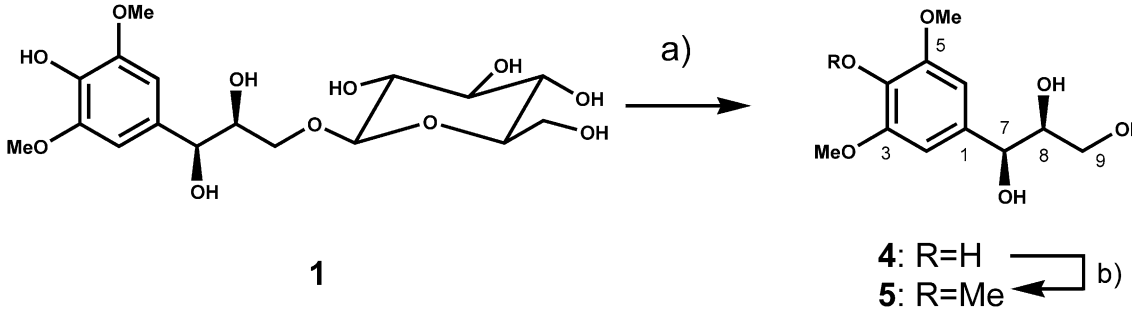
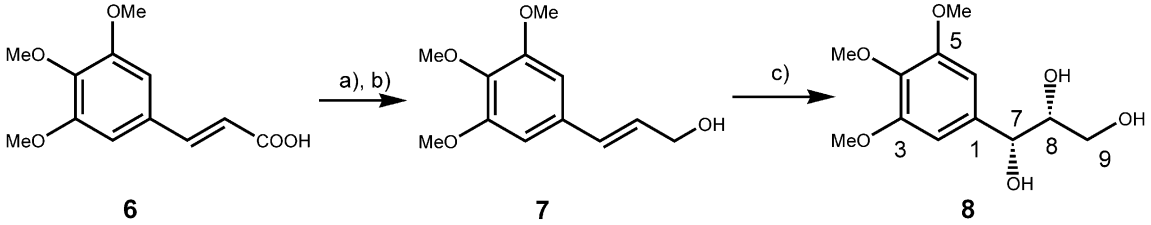


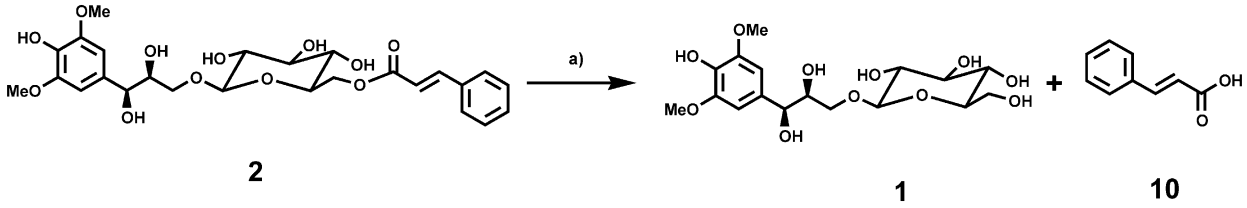
Fig. 2. The important HMBC correlations of compound **2**.



Scheme 1. (a) β-Glucosidase/0.1 N $AcONH_4$–AcOH buffer (pH 5.6), 37 °C (67%) (b) CH_2N_2/MeOH, 0 °C (35%).



Scheme 2. (a) CH_2N_2/MeOH at 0 °C, (b) LAH/THF at 0 °C (28%), (c) AD-mix-β at 4 °C (9%).



Scheme 3. (a) 1% NaOH/EtOH, room temp. (76% for **1**, 74% for **10**).

In our search for α-glucosidase inhibitors from edible plants, compound **1** was rediscovered from hyssop (*H. officinalis*) and we determined its absolute configuration. In addition, a new related compound **2** was identified. It is hoped that these preliminary experiments will provide the basis for further examination of the suitability of hyssop as a medicinal supplement that contributes toward the treatment and prevention of diabetes.

3. Experimental

3.1. General

Fast atom bombardment mass spectra (FAB-MS) were obtained on a Jeol HX-100, and field desorption mass spectra (FD–MS) and electron impact mass spectra (EI-MS) were on Jeol JMS-O1SG-2 and JMS-DX-300 mass spectrometers, respectively. NMR spectra were recorded on a Bruker AM-500 (^{1}H at 500 MHz) and Jeol JNM-EX 270 FT NMR system (^{1}H at 270 MHz; ^{13}C at 67.5 MHz). In the ^{1}H NMR spectra, chemical shifts are reported using TMS as an internal standard. In the ^{13}C NMR spectra, chemical shifts are reported as δ (ppm) values relative to the carbon signals (δ 49.0) and (δ 77.0) of CD_3OD and $CDCl_3$, respectively. IR spectra were on Perkin Elmer System 2000 FT-IR spectrometer.

Medium pressure liquid chromatography (MPLC): EFC-1000 pump (Tokyo Rikakiki, stroke length, 50; stroke rate, 70), Lobar LiChroprep RP-18 (Merck, 440 × 37 mm), UV-absorbance (A_{254nm}); flash column chromatography (FCC): EFC-1000 pump (Tokyo Rikakiki), Wakogel (Wako Pure chem., C-200); Sephadex LH-20 (Amersham Pharmacia Biotech); Diaion HP-20 (Mitsubishi Chem.); TLC, Merck silica gel 60 F_{254} (Art 5554); HPLC : Hitachi liquid chromatograph L-7000 series system.

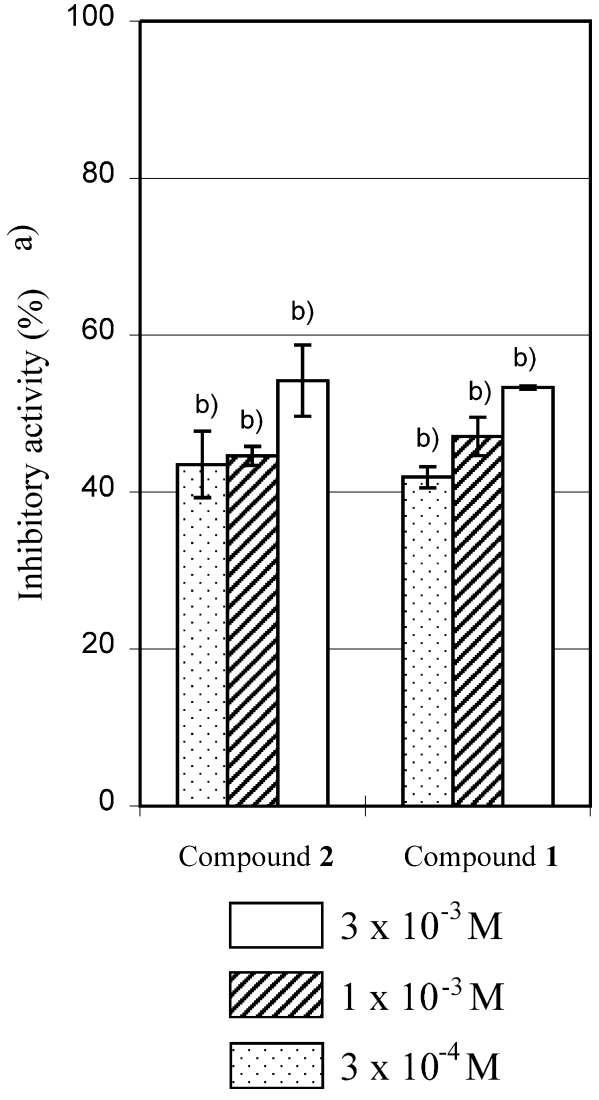


Fig. 3. α-Glucosidase inhibitory activities of **1** and **2** (a) 1-deoxynojirimysin showed its inhibitory activities of 21 and 58% at 1×10^{-8} M and 3×10^{-7} M, respectively; (b) bars represent the means±S.D. of three measurements.

3.2. Plant material

Yarrow (*A. millefolium*) grew wild, and basil (*O. basilicum*), spearmint (*M. spicata*), sage (*S. officinalis*), rocket (*E. vesicaria*), parsley (*P. crispum*), watercress (*N. officinale*), lemon balm (*M. officinalis*), and chervil (*A. cerefolium*) were harvested from the experimental field of the Plant Ecochemicals Research Center. Air-dried aerial parts of oregano (*O. vulgare*), hyssop (*H. officinalis*), chamomile (*M. chamomilla*), tansy (*T. vulgare*) were purchased from Koyu-Seikatsu, Inc. (Kitami, Hokkaido, Japan).

3.3. Chemicals

1-Deoxynojirimycin (**11**) and glucose B test were purchased from Wako pure chemical industries, Ltd. Rat intestinal acetone powder, 3, 4, 5-trimethoxycinnamic acid, and AD-mix-β were purchased from Sigma Aldrich Japan Co., Tokyo.

3.4. Assay for rat intestinal α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was measured according to the described method (Toda et al., 2001). A crude enzyme solution was prepared from rat intestinal acetone powder. Before the amount of glucose derived from sucrose in the reaction mixture was measured, the reaction mixture was passed through an alumina column (1.6 g, ICN Alumina B, ICN Biomedicals GmbH) in order not to hinder the glucose B test by phenolic compounds.

3.5. Extraction and separation

Air-dried leaves of hyssop (1 kg, *H. officinalis*) were soaked in MeOH–H_2O (7:3). After filtration using filter paper (No. 1, Toyo), the filtrate was concentrated in vacuo to about 1.5 l. The resulting extract was partitioned between EtOAc (1 l × 3) and H_2O to afford H_2O soluble and EtOAc soluble layers.

3.5.1. Purification (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**1**)

The H_2O soluble layer was concentrated under reduced pressure to about 500 ml. To the concentrated extract was added a solution of EtOH (2 l), and the mixture was allowed to stand at − 25 °C for 48 h. The supernatant and precipitation were separated with filter paper (No. 1, Toyo). The solution of the supernatant was concentrated in vacuo, and the resultant oil was subjected to Diaion HP-20 (500 g). The resins were successively washed by H_2O (2.5 l), MeOH–H_2O (7:3) (4 l), and MeOH (2 l). The eluent of MeOH–H_2O (7:3), that exhibited α-glucosidase inhibitory activity, was applied to Sephadex LH-20 (MeOH–H_2O 7:3) to yield an active fraction A, which was subjected to MPLC (MeOH–H_2O–HOAc 50:50:0.1) to give an active fraction B. Further purification of fraction B by HPLC (GL Sciences Inc., Inertsil ODS, 20 × 250 mm, MeOH–H_2O–HOAc 15:85:0.1, flow rate: 2 ml/min, A_{254nm}) followed by another purification by HPLC (Kanto chemical Co. Inc., Mightysil RP-18, 4.6 × 250 mm, CH_3CN–H_2O–HOAc 8:92:0.1, flow rate: 1 ml/min, A_{254nm}) afforded (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**1**) (565 mg)

3.5.2. (7*S*, 8*S*)-Syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**1**)

Colorless oil; $[\alpha]_D^{23}$ −17.7° ($c = 0.5$, MeOH); FAB-MS (positive, matrix: glycerol) *m*/*z* (rel. int.): 429 $[M+Na]^+$ (100); FAB-MS (negative, matrix: TEA) *m*/*z* (rel. int.): 405 $[M-H]^-$ (10.8); ^{1}H NMR (270 MHz, CD_3OD): Table 1; ^{13}C NMR (67.5 MHz, CD_3OD): Table 2.

3.5.3. Purification of (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**2**)

The EtOAc-soluble layer was concentrated under reduced pressure, and the resultant black oil was subjected to Diaion HP-20 (500 g). The resins were successively washed by MeOH–H_2O (1:1) (3 l), MeOH–H_2O (7:3) (3 l), and MeOH (3 l). The eluate of MeOH–H_2O (1:1), that showed the inhibitory activities, was applied to a silica gel (Wakogel C-200, Wako Pure Chem., 350 g) CC. The column was successively eluted with $CHCl_3$ (2 l), MeOH–$CHCl_3$ (5:95) (1 l), MeOH–$CHCl_3$ (1:9) (1 l), MeOH–$CHCl_3$ (3:7) (2 l), and MeOH–$CHCl_3$ (4:6) (1 l). The eluate of MeOH–$CHCl_3$ (1:9) and MeOH–$CHCl_3$ (3:7), that exhibited the inhibitory activities, were combined and concentrated in vacuo. The resultant black oil was subjected to Sephadex LH-20 (250 g) with solvent MeOH–$CHCl_3$ (7:3) to afford active fraction C, which was applied to FCC using MeOH–$CHCl_3$ (1:9) to afford active fraction D. Further purification of fraction D by MPLC (MeOH–H_2O–HOAc 50:50:0.1) followed by another purification by HPLC (YMC, YMC-Pack ODS-AM, 300 × 10 mm, MeOH–H_2O–AcOH 40:60:0.1, flow rate: 2.0 ml/min, A_{254nm}) to afford (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**2**) (90 mg).

3.5.4. (7*S*, 8*S*)-Syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**2**)

Colorless oil; $[\alpha]_D^{23}$ − 31.5° ($c = 1.0$, MeOH); FD–MS *m*/*z* (rel. int.): 536 $[M]^+$ (100); ^{1}H NMR (270 MHz, CD_3OD):Table 1; ^{13}C NMR (67.5 MHz, CD_3OD): Table 2.

3.6. Preparation of heptaacetylderivative (**3**)

To a stirred mixture of **1** in pyridine was added a solution of acetic anhydride, and the reaction mixture was further stirred for 24 h at room temperature. The usual work-up was employed followed by the purification of the extract to afford **3** quantitatively.

3.6.1. Heptaacetylderivative (**3**)

FD–MS *m*/*z* (rel. int.) 700 $[M]^+$ (100); HR-FD–MS *m*/*z*: 700.2190 $[M]^+$ (calc. For $C_{31}H_{40}O_{18}$: 700.2214); IR ν_{max} (NaCl) cm^{-1}: 2944, 1755, 1222, 1040; ^{1}H NMR (270 MHz, CD_3OD): δ 6.62 (2H, *s*), 5.92 (1H, *d*, $J = 8.1$ Hz), 5.25 (1H, *m*), 5.22 (1H, *t*, $J = 9.2$, H-3′), 5.06 (1H, t, $J = 9.7$ Hz, H-4′), 5.01 (1H, *dd*, $J = 9.7$, 7.8 Hz, H-2′), 4.52 (1H, *d*, $J = 7.7$ Hz, H-1′), 4.24 (1H, *dd*, $J = 12.2$, 4.7, H-6′a), 4.06 (1H, *dd*, $J = 12.2$, 2.4, H-6′b), 3.83 (3H, *s*), 3.66 (3H, *m*), 2.32 (3H, *s*), 2.15 (3H, *s*), 2.09 (3H, *s*), 2.07 (3H, *s*), 2.06 (3H, *s*), 2.02 (3H, *s*).

3.7. Preparation of hexaacetylderivative (**9**)

To a stirred mixture of **2** in pyridine was added a solution of acetic anhydride, and the reaction mixture was further stirred for 24 h at room temperature. The usual work-up was employed followed by the purification of the extract to afford **9** quantitatively.

3.7.1. Hexaacetylderivative (**9**)

FD–MS *m*/*z* (rel. int.): 788 $[M]^+$ (100), 494 (40); HR-FD–MS *m*/*z*: 788.2508 $[M]^+$ (calc. For $C_{38}H_{44}O_{18}$: 788.2527); IR ν_{max} (NaCl) cm^{-1}: 2944, 1756, 1219, 1038; ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.65 (1H, *d*, $J = 16.0$ Hz), 7.50 (2H, *m*), 7.35 (3H, complex), 6.59 (2H, *s*), 6.40 (1H, *d*, $J = 16.0$ Hz), 5.90 (1H, *d*, $J = 7.9$ Hz), 5.24 (1H, *m*), 5.22 (1H, *br.t*, $J = 9.4$ Hz), 5.07 (1H, *br.t*, $J = 10.0$ Hz), 4.92 (1H, *dd*, $J = 9.5$, 7.8 Hz), 4.52 (1H, *d*, $J = 7.6$

Hz), 4.26 (2H, *m*), 3.78 (6H, *s*), 3.74–3.56 (3H, complex), 2.26 (3H, *s*), 2.12 (3H, *s*), 2.04 (3H, *s*), 2.02 (3H, *s*), 2.00 (3H, *s*), 1.99 (3H, *s*). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 170.0, 169.8, 169.4, 169.2, 168.3, 166.2, 152.1, 145.6, 134.1, 134.0, 130.3, 128.7, 128.1, 117.0, 103.9, 101.0, 74.0, 73.6, 72.6, 71.9, 71.2, 68.6, 67.2, 62.0, 56.2, 21.0, 20.9, 20.7, 20.6, 20.5.

3.8. Preparation of (7S, 8S)-syringoylglycerol (4)

To a stirred mixture of **2** (146 mg, 0.27 mmol) in 3 ml of 0.1 N $AcONH_4$–HOAc buffer (pH 5.6) was added β-glucosidase (5 mg), and the mixture was shaken (100 rpm) at 37 °C for 24 h. The mixture was put directly onto the cartridge column of Bond Elut C_{18}. The column was washed with H_2O (3 ml × 3) and then with MeOH–H_2O (7:3) (3 ml × 3). The volatile components of the MeOH–H_2O (7:3) eluents were removed to give **4** (45 mg, 0.18 mmol, 67%).

3.8.1. (7S, 8S)-Syringoylglycerol (4)

FD–MS *m*/*z* (rel. int.) 244 $[M]^+$ (100); IR ν_{max} (NaCl) cm^{-1}: 3360, 2940, 1614, 1519, 1463, 1428, 1216, 1113, 750; ^{1}H NMR (270 MHz, CD_3OD): δ 6.70 (2H, *s*), 4.55 (1H, *d*, *J*= 5.9 Hz), 3.86 (3H, *s*), 3,69 (1H, *m*), 3.50 (1H, *dd*, *J*= 11.2, 3.7 Hz), 3.30 (1H, *dd*, *J*= 11.2, 6.2 Hz); ^{13}C NMR (67.5 MHz, CD_3OD): δ 148.9, 135.8, 133.9, 105.0, 77.5, 75.5, 64.2, 56.7.

3.9. Preparation of (7S, 8S)-4-O-methylsyringoylglycerol (5)

To a stirred mixture of **4** (16 mg, 0.065 mmol) in MeOH (10 ml) cooled with an ice bath was added excess CH_2N_2. The reaction mixture was further stirred for 1 h at 0 °C. The volatile components were removed under reduced pressure to give a colorless residue, which was purified using preparative TLC (MeOH–$CHCl_3$ 1:9) to afford **5** (5.9 mg, 0.022 mmol, 35%).

3.9.1. (7S, 8S)-4-O-Methylsyringoylglycerol (5)

$[\alpha]_D^{23}$ 21.1° (*c* = 0.59, MeOH) ; EI-MS *m*/*z* (rel. int): 258 (34), 198 (12), 197 (100), 196 (34), 169 (41), 154 (17), 138 (20); HR-EI-MS *m*/*z*: 258.1096 $[M]^+$ (calc. for $C_{12}H_{18}O_6$: 258.1103); IR ν_{max} (NaCl) cm^{-1}: 3383, 2940, 1593, 1506, 1463, 1422, 1328, 1235, 1125, 1005, 754; ^{1}H NMR (CD_3OD, 500 MHz); δ 6.71 (2H, s, H-2′ and -6′), 4.59 (1H, *d*, *J* = 2.9 Hz, H-3), 3.84 (6H, s, C-3′-OMe and C-5′-OMe), 3.74 (3H, s, C-4′-OMe), 3.67 (1H, m, H-2), 3.54 (1H, *dd*, *J* = 6.1, 2.4 Hz, H-1a), 3.40 (1H, *dd*, *J* = 6.1, 3.3 Hz, H-1b).

3.10. Preparation of (2E) 3-(3′, 4′, 5′-trimethoxyphenyl) prop-2-en-1-ol (7)

To a stirred mixture of 3, 4, 5-trimethoxycinnamic acid (**6**, 952 mg, 4 mmol) in MeOH (20 ml) cooled with ice bath was added excess CH_2N_2, and the reaction mixture was further stirred at 0 °C for 1 h. The volatile components were removed under reduced pressure to give a residue. The residue was reduced using LAH (182 mg) in THF (50 ml) to give **7** (250 mg, 28%)

3.10.1. 3-(3′, 4′, 5′-Trimethoxyphenyl) prop-2-en-1-ol (7)

IR ν_{max} (NaCl) cm^{-1}: 3405, 2940, 1584, 1507, 1240, 1127, 1008; EI-MS *m*/*z* (rel. int): 224 (100), 195 (29), 182 (57), 181 (59), 83 (28); ^{1}H NMR (270 MHz, CD_3OD): δ 6.61 (2H, *s*), 6.53 (1H, *d*, *J* = 15.9 Hz), 6.28 (1H, dt, *J* = 15.9, 5.4 Hz), 4.32 (1H, *d*, *J* = 5.4 Hz), 3.87 (6H, *s*), 3.84 (3H, *s*).

3.11. Preparation of (7R, 8R)-4-O-methylsyringoylglycerol (8)

To a stirred mixture of $CH_3SO_2NH_2$ (64 mg, 0.67 mmol) and AD-mix-β (929 mg) in a solution of *t*-BuOH–H_2O (1:1) (3 ml) was added a solution of **7** (150 mg, 0.67 mmol) in a solution of *t*-BuOH–H_2O (1:1) (7 ml). The reaction mixture was stirred at 4 °C for 24 h. To the reaction mixture was added sodium sulfite (640 mg, 5.1 mmol), and the mixture further stirred for 1 h at room temp. The reaction mixture was poured into a mixture of EtOAc (300 ml) and H_2O (150 ml). The EtOAc layer was washed with satd. aq. NaCl (50 ml × 2) and dried over Na_2SO_4. The volatile components of the EtOAc layer was removed under reduced pressure to give a residue, which was purified by preparative TLC (MeOH–$CHCl_3$ 9:41) to afford **8** (15 mg, 0.058 mmol, 9%).

3.11.1. (7R, 8R)-4-O-Methylsyringoylglycerol (8)

$[\alpha]_D^{23}$ −17.0° (*c* = 0.50, MeOH); HR-EI-MS *m*/*z*: 258.1115 $[M]^+$ (calc. for $C_{12}H_{18}O_6$: 258.1103).

3.12. Alkaline hydrolysis of 2

To a stirred solution of 1% NaOH/EtOH (25 ml) was added a solution of **2** (25 mg, 0.046 mmol) in MeOH (10 ml), and the reaction mixture was stirred for 5 h. The reaction mixture was treated with the resins of amberlite 120 B (Organo, 150 g). The resins was washed with MeOH (50 ml). The combined eluents were concentrated under reduced pressure, and the resultant residue was dissolved in 1 ml of MeOH–H_2O (1:9) and put onto the cartridge column of Bond Elut C_{18}. The column was washed with MeOH–H_2O (1:9) (3 ml × 2) and then with MeOH–H_2O (4:1) (3 ml × 2). The eluate of MeOH–H_2O (1:9) was concentrated under reduced pressure to give **1** (14 mg, 0.035 mmol, 76%), and the eluate of MeOH–H_2O (4:1) to afford **10** (50 mg, 0.034 mmol, 74%).

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