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Water-Soluble Constituents of Fennel. II.[1] Four *erythro*-Anethole Glycol Glycosides and Two *p*-Hydroxyphenylpropylene Glycol Glycosides

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Four *erythro*-anethole glycol monoglucosides were isolated from the methanolic extract of fennel, the fruit of *Foeniculum vulgare* MILLER (Umbelliferae). From chemical and spectroscopic evidence, they were characterized as (1′*S*,2′*R*)-*erythro*-anethole glycol 1′-*O*-β-D-glucopyranoside, (1′*R*,2′*S*)-*erythro*-anethole glycol 1′-*O*-β-D-glucopyranoside, (1′*S*,2′*R*)-*erythro*-anethole glycol 2′-*O*-β-D-glucopyranoside, (1′*R*,2′*S*)-*erythro*-anethole glycol 2′-*O*-β-D-glucopyranoside, respectively. Along with these glycosides, two new glycosides of *p*-hydroxyphenylpropylene glycol were obtained and characterized as *threo*-1′-(4-hydroxyphenyl)propane-1′,2′-diol 4-*O*-β-D-glucopyranoside (a mixture of two stereoisomeric forms) and 1′-(4-hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-glucopyranoside (an epimeric mixture at C-2′) from spectral evidence.

This is the first report of the isolation of all four *O*-β-D-glucopyranosides which have the glucosyl unit attached to the hydroxy group of the *erythro*-glycol function of individual stereoisomeric aglycones.

Key words fennel; *Foeniculum vulgare* fruit; four *erythro*-anethole glycol glucoside; phenylpropylene glycol glycoside; ^{13}C-NMR

Fennel [Japanese name "Uikyoh," the fruit of *Foeniculum vulgare* MILLER (Umbelliferae)] is a well known aromatic herb and its fruit is used worldwide as an important medicine and spice. Fennel contains an essential oil accounting for 3—8% of its total weight, and anethole, estragole and *p*-anisaldehyde have been identified as the main components of this oil.[2] As anethole-related compounds, we have already reported the isolation of *erythro*- and *threo*-anethole glycols (**1** and **1′**).[3] Herein, we describe the isolation and structure elucidation of four new anethole glycol glucosides and two new *p*-hydroxypenylpropylene glycol glycosides from fennel.

The methanolic extract of commercial fennel was dissolved in water and successively extracted with ether and ethyl acetate. The aqueous layer was evaporated and the residue was chromatographed on Amberlite XAD-II to give water and methanol eluates. The methanol eluate was subjected to a combination of Sephadex LH-20, silica gel and Lobar RP-8 column chromatography to obtain two fractions consisting of an anethole glycol glucoside mixture and a *p*-hydroxyphenylpropylene glycol glucoside mixture. Finally, the mixtures were separated into four and two components, respectively, by HPLC using reversed-phase and carbohydrate analysis columns as described in the Experimental section.

Glycosides I (**2**, $C_{16}H_{24}O_8$, $[\alpha]_D^{26}$ +13.8°) and II (**3**, $C_{16}H_{24}O_8$, $[\alpha]_D^{26}$ −61.0°) showed $[M+Na]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 367 and 165 in the positive FAB-MS. The mixture of **2** and **3** was hydrolyzed with hesperidinase and, from the hydrolyzed mixture, D-glucose and an aglycone ($C_{10}H_{14}O_3$) identified as **1** were obtained. The ^{1}H- and ^{13}C-NMR chemical shifts (Tables 1 and 2) of **2** and **3** indicated them to be stereoisomeric in nature and monoglucosides of **1**. The fact that the β-glucosyl units are attached to the individual aglycones by the hydroxyl groups at C-1′ was evident from the downfield shift of the C-1′ signals when compared with those of **1** [**1**: δ 78.09, **2**: δ 86.03, **3**: δ 83.39], and the observed nuclear Overhauser effect (NOE) interactions between the anomeric proton signals and H-1′ signals in their nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectra. Since the $[\alpha]_D$ values of **2** (+13.8°) and **3** (−61.0°) were found to have opposite signs, **2** and **3** can be represented by (+)- and (−)-*erythro*-anethole glycol 1′-*O*-β-D-glucopyranoside, respectively.

Glycoside III (**4**, $C_{16}H_{24}O_8$, $[\alpha]_D^{23}$ −15.0°) and IV (**5**, $C_{16}H_{24}O_8$, $[\alpha]_D^{23}$ −28.3°) showed $[M+Na]^+$, $[M-H_2O+H]^+$ and $[M-C_6H_{12}O_6+H]+$ ion peaks at *m/z* 367, 327 and 165 in the positive FAB-MS. Enzymatic hydrolysis of a mixture of **4** and **5** also gave **1** and D-glucose. The ^{1}H- and ^{13}C- NMR chemical shifts (Tables 1 and 2) of **4** and **5** also showed that both compounds were monoglucosides of **1**. The position of the β-glucosyl unit was proved to be C-2′ by the downfield shift of the C-2′ signals when compared with those of **1** [**1**: δ 72.12, **4**: δ 80.42, **5**: δ 80.64]. Each $[M]_D$ value and the values of $[M]_D$−methyl β-D-glucopyranoside (−62°)[4] [**4** (−51°; Δ+11°); **5** (−96°; Δ−34°)] suggested that the aglycones of their glucosides should be (+)- and (−)-forms. Thus, **4** and **5** may be represented as (+)- and (−)-*erythro*-anethole glycol 2′-*O*-β-D-glucopyranoside, respectively.

As the (1′*R*, 2′*S*) form of *erythro*-phenylpropylene glycol ($[\alpha]_D^{21}$ −38.6°) had a negative $[\alpha]_D$ value,[5] the absolute stereochemistry of C-1′ and C-2′ of (−)-*erythro*-anethole glycol should be *R* and *S*, and so, (+)-*erythro*-anethole glycol should be *S* and *R*. Furthermore, their ^{13}C-NMR glycosylation shift also supports this stereochemistry. The values of the upfield glycosylation shift of the β-pro-*S* side and β′-pro-*R* side carbons of the secondary alcohols to which β-D-glucopyranose is attached are known to reflect the absolute configuration of the alcohol, and the β-pro-*S* side carbon showed a larger shift than the β′-pro-*R* side carbon.[6] Comparison of the C-1 and C-2′ chemical shifts between a pair of stereoisomeric 1′-*O*-glucosides [C-1 (**2**: δ 133.04 (pro-*R*), **3**: δ 131.68 (pro-*S*)); C-2′ (**2**: δ 70.56 (pro-*S*), **3**: δ 70.74 (pro-*R*))] agreed well with the 1′*S* configuration for **2** and 1′*R* configuration for **3**. Comparison of the C-1′ and C-3′ chemical shifts between a pair of stereoisomeric 2′-*O*-glucosides [C-1′ (**4**: δ 75.73 (pro-*R*), **5**: δ 74.77 (pro-*S*)); C-2′ (**4**: δ 14.15 (pro-*S*), **5**: δ 16.25 (pro-*R*))] also agreed well with the 2′*R* configuration for **4** and 2′*S* configuration for **5**. Therefore, **2**, **3**, **4** and

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Table 1. ^{1}H-NMR Chemical Shifts of **2**—**5** (in Pyridine-d_5, 500 MHz)

	2	**3**	**4**	**5**
H-2, 6	7.62 2H, d (8.5)	7.77 2H, d (8.5)	7.64 2H, d (8.5)	7.68 2H, d (8.5)
H-3, 5	6.95 2H, d (8.5)	6.95 2H, d (8.5)	6.99 2H, d (8.5)	7.02 2H, d (8.5)
H-1′	5.23 1H, d (4.0)	5.29 1H, d (5.0)	5.43 1H, d (3.0)	5.35 1H, d (3.0)
H-2′	4.51 1H, qd (4.0, 6.0)	4.40 1H, qd (5.0, 6.0)	4.49 1H, qd (3.0, 6.5)	4.52 1H, qd (3.0, 6.5)
H_3-3′	1.40 3H, d (6.0)	1.41 3H, d (6.0)	1.36 3H, d (6.5)	1.37 3H, d (6.5)
OCH_3	3.65 3H, s	3.63 3H, s	3.67 3H, s	3.66 3H, s
Glc-1	5.36 1H, d (8.0)	4.92 1H, d (7.5)	5.08 1H, d (7.5)	5.28 1H, d (7.5)

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses].

Table 2. ^{13}C-NMR Chemical Shifts of **1**—**5** (in Pyridine-d_5, 125 MHz)

	1$^{a)}$	**2**	**3**	**4**	**5**
C-1	136.61	133.04 (−3.6)	131.68 (−4.9)	135.36 (−1.3)	134.92 (−1.7)
C-2,6	128.78	129.14 (+0.4)	130.43 (+1.7)	128.47 (−0.3)	128.61 (−0.2)
C-3,5	113.77	113.68 (−0.1)	113.85 (+0.1)	113.76 (±0.0)	113.88 (+0.1)
C-4	159.15	159.31 (+0.2)	159.63 (+0.5)	159.05 (−0.1)	159.18 (±0.0)
C-1′	78.09	86.03 (+7.9)	83.39 (+5.3)	75.73 (−2.4)	74.77 (−3.3)
C-2′	72.12	70.56 (−1.6)	70.74 (−1.4)	80.42 (+8.3)	80.64 (+8.5)
C-3′	18.98	18.21 (−0.8)	19.81 (+0.8)	14.15 (−4.8)	16.25 (−2.7)
OCH_3	55.14	55.14 (±0.0)	55.08 (−0.1)	55.13 (±0.0)	55.13 (±0.0)
Glc-1		105.07	101.46	103.82	104.24
Glc-2		76.10	75.43	75.10	75.78
Glc-3		78.71	78.64	78.59	78.79
Glc-4		71.59	71.88	71.86	71.64
Glc-5		78.71	78.53	78.53	78.58
Glc-6		62.61	62.83	62.88	62.78

δ in ppm from TMS. *a*) Measured at 67.5 MHz. $\Delta\delta$ ($\delta_{\text{glucoside}}-\delta_{\text{aglycone}}$) are given in parentheses.

Table 3. ^{1}H-NMR Chemical Shifts of **6** and **7** (in Pyridine-d_5, 500 MHz)

	6a	**6b**	**7**
H-2,6	7.61 2H, d (8.5)	7.61 2H, d (8.5)	7.39 2H, d (8.5)
H-3,5	7.42 2H, d (8.5)	7.42 2H, d (8.5)	7.34 2H, d (8.5)
H-1′	4.82 1H, d (7.0)	4.82 1H, d (7.0)	3.02 1H, dd (6.5, 13.5) [3.03 1H, dd (6.0, 13.5)]
	—	—	3.15 1H, dd (6.5, 13.5) [3.14 1H, dd (6.0, 13.5)]
H-2′	4.25 1H, m	4.25 1H, m	4.33 1H, m
H_3-3′	1.29 3H, d (6.5)	1.30 3H, d (6.5)	4.00 2H, dd (2.5, 6.0)
Glc-1	5.64 1H, d (7.5)	5.65 1H, d (7.0)	5.61 1H, d (7.5)

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. Minor epimeric component is given in brackets.

5 were characterized as (1′*S*,2′*R*)-*erythro*-anethole glycol 1′-*O*-β-D-glucopyranoside, (1′*R*,2′*S*)-*erythro*-anethole glycol 1′-*O*-β-D-glucopyranoside, (1′*S*,2′*R*)-*erythro*-anethole glycol 2′-*O*-β-D-glucopyranoside and (1′*R*,2′*S*)-*erythro*-anethole glycol 2′-*O*-β-D-glucopyranoside, respectively.

This is the first report of the isolation of all four *O*-β-D-glucopyranosides which have the glucosyl unit attached to the hydroxy group of the *erythro*-glycol function of individual stereoisomeric aglycones. In contrast to the *erythro*-forms, the *threo*-form of the anethole glycol glycoside was not found in this extract.

Glycoside V (**6**, $C_{15}H_{22}O_8$, $[\alpha]_D^{23}$ −48.5°) showed one peak on HPLC, but it was suggested to be an equivalent mixture of two diastereomeric compounds from the NMR spectral data (Tables 3 and 4). Its positive FAB-MS showed $[M+K]^+$, $[M+Na]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 369, 353 and 151. The ^{1}H- and ^{13}C-NMR data for **6** showed the presence of one β-glucopyranosyl, one 1,4-disubstituted benzene and one 1′,2′-dihydroxypropyl group. The position of the

Table 4. ^{13}C-NMR Chemical Shifts of **6** and **7** (in Pyridine-d_5, 125 MHz)

	6a	**6b**	**7**
C-1	137.56	137.61	133.82 [133.80]
C-2,6	128.90	128.90	131.07
C-3,5	116.47	116.52	116.74
C-4	157.94	157.96	157.09
C-1′	79.16	79.08	40.31
C-2′	72.42	72.42	74.09
C-3′	19.68	19.70	66.74 [66.73]
Glc-1	102.33	102.33	102.38
Glc-2	75.00	75.00	75.01
Glc-3	78.55	78.55	78.55
Glc-4	71.27	71.27	71.28
Glc-5	78.85	78.85	78.82
Glc-6	62.33	62.32	62.34

δ in ppm from TMS. Minor epimeric component is given in brackets.

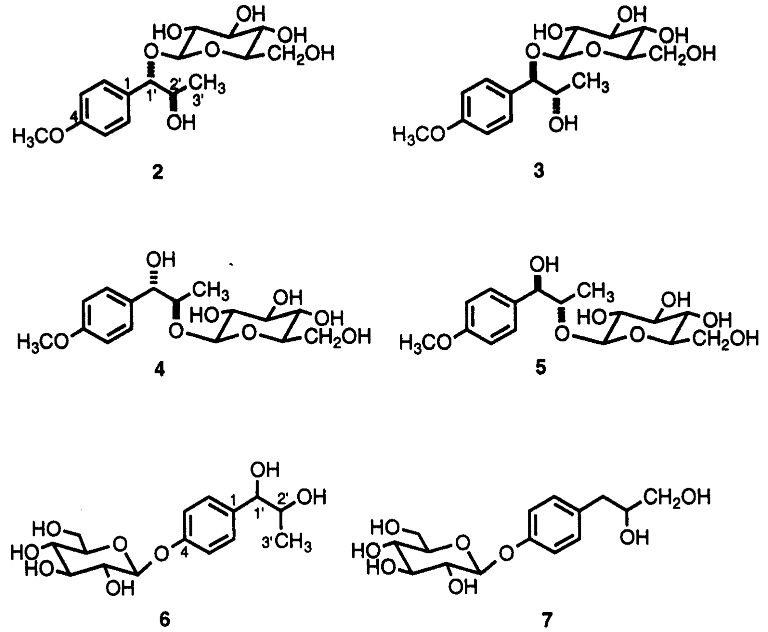

Chart 1. Structures of 2—7

glucosyl unit was verified to be C-4 of the benzene ring from the observed NOE interaction between the signal of the glucosyl anomeric proton and H-3 in its NOESY spectrum. By comparison of its ^{1}H- and ^{13}C-NMR data with those of **1** and **1′** [H-1′ (**6**: δ 4.82 , d, J=7.0 Hz, **1**: δ 5.07, d, J=4.5 Hz, **1′**: δ 4.84, d, J=7.0 Hz); H_3-3′ (**6**: δ 1.29 and 1.30, **1**: δ 1.54, **1′**: δ 1.32); C-1′ (**6**: δ 79.16 and 79.08, **1**: δ 78.09, **1′**: δ 79.19); C-2′ (**6**: δ 72.42, **1**: δ 72.12, **1′**: δ 72.49); C-3′ (**6**: δ 19.68 and 19.70, **1**: δ 18.98, **1′**: δ 19.70)], the aglycone of **6** was indicated to be *threo*-1′-(4-hydroxyphenyl)propane-1′,2′-diol. Therefore, **6** is an equivalent mixture of two stereoisomeric *threo*-1′-(4-hydroxyphenyl)propane-1′,2′-diol 4-*O*-β-D-glucopyranosides.

Glycoside VI (**7**, $C_{15}H_{22}O_8$, $[\alpha]_D^{23}$ −57.0°) was also found to be a mixture of two diastereomeric compounds (about 3 : 1) from NMR spectral data (Tables 3 and 4). Its positive FAB-MS showed $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$ ion peaks at *m/z* 369, 353 and 331, and the ^{1}H- and ^{13}C-NMR data for **7** showed the presence of one β-glucopyranosyl, one 1,4-disubstituted benzene and one 2′,3′-dihydroxypropyl group. The NOE interaction between the signal of the glucosyl anomeric proton and H-3 in its NOESY spectrum suggested that the glucosyl unit was attached to the C-4. Since the glucose was considered to be the same D-form as the other glucosides, **7** could be represented as an epimeric mixture of 1′-(4-hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-glucopyranoside at C-2′.

Although attempts to separate these isomers were unsuccessful, **6** and **7** were also shown to be new compounds.

Experimental

The instruments used and the experimental conditions for obtaining spectral data and for chromatography were the same as in the preceding paper.[1] FAB-MS were recorded using glycerol or *p*-nitrobenzyl alcohol (NBA) as matrix.

Extraction and Separation of 2—7 Commercial fennel (purchased from Kinokuniya Chinese Medicine Pharmacy, Ltd., lot. No. A0CJ0D28J; 2.0 kg) was extracted with methanol (10 l) at room temperature. After evaporation of the solvent, the residue (329.4 g) was partitioned into ether–water and then ethyl acetate–water. The aqueous portion was chromatographed on Amberlite XAD-II ($H_2O \rightarrow$MeOH). The methanol eluate (29.5 g) was chromatographed on Sephadex LH-20 (MeOH) to give seven fractions (frs. A—G). Fraction C (16.9 g) was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1)→MeOH] to give fifteen fractions (frs. C_1—C_{15}). Fraction C_6 (1.9 g) was subjected to a Lobar RP-8 column [CH_3CN–H_2O (3 : 17)] to give thirteen fractions (frs. $C_{6\text{-}1}$—$C_{6\text{-}13}$). Fraction $C_{6\text{-}6}$ was subjected to HPLC [octadecyl silica (ODS); CH_3CN–H_2O (1 : 7)] to give **3** (5 mg) and **2** (11 mg). Fraction $C_{6\text{-}8}$ was acetylated with Ac_2O and pyridine and the acetylated fraction was subjected to HPLC [ODS; CH_3CN–H_2O (3 : 2)] to give five fractions (frs. $C_{6\text{-}8\text{-}1}$—$C_{6\text{-}8\text{-}5}$). Fraction $C_{6\text{-}8\text{-}2}$ was deacetylated by heating in a water bath with 15% NH_4OH–MeOH for 4 h to give a mixture of **4** and **5** (16 mg). A portion of this mixture (12 mg) was subjected to HPLC [carbohydrate analysis; CH_3CN–H_2O (14 : 1)] to give **5** (4 mg) and **4** (6 mg) in pure form. Fraction C_{10} (0.4 g) was subjected to chromatography on a Lobar RP-8 column [MeOH–H_2O (1 : 4)] to give seven fractions (frs. $C_{10\text{-}1}$—$C_{10\text{-}7}$). Fraction $C_{10\text{-}2}$ was subjected to HPLC [carbohydrate analysis; CH_3CN–H_2O (19 : 1)] to give **6** (4 mg) and **7** (3 mg).

Enzymatic Hydrolysis of a mixture of 2 and 3, and 4 and 5 A mixture of **2** and **3** (4 mg) and a mixture of **4** and **5** (4 mg) were each dissolved in water (5 ml) with crude hesperidinase (3 mg) and shaken in a water bath at 37 °C for 48 h. Each mixture was evaporated *in vacuo* to dryness and the residues were chromatographed on silica gel [$CHCl_3$–MeOH (19 : 1→7 : 3)] to give **1** (2 mg) and sugar fractions, respectively. **1** was identified as *erythro*-anethole glycol from ^{1}H- and ^{13}C-NMR data. The sugar fraction was passed through a Sephadex LH-20 (MeOH) to give a syrup, and HPLC [column; carbohydrate analysis, detector; JASCO RI-930 detector and JASCO OR-990 chiral detector, solv.; CH_3CN–H_2O (17 : 3), 2 ml/min; t_R 4.53 min)] showed the presence of D-glucose.

(1′*S*,2′*R*)-*erythro*-Anethole Glycol 1′-*O*-β-D-Glucopyranoside (2) An amorphous powder, $[\alpha]_D^{26}$ +13.8°(c=0.9, MeOH). Positive FAB-MS *m/z* (glycerol): 383 $[M+K]^+$, 367 $[M+Na]^+$, 345.1535 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_8$: 345.1550), 327 $[M-H_2O+H]^+$, 165 $[M-C_6H_{12}O_6+H]^+$ (base).

(1′*R*,2′*S*)-*erythro*-Anethole Glycol 1′-*O*-β-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{26}$ −61.0°(c=0.3, MeOH). Positive FAB-MS *m/z* (glycerol): 689 $[2M+H]^+$, 367.1382 $[M+Na]^+$ (Calcd for $C_{16}H_{24}O_8Na$: 367.1369), 327 $[M-H_2O+H]^+$, 165 $[M-C_6H_{12}O_6+H]^+$ (base).

(1′*S*,2′*R*)-*erythro*-Anethole Glycol 2′-*O*-β-D-Glucopyranoside (4) An amorphous powder, $[\alpha]_D^{23}$ −15.0°(c=0.5, MeOH). Positive FAB-MS *m/z* (glycerol): 689 $[2M+H]^+$, 367.1367 $[M+Na]^+$ (Calcd for $C_{16}H_{24}O_8Na$: 367.1369), 327 $[M-H_2O+H]^+$, 165 $[M-C_6H_{12}O_6+H]^+$ (base).

(1′*R*,2′*S*)-*erythro*-Anethole Glycol 2′-*O*-β-D-Glucopyranoside (5) An amorphous powder, $[\alpha]_D^{23}$ −28.3°(c=0.3, MeOH). Positive FAB-MS *m/z* (glycerol): 689 $[2M+H]^+$, 367.1383 $[M+Na]^+$ (Calcd for $C_{16}H_{24}O_8Na$: 367.1369), 327 $[M-H_2O+H]^+$, 165 $[M-C_6H_{12}O_6+H]^+$ (base).

***threo*-1′-(4-Hydroxyphenyl)propane-1′,2′-diol 4-*O*-β-D-Glucopyranoside (6)** An amorphous powder, $[\alpha]_D^{23}$ −48.5° (c=0.2, MeOH). Positive FAB-

MS *m/z* (NBA): 369 $[M+K]^+$, 353.1222 $[M+Na]^+$ (base, Calcd for $C_{15}H_{22}O_8Na$: 353.1212), 151 $[M-C_6H_{12}O_6+H]^+$.

1′-(4-Hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-Glucopyranoside (7) An amorphous powder, $[\alpha]_D^{23}-57.0°$ (c=0.2, MeOH). Positive FAB-MS *m/z* (NBA): 369 $[M+K]^+$, 353.1186 $[M+Na]^+$ (base, Calcd for $C_{15}H_{22}O_8Na$: 353.1212), 331 $[M+H]^+$.

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