Constituents of Fennel. X. New Chromanone and Phenylethanoid Glycosides, and *threo*-Epoxyanethole

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From the water-soluble portion of the methanol extract of the herbal medicine fennel, a new chromanone glycoside and a new phenylethanoid glycoside were isolated, and their structures were determined by spectral methods. An optical isomeric mixture of *threo*-epoxyanethole was obtained from the ether-soluble portion, and it was considered to be an auto-oxidation product of *trans*-anethole.

Key words fennel; *Foeniculum vulgare* fruit; chromanone glycoside; *thero*-epoxyanethole; phenylethanoid glycoside; auto-oxidation product

We have exhaustively investigated the constituents of the water-soluble portion of fennel, the fruit of *Foeniculum vulgare* MILLER (Umbelliferae), and reported the isolation and characterization of alkyl glycosides,[1] aromatic compound glycosides,[2] monoterpenoid glycosides of various types,[3] glucides and nucleosides.[4] Herein, we describe the isolation and structure elucidation of chromanone derivative and phenylethanoid glycosides from the water-soluble fraction. We also examined the constituents of the ether-soluble portion, and a *threo*-epoxyanethole was obtained together with sterols and a triterpenoid.

The methanolic extract of commercial fennel was treated as described in the Experimental section, and from the water-soluble portion, glycosides **1** to **6** were isolated.

Glycoside **1** ($C_{20}H_{26}O_{10}$, an amorphous powder, $[\alpha]_D^{22}$ −68.0°) showed $[M+K]^+$, $[M+Na]^+$, $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m/z* 465, 449, 427 and 265 in the positive FAB-MS, and acid hydrolysis of **1** gave D-glucose as a sugar component. The ^1H-, ^{13}C- and ^{13}C–^1H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2) showed the presence of one β-D-glucopyranosyl, 1,2,4,5-tetrasubstituted benzene, gem-dimethyl groups, three methylenes, one carboxyl group, and one carbonyl carbon. The analysis of heteronuclear multiple-bond correlation (HMBC) spectral and ^1H–^1H COSY spectral data (Fig. 1, shown in heavy lines and dotted line) suggested that the aglycone of **1** was a chromanone derivative having two *tert*-methyls at C-2, a carboxyethyl group at C-6 and a hydroxyl group at C-7. The location of the glucosyl unit was determined to be C-7 by correlation between C-7 and the glucosyl H-1 signals in the HMBC spectrum. Therefore, **1** was characterized as 6-carboxyethyl-7-hydroxy-2,2-dimethylchromanone 7-*O*-β-D-glucopyranoside.

Glycoside **2** ($C_{17}H_{20}O_9$, an amorphous powder, $[\alpha]_D^{22}$ −44.0°) was identified as cnidioside A by direct comparison with an authentic sample.[5]

Glycoside **3** ($C_{16}H_{24}O_9$, an amorphous powder, $[\alpha]_D^{22}$ −87.0°) showed $[M+H]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 361 and 181 in the positive FAB-MS. The NMR revealed **3** to have one β-D-glucopyranosyl, one 1,3,4-trisubstituted benzene ring, one dihydroxyethyl and two methoxyl groups. Since the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum showed the following cross-peaks: H-1′/H-2, H-1′/H-6, and H-1′/glucosyl H-1 (Fig. 2), **3** was suggested to be 1′-(3,4-dimethoxyphenyl)-ethane-1′,2′-diol 1′-*O*-β-D-glucopyranoside. The absolute configuration at C-1′ was deduced to be *R* from its $[M]_D$ value (−313°), which was negative as was (1*R*′)-1′-(3-hydroxy-4-methoxyphenyl)ethane-1′,2′-diol (−42°)[2a] when calculated using the value for methyl β-D-glucopyranoside (−62°, **3**−methyl β-D-glucopyranoside=−251°).[6] Comparison of the chemical shift of glucosyl C-1 of **3** (δ 101.85) with those of *erythro*-anethole glycol 1′-*O*-β-D-glucopyranosides (1*R* form, δ 101.46 and 1*S* form, δ 105.07)[2b] also supported this conclusion. From these facts, **3** was characterized as (1′*R*)-1′-(3,4-dimethoxyphenyl)ethane-1′,2′-diol 1′-*O*-β-D-glucopyranoside.

Glycoside **4** (an amorphous powder, $[\alpha]_D^{24}$ −45.0°), **5** (an amorphous powder, $[\alpha]_D^{22}$ −43.5°) and **6** (an amorphous

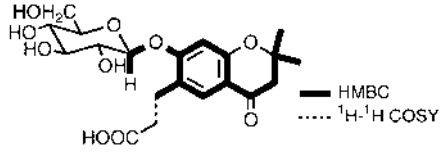

Fig. 1. Partial Structure of **1** Solved by HMBC and ^1H–^1H COSY Spectra

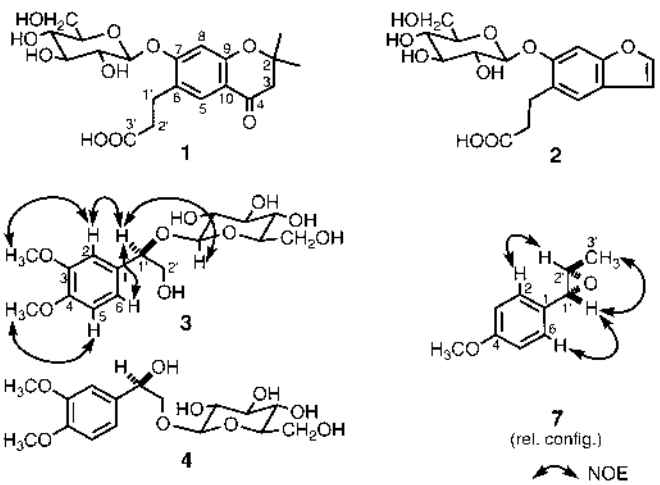

Fig. 2. Structures of **1**—**4** and **7**, and NOE Interactions Observed in the NOESY Spectra of **3** and **7**

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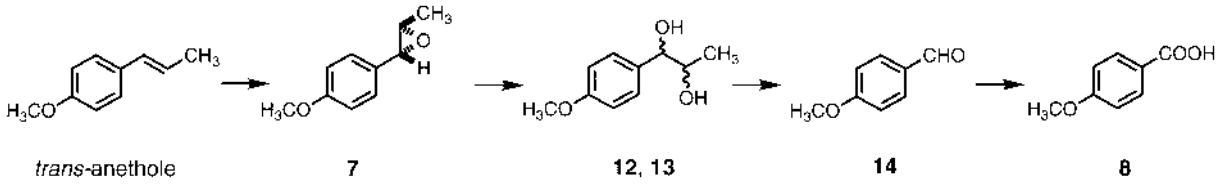

Chart 1. Auto-oxidation Process of *trans*-Anethole

Table 1. ^{1}H-NMR Chemical Shifts of **1**, **3**, **4** and **7** (at 500 MHz)

	1[a]		**3**[b]	**4**[b]	**7**[c]
H_2-3	2.67 d (1.5)	H-2	7.59[d]	7.37 d (2.0)	7.33 d (8.5)
H-5	7.61 s	H-3	—	—	6.90 d (8.5)
H-8	6.64 s	H-5	6.90 d (8.0)	6.95 d (8.5)	7.01 d (8.0)
CH_3	1.41 s	H-6	7.24 dd (2.0, 8.0)	7.24 dd (2.0, 8.5)	6.90 d (8.5)
	1.42 s	H-1′	5.58 dd (4.5, 7.0)	5.39 dd (3.5, 8.5)	4.27 d (9.0)
H_2-1′	2.88 m	H-2′	4.15 dd (4.5, 11.5)	4.30 dd (3.5, 11.0)	3.73 dq (6.0, 9.0)
H_2-2′	2.37 ddd (6.0, 12.0, 12.0)		4.31 dd (7.0, 11.5)	4.46 dd (8.5, 11.0)	—
	2.44 ddd (6.0, 12.0, 12.0)	H_3-3′	—	—	1.00 d (6.0)
Glc-1	4.95 d (7.5)	3-OCH_3	3.76 s	3.74 s	—
		4-OCH_3	3.76 s	3.72 s	3.80 s
		Glc-1	5.01 d (7.5)	5.08 d (7.5)	—

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. Measured in *a*) CD_3OD, *b*) pyridine-d_5, *c*) $CDCl_3$. *d*) Signal is overlapped with pyridine-d_5.

powder, $[\alpha]_D^{22}$ −49.0°) were identified as 1′-(3,4-dimethoxyphenyl)ethane-1′,2′-diol 2′-*O*-β-D-glucopyranoside,[7] β-sitosteryl β-D-glucopyranoside and stigmasteryl β-D-glucopyranoside from the results of NMR analysis (Tables 1 and 2). The absolute configuration at C-1′ of **4** was shown to be *R* for the same reason as described for **3** ($[M]_D$ value of **4**−methyl β-D-glucopyranoside=−100°).

We also investigated the ether-soluble portion of the fruit in the hope of isolating anethole related compounds, and compounds **7** to **11** were obtained as described in Experimental.

Compound **7** ($C_{10}H_{12}O_2$, mp 182—184 °C, $[\alpha]_D^{21}$ 0°) showed $[M+H]^+$ ion peaks at *m/z* 165 in the positive FAB-MS and chemical ionization (CI)-MS spectra. Comparison of the ^{1}H- and ^{13}C-NMR (Tables 1 and 2) with those of *erythro*- and *threo*-anethole (**12** and **13**),[2b] and the molecular formula revealed that **7** is an oxidation product of anethole with an epoxy ring between C-1′ and C-2′. The following NOE interactions were observed: H-1′/H-6, H-2′/H-2 and H-1′/H_3-3′ in the NOESY spectrum (Fig. 2), suggesting that the stereochemical relation between C-1′ and C-2′ of **7** should be *threo*. Thus, **7** was characterized as *threo*-epoxyanethole. As **7** has no optical rotation, it was considered to be an equivalent mixture of optical isomers the same as **12** and **13**.

Compound **8** ($C_8H_8O_3$, mp 185—186 °C, $[\alpha]_D^{21}$ 0°), **9** ($C_{29}H_{50}O$, mp 137—139 °C, $[\alpha]_D^{22}$ −31.0°), **10** ($C_{29}H_{48}O$, mp 167—169 °C, $[\alpha]_D^{22}$ −47.0°) and **11** ($C_{30}H_{48}O_3$, mp >300 °C, $[\alpha]_D^{21}$ +85.0°) were identified as *p*-anisic acid, β-sitosterol, stigmasterol and oleanolic acid, respectively.

Fennel contains 3—8% essential oil comprising 57—82% anethole and 6—27% *p*-ansaldehyde (**14**),[8] and **14** is regarded as a compound of an auto-oxidation product of anethole while the fennel is preserved.[9] Thus, the composition of **14** is believed useful for characterizing the quality of medicine.[10] As compounds **7**, **8**, **12** and **13**, which were obtained as anethole-related constituents of fennel,[11] are also regarded as auto-oxidation products of anethole, the oxidation process is proposed as shown in Chart 1.

Table 2. ^{13}C-NMR Chemical Shifts of **1**, **3**, **4** and **7** (at 125 MHz)

	1[a]	**3**[b]	**4**[b]	**7**[c]
C-1	—	132.98	135.15	131.18
C-2	80.68	111.99	111.19	128.75
C-3	49.29	149.43[d]	149.18[d]	113.87
C-4	193.75	150.02[d]	149.44[d]	159.53
C-5	128.08	112.26	112.34	113.87
C-6	126.35	120.54	119.18	128.75
C-7	163.75	—	—	—
C-8	104.17	—	—	—
C-9	115.68	—	—	—
C-10	162.10	—	—	—
CH_3	26.70	—	—	—
	26.84	—	—	—
C-1′	28.13	81.10	72.71	84.11
C-2′	39.8 (br)	67.81	76.80	76.83
C-3′	182.5 (br)	—	—	17.27
3-OCH_3	—	55.72	55.79	—
4-OCH_3	—	55.93	55.98	56.06
Glc-1	101.92	101.85	104.97	
Glc-2	74.62	75.36	75.21	
Glc-3	78.29	78.60	78.78	
Glc-4	71.23	71.75	71.66	
Glc-5	77.74	78.60	78.60	
Glc-6	62.46	62.65	62.69	

δ in ppm from TMS. Measured in *a*) CD_3OD, *b*) pyridine-d_5, *c*) $CDCl_3$. *d*) Assignments may be interchanged in each column.

Experimental

Alumina column chromatography was carried out using neutral aluminum oxide (grade III, Woelm). CI-MS was taken on a JEOL JMS D-300 spectrometer. The other instruments used and the experimental conditions for obtaining spectral data and for chromatography were the same as described in the preceding paper.[1]

Extraction and Isolation of 1 to 13 As reported in the previous paper, commercial fennel (2.0 kg) was extracted with methanol. The methanol extract (329.4 g) was partitioned into ether–water, then ethyl acetate–water,

and the thus-obtained aqueous portion was subjected to Amberlite XAD-II ($H_2O \rightarrow$MeOH). The methanol eluate (29.5 g) was chromatographed over Sephadex LH-20 (MeOH) to give seven fractions (frs. A—G). Fraction C (16.9 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1) $\rightarrow$MeOH] to give fifteen fractions (frs. C_1—C_{15}). Fraction C_2 (1.1 g) was treated with MeOH–H_2O (1 : 1), and a part of the insoluble portion (230 $\rightarrow$20 mg) was subjected to HPLC [symmetryprep C_8, MeOH] to give **6** (8 mg) and **5** (12 mg). Fraction C_7 (0.7 g) was subjected to a Lobar RP-8 column [CH_3CN–H_2O (1 : 10 $\rightarrow$ 3 : 17)] to give nine fractions (frs. $C_{7\text{-}1}$—$C_{7\text{-}9}$). Fraction $C_{7—5}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (19 : 1)] to give three fractions (frs. $C_{7\text{-}5\text{-}1}$—$C_{7\text{-}5\text{-}3}$). Fraction $C_{7\text{-}5\text{-}3}$ was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [symmetryprep C_{18}, CH_3CN–H_2O (9 : 11)] to give two fractions. Each fraction was deacetylated by heating with 20% NH_4OH–MeOH for 2 h in a water bath to give **3** (5 mg) and **4** (2 mg) in pure form. Fraction D (1.9 g) was chromatographed over Sephadex LH-20 (MeOH) to give six fractions (frs. D_1—D_6). Fraction D_4 (0.1 g) was subjected to a Lobar RP-8 column [MeOH–H_2O (1 : 4)] and HPLC [ODS, MeOH–H_2O (1 : 4)] to give **1** (22 mg). Fraction D_5 (0.1 g) was subjected to a Lobar RP-8 column [MeOH–H_2O (3 : 17)] to give **2** (10 mg). The ether-soluble portion (74.3 g in 225.6 g) was chromatographed over silica gel [hexane $\rightarrow$ hexane–EtOAc (9 : 1 $\rightarrow$ 4 : 1 $\rightarrow$ 3 : 2) $\rightarrow$ acetone $\rightarrow$ MeOH] to give fourteen fractions (frs. N_1—N_{14}). Fraction N_4 (2.0 g) was chromatographed over silica gel [$CHCl_3$–$CHCl_3$–MeOH (9 : 1)] to give five fractions (frs. $N_{4\text{-}1}$—$N_{4\text{-}5}$). Fractions $N_{4\text{-}3}$ (0.3 g) and $N_{4\text{-}4}$ (0.9 g) were individually chromatographed over aluminum oxide [hexane–EtOAc (9 : 1)] to give **8** (30 mg) from fr. $N_{4\text{-}3}$, and **7** (45 mg) from fr. $N_{4\text{-}4}$. Fraction N_7 (2.7 g) was chromatographed over silica gel [hexane–EtOAc (4 : 1 $\rightarrow$ 7 : 3) $\rightarrow$ EtOAc] and HPLC [symmetryprep C_8, MeOH] to give **10** (40 mg) and **9** (30 mg). Fraction N_{10} (3.9 g) was chromatographed over silica gel [$CHCl_3 \rightarrow CHCl_3$–MeOH (9 : 1)] and Sephadex LH-20 [MeOH] to give **11** (90 mg). The ethyl acetate-soluble portion (2.8 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1) $\rightarrow$ MeOH] to give five fractions (frs. O_1—O_5). Fraction O_2 (0.6 g) was subjected to a Lobar RP-8 column [MeOH–H_2O (1 : 1)] and HPLC [symmetryprep C_8, MeOH–H_2O (1 : 1)] to give **13** (60 mg) and **12** (140 mg). Fraction O_3 (0.3 g) was treated with MeOH–H_2O (1 : 1), and a part of the insoluble portion (120 $\rightarrow$ 12 mg) was subjected to HPLC [symmetryprep C_8, MeOH] to give **6** (5 mg) and **5** (7 mg).

The following compounds were identified by comparison with authentic compounds.

Cnidioside A (**2**), β-sitosteryl β-D-glucopyranoside (**5**), stigmasteryl β-D-glucopyranoside (**6**), *p*-anisic acid (**8**), β-sitosterol (**9**), stigmasterol (**10**), oleanolic acid (**11**), *threo*-anethole glycol (**12**), and *erythro*-anethole glycol (**13**).

6-Carboxyethyl-7-hydroxy-2,2-dimethylchromanone 7-*O*-β-D-Glucopyranoside (1) An amorphous powder, $[\alpha]_D^{22}$ −68.0° (*c*=0.8, MeOH). Positive FAB-MS *m/z*: 465 $[M+K]^+$, 449.1458 $[M+Na]^+$ (Calcd for $C_{20}H_{26}NaO_{10}$: 449.1424), 427.1612 $[M+H]^+$ (Calcd for $C_{20}H_{27}O_{10}$: 427.1604), 265 $[M-C_6H_{10}O_5+H]^+$ (base). HMBC (in CD_3OD, 500 MHz): H_2-3/C-2, -4, $\underline{C}H_3$a, $\underline{C}H_3$b; H-5/C-4, -7, -10, -1′; H-8/C-6, -7, -9, -10; $\underline{C}H_3$a/C-2, -3, -$\underline{C}H_3$b; $\underline{C}H_3$b/C-2, -3, -$\underline{C}H_3$a; H_2-1′/C-5, -6, -7, -2′; H-Glc-1/C-7.

Acid Hydrolysis of 1 Glycoside **1** (8 mg) was dissolved in aq. 2 N H_2SO_4 and heated in a water bath for 3 h. The reaction mixture of hydrolysate was neutralized with $NaHCO_3$, the salt was filtered off, and the filtrate was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)]. The sugar fraction was subjected to HPLC [column, carbohydrate analysis (Waters, size, 3.9×300 mm); detector, JASCO RI-930 detector; solv., CH_3CN–H_2O (17 : 3), 2 ml/min; t_R 4.5 min (same location as that of D-glucose)].

(1′*R*)-1′-(3,4-Dimethoxyphenyl)ethane-1′,2′-diol 1′-*O*-β-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{24}$ −87.0° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 453 $[M+H+92\ (glycerol)]^+$, 361.1512 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_9$: 361.1499), 181 $[M-C_6H_{12}O_6+H]^+$ (base).

(1′*R*)-1′-(3,4-Dimethoxyphenyl)ethane-1′,2′-diol 2′-*O*-β-D-Glucopyranoside (4) An amorphous powder, $[\alpha]_D^{24}$ −45.0° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 453 $[M+H+92\ (glycerol)]^+$, 383.1329 $[M+Na]^+$ (Calcd for $C_{16}H_{24}NaO_9$: 383.1318), 181 $[M-C_6H_{12}O_6+H]^+$ (base).

***threo*-Epoxyanethole (7)** Colorless needles, mp 182—184 °C, $[\alpha]_D^{21}$ 0° (*c*=1.8, $CHCl_3$). Positive FAB-MS *m/z*: 329 $[2M+H]^+$, 187 $[M+Na]^+$, 165.0904 $[M+H]^+$ (base, Calcd for $C_{10}H_{12}O_2$: 165.0916). CI-MS *m/z*: 329 $[2M+H]^+$, 165 $[M+H]^+$ (base).

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