Water-Soluble Constituents of Fennel. I. Alkyl Glycosides

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From the water-soluble portion of the methanol extract of the herbal medicine fennel [fruit of *Foeniculum vulgare* MILLER (Umbelliferae)], one ethyl, two propyl, two butyl and four isopentyl (hemiterpenoid) glycosides were obtained. Of these glycosides, six were new.

Key words fennel; *Foeniculum vulgare* fruit; Umbelliferae; alkyl glycoside; isopentyl glycoside

Fennel [Japanese name "Uikyoh," the fruit of *Foeniculum vulgare* MILLER (Umbelliferae)] is cultivated in many parts of the world, and is a popular aromatic herb. Its fruit has been used as a medicine prescribed for carminative stomach ailments and as an important spice. Most of the studies on this fruit were concerned with the essential oils,[1] and no report had been published about the water-soluble constituents until a report of the isolation of stilbene-trimer glycosides and monoterpenoid glycosides.[2] As we are interested in the constituents of the water-soluble portion of this fruit, we undertook a detailed investigation on this material. Up to now, we have isolated more than one hundred compounds, which will be described in the following series of papers. In this paper, we discuss simple alkyl glycoside derivatives.

The methanolic extract of commercial fennel was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was chromatographed on Amberlite XAD-II to give water and methanol fractions which were subjected to Sephadex LH-20, silica gel and Lobar RP-8 column chromatography. The alkyl glycosides were concentrated into the relatively rapidly eluting fractions when passed through the Lobar RP-8 column. Finally, HPLC was used for the purification of nine glycosides (**1**—**9**), whose NMR data are listed in Tables 1 and 2. All of them, except **9**, were derivatives of β-D-glucopyranoside as shown by their ^{13}C-NMR data (Table 2), and their formulae were suggested from the accurate mass number of the $[M+H]^+$ or $[M+Na]^+$ ion peaks in high-resolution positive FAB-MS.

Glycoside **1** ($C_8H_{16}O_6$, a colorless syrup, $[\alpha]_D^{23}$ −26.4°) was identified as ethyl β-D-glucopyranoside.[3] As we did not use ethanol in the isolation process, the possibility of **1** being an artifact can be ruled out.

Glycoside **2** ($C_9H_{18}O_6$, mp 129—131 °C, $[\alpha]_D^{23}$ −35.6°) was characterized as isopropyl β-D-glucopyranoside.[4] This is the first example of its isolation from natural sources.

Glycoside **3** ($C_9H_{18}O_7$, an amorphous powder, $[\alpha]_D^{23}$ −21.0°) showed one peak by HPLC, but this was a mixture of two isomeric compounds in a ratio of about 3 : 1 from the NMR spectral data (Tables 1 and 2). It contained one *sec*-methyl, one hydroxymethyl and one hydroxymethine group. The compound was, thus, the glucoside of propane-1,2-diol ($C_3H_8O_2$). From the observed nuclear Overhauser effect (NOE) interaction between its anomeric proton signal and the H_2-1 signal in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum, the glucosyl unit was attached to the primary alcohol of the aglycone. Therefore, **3** was an epimeric mixture of propane-1,2-diol 1-*O*-β-D-glucopyranosides.[5]

Glycoside **4** ($C_{10}H_{20}O_7$, an amorphous powder, $[\alpha]_D^{23}$ −32.0°) was also found to be a mixture of two isomeric compounds (about 1 : 1) from the NMR spectral data (Tables 1 and 2). It contained two *sec*-methyl and two hydroxymethine groups. Then, the aglycone of **4** was found to be butane-2,3-diol ($C_4H_{10}O_2$). Therefore, **4** was an isomeric mixture of butane-2,3-diol 2-*O*-β-D-glucopyranosides. Attempts to separate these isomers were unsuccessful.

Glycoside **5** ($C_{11}H_{22}O_6$, mp 86—88 °C, $[\alpha]_D^{23}$ −33.0°) and glycoside **6** ($C_{11}H_{22}O_6$, an amorphous powder, $[\alpha]_D^{22}$ −24.7°) were obtained as a binary mixture and they were separated in pure form by repeated HPLC using a carbohydrate analysis column. The major component **5** had two *sec*-methyl, one methylene, one methine and one hydroxymethyl group. Thus, the aglycone was an isopentanol ($C_5H_{12}O$) with the hydroxyl group at C-1, and **5** was characterized as 3-methylbutan-1-ol β-D-glucopyranoside (isopentyl β-D-glucopyranoside). The minor component **6** was identified as (2*S*)-2-methylbutan-1-ol β-D-glucopyranoside which had already been isolated from the leaves of *Bystropogon plumosus*,[6] a plant native to the Canary Islands, by comparison of NMR and $[\alpha]_D$ data.

Glycoside **7** ($C_{11}H_{20}O_6$, mp 55—56 °C, $[\alpha]_D^{22}$ −15.8°) and glycoside **8** ($C_{11}H_{20}O_6$, mp 68—70 °C, $[\alpha]_D^{21}$ −23.6°) were also obtained as a binary mixture, which could be separated by HPLC using an octadecyl silica (ODS) column. Glycoside **7** had one *tert*-methyl, one *sec*-methyl, one trisubstituted double bond and one hydroxymethyl group. The compound was, thus, the glucoside of Δ^2-isopentenyl alcohol ($C_5H_{10}O$). As NOE interactions between the *tert*-methyl and *sec*-methyl (H_3-5 and H_3-4), and between the hydroxymethyl and vinyl proton (H_2-1 and H-3) were observed in its NOESY spectrum, the configuration of the double bond should be *E*. Therefore, **7** was characterized as (2*E*)-2-methyl-2-buten-1-ol β-D-glucopyranoside. Glycoside **8** was identified as 3-methyl-2-buten-1-ol β-D-glucopyranoside which had been reported as a fragrance precursor of the flower buds of *Citrus unshiu*.[7]

Glycoside **9** ($C_{15}H_{28}O_{11}$, an amorphous powder, $[\alpha]_D^{23}$ −46.0°) showed an $[M+H]^+$ ion peak at *m/z* 385 in the positive FAB-MS, and comparison of its ^{1}H- and ^{13}C-NMR spectral data with those of **4** suggested that it is a β-apiofuranoside[8] of **4**. The position of the apiofuranosyl unit was at the C-6 of the glucose from the downfield shift of the glucosyl C-6 carbon (6 ppm). Since this apiose was considered to be the same D-form as other apiosides, **9** was concluded to be butane-1,2-diol 2-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside. However, the absolute configuration of the aglycone of **9** could not be assigned.

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Table 1. ^{1}H-NMR Chemical Shifts of **1**—**9** (in Pyridine-d_5, 500 MHz)

	1	**2**	**3a**	**3b**
H-1	3.69 1H, dd (7.0, 9.5)	1.21 3H, d (6.0)	3.82 1H, dd (7.0, 10.0)	[3.84 1H, dd (4.0, 10.5)]
	4.10 1H, dd (7.0, 9.5)	—	4.15 1H, dd (4.5, 10.0)	[4.17 1H, dd (6.5, 10.5)]
H-2	1.19 3H, t (7.0)	3.95 1H, m	4.31 1H, m	[4.31 1H, m]
H-3	—	1.26 3H, d (6.0)	1.29 3H, d (6.5)	[1.32 3H, d (6.5)]
Glc-1	4.83 1H, d (8.0)	4.90 1H, d (8.0)	4.97 1H, d (7.5)	[4.95 1H, d (7.5)]

	4a	**4b**	**9**
H-1	1.43 3H, d (6.5)	[1.39 3H, d (6.5)]	1.39 3H, d (6.5)
H-2	4.12 1H, dd (4.0, 6.5)	[4.16 1H, dd (4.0, 6.5)]	3.87 1H, qd (6.5, 6.5)
H-3	4.14 1H, dd (4.0, 6.5)	[4.17 1H, dd (4.0, 6.5)]	3.97 1H, qd (6.5, 6.5)
H-4	1.33 3H, d (6.5)	[1.39 3H, d (6.5)]	1.24 3H, d (6.5)
Glc-1	5.13 1H, d (8.0)	[4.98 1H, d (7.5)]	5.05 1H, d (7.5)
Api-1	—	—	5.81 1H, d (2.5)

	5	**6**	**7**	**8**
H-1	3.68 1H, dt (7.0, 9.5)	3.42 1H, dd (7.0, 9.5)	4.21 1H, d (11.5)	4.38 1H, d (12.0)
	4.14 1H, dt (7.0, 9.5)	3.68 1H, dd (7.0, 9.5)	4.46 1H, d (11.5)	4.59 1H, d (12.0)
H-2	1.54 2H, dd (7.0, 7.0)	1.50 1H, m	—	5.53 1H, qt (1.5, 7.5)
H-3	1.69 1H, m	1.09 2H, m	5.60 1H, qq (1.5, 7.0)	—
H-4	0.79[a] 3H, d (6.5)	0.78 3H, t (7.0)	1.52 3H, qd (1.5, 7.0)	1.61 3H, s
H-5	0.78[a] 3H, d (6.5)	0.92 3H, d (6.5)	1.70 3H, qd (1.5, 1.5)	1.54 3H, s
Glc-1	4.85 1H, d (8.0)	4.83 1H, d (7.5)	4.89 1H, d (7.5)	4.92 1H, d (7.5)

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. *a*) Assignments may be interchanged. Epimeric components are given in brackets.

Table 2. ^{13}C-NMR Chemical Shifts of **1**—**9** (in Pyridine-d_5, 125 MHz)

	1[a]	**2**	**3a**	**3b**	**4a**	**4b**	**9**
C-1	64.94	23.90	76.72	[76.54]	17.12	[15.47]	18.35
C-2	15.52	70.77	66.73	[66.40]	80.82	[79.87]	83.57
C-3		22.05	20.08	[20.22]	70.16	[69.48]	71.60
C-4					19.21	[18.66]	18.77
Glc-1	104.45	102.66	105.46	[105.06]	104.62	[103.21]	105.94
Glc-2	75.21	75.26	75.37	[75.23]	75.73	[75.11]	75.61
Glc-3	78.50[b]	78.43[b]	78.57	[78.57]	78.58	[78.50]	78.59
Glc-4	71.68	71.71	71.67	[71.66]	71.87	[71.68]	71.87
Glc-5	78.60[b]	78.58[b]	78.65	[78.65]	78.63	[78.63]	77.18
Glc-6	62.80	62.86	62.77	[62.70]	62.86	[62.81]	68.94
Api-1							111.16
Api-2							77.83
Api-3							80.53
Api-4							75.08
Api-5							65.65

	5	**6**	**7**	**8**
C-1	68.16	74.99	74.93	65.72
C-2	38.97	35.52	133.31	121.92
C-3	25.14	26.40	122.26	135.87
C-4	22.72[c]	11.43	13.15	25.64
C-5	22.66[c]	16.71	13.84	17.93
Glc-1	104.78	105.12	103.47	103.54
Glc-2	75.27	75.29	75.25	75.26
Glc-3	78.64	78.66	78.66	78.67
Glc-4	71.75	71.78	71.75	71.79
Glc-5	78.54	78.55	78.52	78.57
Glc-6	62.87	62.89	62.84	62.87

δ in ppm from TMS. *a*) Measured at 67.5 MHz. *b,c*) Assignments may be interchanged. Epimeric components are given in brackets.

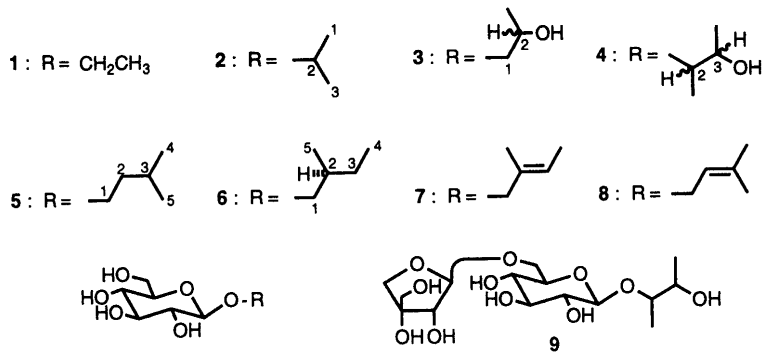

Chart 1. Structures of **1**—**9**

The presence of the above simple alkyl glycosides suggests that the corresponding alcohols may also be components of the essential oil from this crude drug.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. FAB-MS were recorded with a JEOL HX-110 spectrometer using glycerol as matrix. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM GX-270 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ units. ^{13}C–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-bond correlation (HMBC) and NOESY spectra were obtained with the usual pulse sequence and data processing was performed with standard JEOL software. Column chromatography was carried out under TLC monitoring using Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μm, Pharmacia), a Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck 5721) and spots were detected with *p*-anisaldehyde–H_2SO_4 reagent. HPLC separation was carried out on a JASCO chromatograph (980-system) with a JASCO RI-930 detector, and ODS-3251-D (Senshu pak; column size, 8×250 mm) and carbohydrate analysis (Waters; column size, 3.9×300 mm) columns.

Extraction and Separation of Alkyl Glycosides 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 successively partitioned between ether–water and ethyl acetate–water. Removal of the solvent from each phase gave the ether (225.6 g), ethyl acetate (2.8 g) and aqueous (101.0 g) extracts. The aqueous extract was chromatographed over 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) → MeOH] to give fifteen fractions (frs. C_1—C_{15}). Fraction C_5 (1.7 g) was passed through a Lobar RP-8 column [CH_3CN–H_2O (3 : 17)] to give twelve fractions (frs. $C_{5\text{-}1}$—$C_{5\text{-}12}$).

Fraction $C_{5\text{-}5}$ was subjected to HPLC [ODS, CH_3CN–H_2O (1 : 9)] to give a mixture of **8** and **7** (10 mg) and fr. $C_{5\text{-}5\text{-}2}$. This mixture was subjected to HPLC [ODS, CH_3CN–H_2O (3 : 97)] to give **8** (4 mg) and **7** (4 mg) in pure form. Fraction $C_{5\text{-}7}$ was acetylated with Ac_2O and pyridine, and the acetylated compounds were subjected to HPLC [ODS, CH_3CN–H_2O (1 : 1)] to give four fractions (frs. $C_{5\text{-}7\text{-}1}$—$C_{5\text{-}7\text{-}4}$). Fraction $C_{5\text{-}7\text{-}4}$ was hydrolyzed by heating in a water bath with 5% NH_4OH–MeOH for 2 h, and then subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (97 : 3)] to give **6** (5 mg) and **5** (15 mg) in pure form. Fraction C_6 (1.9 g) was passed through 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 $C_{6\text{-}2}$ was subjected to HPLC [ODS, CH_3CN–H_2O (3 : 97)] to give **2** (60 mg). Fraction C_9 (1.3 g) was passed through a Lobar RP-8 column [MeOH–H_2O (3 : 17 → 1 : 4)] to give eleven fractions (frs. $C_{9\text{-}1}$—$C_{9\text{-}11}$). Fraction $C_{9\text{-}2}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (24 : 1)] to give **3** (7 mg). Fraction $C_{9\text{-}3}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (24 : 1)] to glve **4** (4 mg). Fraction C_{10} (0.4 g) was passed through 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 **9** (3 mg).

The H_2O eluate (71.5 g) was chromatographed over Sephadex LH-20 (MeOH) to give six fractions (frs. H—M). Fraction I (56.9 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (17 : 3 : 0.2 → 4 : 1 : 0.1 → 7 : 3 : 0.5) → MeOH] to give twelve fractions (frs. I_1—I_{12}). Fraction I_6 (0.7 g) was passed through a Lobar RP-8 column [CH_3CN–H_2O (99 : 1 → 19 : 1)] to give seven fractions (frs. $I_{6\text{-}1}$—$I_{6\text{-}7}$). Fraction $I_{6\text{-}4}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (19 : 1)] to give **1** (20 mg).

Ethyl β-D-Glucopyranoside (1) A colorless syrup, $[\alpha]_D^{23}$ −26.4° (*c*=0.8, MeOH), [lit.[3]; $[\alpha]_D$ −29.6° (MeOH)]. Positive FAB-MS *m/z*: 417 $[2M+H]^+$, 247 $[M+K]^+$, 231 $[M+Na]^+$, 209.1022 $[M+H]^+$ (base, Calcd for $C_8H_{17}O_6$: 209.1025), 163 $[M-C_2H_5OH+H]^+$.

Isopropyl β-D-Glucopyranoside (2) Colorless needles (MeOH), mp 129—131 °C, $[\alpha]_D^{23}$ −35.6° (*c*=1.1, MeOH). Positive FAB-MS *m/z*: 445 $[2M+H]^+$, 245 $[M+Na]^+$, 223.1162 $[M+H]^+$ (base, Calcd for $C_9H_{19}O_6$: 223.1182), 43 $[M-C_6H_{12}O_6+H]^+$.

Propane-1,2-diol 1-*O*-β-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{23}$ −21.0° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 239.1139 $[M+H]^+$ (base, Calcd for $C_9H_{19}O_7$: 239.1130), 207 $[M-CH_3OH+H]^+$.

Butane-2,3-diol 2-*O*-β-D-Glucopyranoside (4) An amorphous powder, $[\alpha]_D^{23}$ −32.0° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 275 $[M+Na]^+$, 253.1268 $[M+H]^+$ (base, Calcd for $C_{10}H_{20}O_7$: 253.1287).

3-Methylbutan-1-ol β-D-Glucopyranoside (5) Colorless needles (MeOH), mp 86—88 °C, $[\alpha]_D^{23}$ −33.0° (*c*=0.7, MeOH). Positive FAB-MS *m/z*: 523 $[2M+Na]^+$, 501 $[2M+H]^+$, 273.1324 $[M+Na]^+$ (base, Calcd for $C_{11}H_{22}O_6Na$: 273.1314), 251 $[M+H]^+$, 71 $[M-C_6H_{12}O_6+H]^+$.

(2*S*)-2-Methylbutan-1-ol β-D-Glucopyranoside (6) An amorphous powder, $[\alpha]_D^{22}$ −24.7° (*c*=0.2, MeOH), [lit.[6]; $[\alpha]_D$ −22.2° (MeOH)]. Positive FAB-MS *m/z*: 523 $[2M+Na]^+$, 501 $[2M+H]^+$, 273 $[M+Na]^+$ (base), 251.1516 $[M+H]^+$ (Calcd for $C_{11}H_{23}O_6$: 251.1495), 71 $[M-C_6H_{12}O_6+H]^+$.

(2*E*)-2-Methyl-2-buten-1-ol β-D-Glucopyranoside (7) Colorless needles (MeOH), mp 55—56 °C, $[\alpha]_D^{22}$ −15.8° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 287 $[M+K]^+$ (base), 271 $[M+Na]^+$, 249.1335 $[M+H]^+$ (Calcd for $C_{11}H_{21}O_6$: 249.1338), 69 $[M-C_6H_{12}O_6+H]^+$.

3-Methyl-2-buten-1-ol β-D-Glucopyranoside (8) Colorless needles (MeOH), mp 68—70 °C, $[\alpha]_D^{21}$ −23.6° (*c*=0.2, MeOH), [lit.[7]; $[\alpha]_D$ −20.0° (MeOH)]. Positive FAB-MS *m/z*: 519 $[2M+Na]^+$, 497 $[2M+H]^+$, 287 $[M+K]^+$, 271 $[M+Na]^+$ (base), 249.1341 $[M+H]^+$ (Calcd for $C_{11}H_{21}O_6$: 249.1338), 69 $[M-C_6H_{12}O_6+H]^+$.

Butane-2,3-diol 2-*O*-β-D-Apiofuranosyl-(1→6)-β-D-glucopyranoside (9) An amorphous powder, $[\alpha]_D^{23}$ −46.0° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 407 $[M+Na]^+$, 385.1715 $[M+H]^+$ (Calcd for $C_{15}H_{29}O_{11}$: 385.1709), 315 $[M-C_4H_8O+H]^+$ (base).

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