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; threo-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 (C₉H₁₀O₉, an amorphous powder, [α]D²² −68.0°) showed [M+K]+, [M+Na]+, [M+H]+ and [M−C₆H₄O₃+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-, ¹³C- and ¹H–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 methyl-ones, 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 (C₁₇H₂₀O₉, an amorphous powder, [α]D²² −44.0°) was identified as cnidioside A by direct comparison with an authentic sample.⁵

Glycoside 3 (C₂₂H₂₄O₁₀, an amorphous powder, [α]D²² −87.0°) showed [M+H]+ and [M−C₆H₁₂O₆+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]+ value (−313°), which was negative as was (1R')-1'(3-hydroxy-4-methoxyphenyl)ethane-1',2'-diol (−42°)⁶ when calculated using the value for methyl β-D-glucopyranoside (−62°). 3–methyl β-D-glucopyranoside = −251°).⁶ Comparision of the chemical shift of glucosyl C-1 of 3 (δ 101.85) with those of erythro-anethole glycol 1'-O-β-D-glucopyranosides (1R form, δ 101.46 and 1S form, δ 105.07)⁶ 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, [α]D²² −45.0°), 5 (an amorphous powder, [α]D²² −43.5°) and 6 (an amorphous

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

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powder, \([\delta]_{D}^{2} \approx -49.00\) were identified as 1’-(3,4-dimethoxyphenyl)ethane-1’,2’,3’-diol 2’,3’,4’-O-\(\beta\)-D-glucopyranoside, \(\beta\)-sitosterol \(\beta\)-D-glucopyranoside and stigmasteryl \(\beta\)-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 \(\beta\)-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_{30}H_{48}O_{3}, mp 182–184°C, \([\delta]_{D}^{2} 0°\)) 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), \(^{2h}\) 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’ 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_{10}H_{12}O_{3}, mp 185–186°C, \([\delta]_{D}^{2} 0°\)), 9 (C_{39}H_{50}O, mp 137–139°C, \([\delta]_{D}^{2} -31.00\)), 10 (C_{30}H_{48}O, mp 167–169°C, \([\delta]_{D}^{2} -47.00\)) and 11 (C_{39}H_{50}O, mp >300°C, \([\delta]_{D}^{2} +85.00\)) were identified as p-anisic acid, \(\beta\)-sitosterol, stigmastanol and oleanolic acid, respectively.

Fennel contains 3–9% essential oil comprising 57–82% anethole and 6–27% p-anisaldehyde (14), \(^{3j}\) and 14 is regarded as a compound of an auto-oxidation product of anethole while the fennel is preserved. \(^{3j}\) 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.

**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.\(^{11}\)

**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,
The sugar fraction was subjected to HPLC [column, carbohydrate analysis; Curro P., Micali G., Lauzza F., Pharm. Acta Helv., 77, 335—339 (2002)].

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References and Notes

11) Fennel used in this experiment (purchased from Kinokuniya Chinese Medicine Pharmacy, Ltd., lot. No. AOCl40283) contained 3.8% of essential oil, and the ratio of anethole and 14 was 17:3 by HPLC analysis; Curro P., Micali G., Lauzza F., J. Chromatogr., 404, 273—278 (1987).