PERGAMON

Phytochemistry 61 (2002) 455–459

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Water-soluble constituents of caraway: aromatic compound, aromatic compound glucoside and glucides

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Received 29 January 2002; received in revised form 3 July 2002

Abstract

From the water-soluble portion of the methanolic extract of caraway (fruit of *Carum carvi* L.), an aromatic compound, an aromatic compound glucoside and a glucide were isolated together with 16 known compounds. Their structures were clarified as 2-methoxy-2-(4′-hydroxyphenyl)ethanol, junipediol A 2-*O*-β-D-glucopyranoside and L-fucitol, respectively.

Keywords: Caraway; *Carum carvi* fruit; Umbelliferae; 2-Methoxy-2-(4′-hydroxyphenyl)ethanol; Junipediol A 4-*O*-β-D-glucopyranoside; L-Fucitol

1. Introduction

In previous papers (Matsumura et al., 2001, 2002), the isolation and characterization of 19 monoterpenoids, including eight stereoisomers of *p*-menthane-2,8,9-triol and five stereoisomers of *p*-menthane-1,2,8,9-triol, and eleven glucosides from the methanolic extract of caraway [fruit of *Carum carvi* L.; Umbelliferae], which has been used as a popular aromatic herb and medicine, were reported. In continuation of these studies on the water-soluble constituents of spices, the isolation and structure elucidation of aromatic compounds, aromatic compound glucosides, alkyl glycosides, glucides and nucleoside were undertaken.

2. Results and discussion

Commercial caraway was extracted with MeOH–H_2O (70:30), and the corresponding extract was partitioned into ether-water and ethyl acetate-water, successively. The aqueous layer was applied to an Amberlite XAD-II column to give aqueous and methanol eluate fractions. The fractions were subjected to Sephadex LH-20, silica gel and Lobar RP-8 column chromatography, and finally, HPLC was used for final purification. In this way, one aromatic compound (**1**), five aromatic compound glucosides (**2**–**6**), one alkyl glucoside (**7**), and uridine (**8**) were isolated from the methanol eluate fraction, as well as 11 glucides (**9**–**19**) were isolated from the water eluate fraction. Among them, **1**, **2** and **14** were new. Molecular formulae of the new compounds were suggested from the accurate mass number of $[M+H]^+$ or $[M+Na]^+$ ion peaks in the high-resolution positive FAB–MS.

Aromatic compound **1** ($C_9H_{12}O_3$, an amorphous powder, $[\alpha]_D^{22}$ −15°) showed an $[M+Na]^+$ ion peak at *m*/*z* 191 in the positive FAB–MS, and the ^1H and ^{13}C NMR spectral data indicated the presence of one 1,4-disubstituted benzene ring, one *sec*-hydroxymethyl, one oxygenated methine and one methoxyl group. Thus, **1** was suggested to be a methyl ether of 2-hydroxy-2-(4′-hydroxyphenyl)ethanol. The position of the methoxyl group was found to be C-2 from the H–C long-range correlation between the methoxyl proton and C-2 carbon in the HMBC spectrum. Since commercial (2*R*)-2-methoxy-2-phenylethanol showed rather large negative optical rotation ($[\alpha]_D^{22}$ −119° for 2*R*-form; $[\alpha]_D^{22}$ +120° for 2*S*-form) than observed for **1**, **1** was considered to be an epimeric mixture of 2-methoxy-2-(4′-hydroxyphenyl)ethanol.

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0031-9422/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0031-9422(02)00288-1

Aromatic compound glucoside **2** ($C_{16}H_{24}O_9$, an amorphous powder, $[\alpha]_D^{25}$ −34°) revealed $[M+Na]^+$, $[M+H]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 383, 361 and 181 in the positive FAB–MS. The ^1H and ^{13}C NMR spectral data showed the presence of one β-glucopyranosyl group, one 1,3,4-trisubstituted benzene ring, one 1,3-dihydroxyisopropyl group and one methoxyl group. Enzymatic hydrolysis of **2** gave junipediol A [2-(3′-methoxy-4′-hydroxyphenyl)propane-1,3-diol] (Comte et al., 1997) and D-glucose, and **2** was suggested to be a monoglucoside of junipediol A. The position of the β-glucosyl unit was proven to be at C-4 from the NOE interaction observed between the glucosyl H-1/H-5 in the NOESY spectrum (Fig. 1) and the HNBC correlation of glucosyl H-1/C-4. Thus, **2** was characterized as junipediol A 4-*O*-β-D-glucopyranoside.

Glucide **14** ($C_6H_{14}O_5$, an amorphous powder, $[\alpha]_D^{21}$ −5°) consisted of one *sec*-methyl, one hydroxylated methylene, four hydroxylated methines, and was suggested to be 1-deoxyhexitol. As L-fucitol (1-deoxy-D-galactitol), which was obtained by the $NaBH_4$ reduction of L-fucose, showed identical spectral data and optical rotation value with **14**, glucide **14** was identified as L-fucitol. Compound **14** is a new natural-occurring product.

Aromatic compound glucosides **3**–**6**, alkyl glucoside **7**, glucides **9**–**19** were identified as coniferin (Sugiyama et al., 1993), syringin (Kitajima et al., 1998a), benzyl β-D-glucopyranoside (Kitajima et al., 1998a), phenetyl β-D-glucopyranoside (Kitajima et al., 1998a), 3-methylbutyl β-D-glucopyranoside (Kitajima et al., 1998b), glycerol 2-*O*-α-L-fucopyranoside (Kitajima et al., 2001), *meso*-erythritol (Kitajima et al., 1999), D-threitol (Kitajima et al., 1999), 1-deoxy-D-ribitol (Kitajima et al., 1999), (3*R*)-hydroxymethylbutane-1,2,3,4-tetrol (Kitajima et al., 1998c, 1999), 1-deoxy-D-glucitol (Kitajima et al., 1999), D-glucitol (the authentic sample was prepared by $NaBH_4$ reduction of D-glucose), *meso*-galactitol (the authentic sample was prepared by $NaBH_4$ reduction of D-galactose), D-mannitol and D-fructose by direct comparison with authentic samples, respectively.

The fruit of caraway contains 3–7% of essential oil, which consists of D-carvone (main: 50–60%), L-limonene and other monoterpenoids (Salveson and Svendsen, 1976), but no aromatic compound was reported as a constituent of this oil. On the other hand, from the water-soluble portion of the methanolic extract of caraway, nineteen monoterpenoid alcohols (yields from the Amberlite XAD-II column methanol eluate: 2.63%) and an aromatic compound (yield: 0.01%) were obtained as free form, and eleven monoterpenoid alcohols (yield: 3.69%) and five aromatic compounds (yield: 1.00%) were obtained in glucosyl form. Consequently, the aromatic compounds of this fruit are considered to be inclined to exist as glucosides rather than in free form.

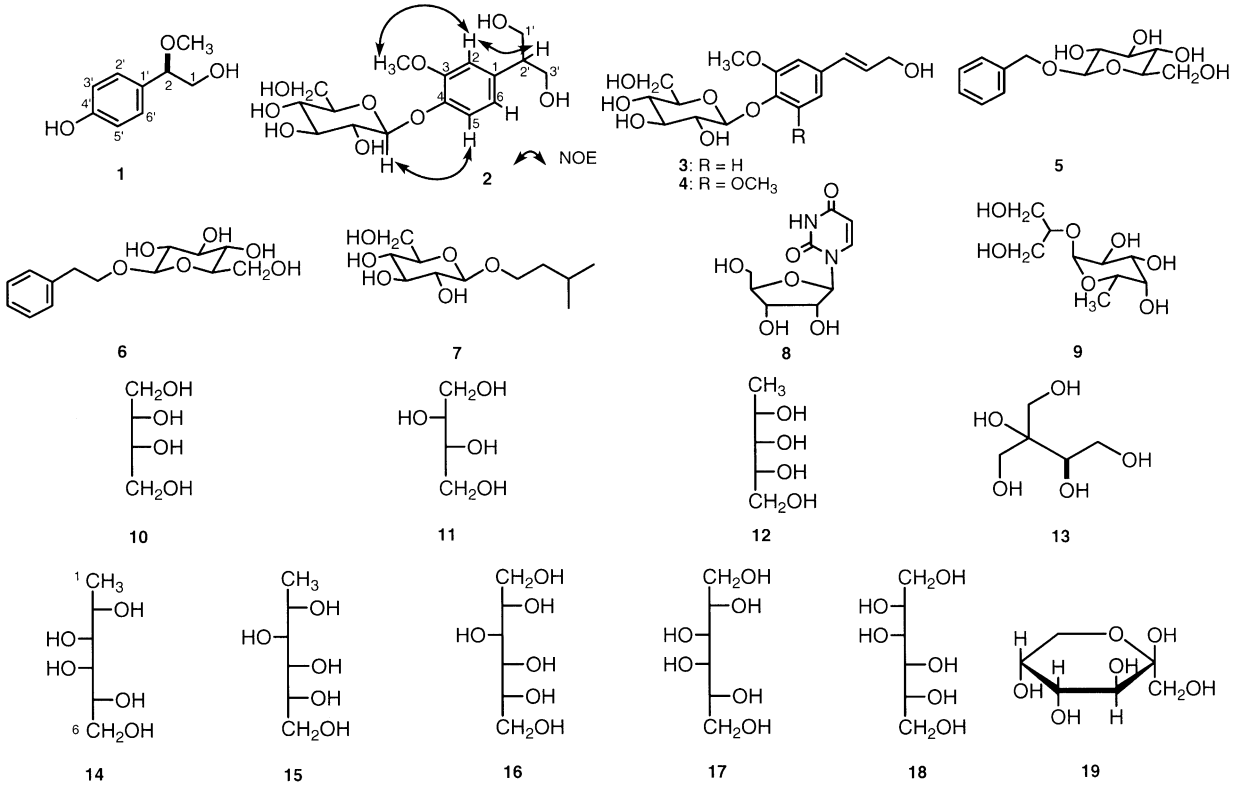

Fig. 1. Structures of **1**–**19**, and NOE interactions observed in the NOESY spectrum of **2**.

3. Experimental

3.1. General

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 obtained using Jeol JNM GX-270 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were tabulated as δ value. ^{1}H–^{13}C COSY, HMBC and NOESY spectra were obtained with the standard pulse sequence, and data processing was performed with standard JEOL software. CC was carried out under TLC monitoring using Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 μm, Pharmacia), Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck 5721) and compounds were detected with *p*-anisaldehyde-H_2SO_4 reagent. Optical rotations were measured in MeOH, except **3** and **4** (pyridine) and **12**, **14** and **15** (H_2O). HPLC separation was carried out with Symmetryprep C_{18} 7μm [Waters; 7.8 × 300 mm; ODS], carbohydrate analysis [Waters; 3.9 × 300 mm; CHA] and Wakobeads T-100s [Wako; 6.0 × 150 mm; WBT] columns. (2*R*)- and (2*S*)-2-methoxy-2-phenylethanol were purchased from Aldrich Chemical Co. Acetylation was done in the usual way using Ac_2O and pyridine. No acetoxyl group had been detected by the NMR spectral analysis of the materials prior to acetylation.

3.2. Extraction and separation

Commercial caraway (fruit of *Carum carvi* L.; purchased from Asaoka Spices Ltd., Lot. No. 93010; 2 kg) was extracted with MeOH–H_2O 70:30, 4 l × 2), and the extract was partitioned into ether–water and EtOAc–water, respectively. The aqueous portion was subjected to Amberlite XAD-II ($H_2O \rightarrow$MeOH) chromatography to give water eluate (170.2 g) and MeOH eluate (27.6 g) fractions.

The MeOH eluate was subjected to Sephadex LH-20 (MeOH) to give eight fractions (frs. A–H). Fraction B (18.9 g) was applied to silica gel [eluted with $CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→7:3:0.5)→MeOH] to give 14 fractions (frs. B_1–B_{14}). Fraction B_3 (1.70 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give nine fractions (frs. B_{3-1}–B_{3-9}), and fr. B_{3-7} was subjected to HPLC [ODS, MeOH–H_2O (1:7)] and silica gel [$CHCl_3$–MeOH–H_2O (9:1:0.1)] to give **6** (2 mg). Fraction B_4 (0.82 g) was subjected to Lobar RP-8 column chromatography [eluted with MeCN-H_2O (3:17)], HPLC [CHA, MeCN–H_2O (19:1)] and Sephadex LH-20 (MeOH) to give **7** (5 mg) and **5** (5 mg). Fraction B_5 (0.44 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [carbohydrate analysis (CHA), MeCN–H_2O (19:1)] to give **3** (146 mg). Fraction B_6 (0.94 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give eight fractions (frs. B_{6-1}–B_{6-8}). Fraction B_{6-4} was subjected to HPLC [CHA, MeCN–H_2O (9:1)] to give **4** (50 mg). Fraction B_{12} (0.85 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [CHA, MeCN–H_2O (9:1)] to give **2** (71 mg). Fraction C (1.57 g) was subjected to silica gel chromatography [eluted with $CHCl_3$-MeOH-H_2O (4:1:0.1)→MeOH] to give eight fractions (frs. C_1–C_8). Fraction C_2 (0.08 g) was subjected to HPLC [CHA, MeCN–H_2O (19:1)] to give **1** (2 mg), and fr. C_4 (0.58 g) was subjected to Sephadex LH-20 (MeOH) and HPLC [ODS, MeCN–H_2O (1:39)] to give **8** (30 mg).

The water eluate fraction was subjected to Sephadex LH-20 (MeOH) to give four fractions (frs. I–L). Fraction J (23.36 g) was applied to silica gel [eluted with $CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→7:3:0.5)→MeOH] to give 25 fractions (frs. J_1–J_{24}). Fraction J_{17} (0.26 g) was passed through a Lobar RP-8 column (H_2O), HPLC [CHA, MeOH–H_2O (49:1)]. The so-obtained glucide fraction was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, MeCN–H_2O (1:1)] to give two components. These two components were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h. Then, **10** (45 mg) and **11** (23 mg) were isolated after Sephadex LH-20 (MeOH) CC. Fraction J_{18} (0.29 g) was subjected to a Lobar RP-8 column (H_2O) to give five fractions (frs. J_{18-1}–J_{18-5}). Fraction J_{18-2} was subjected to HPLC [CHA, MeCN–H_2O (19:1)] to give **13** (2 mg) and **15** (7 mg), fr. J_{18-3} was subjected to HPLC [CHA, MeCN–H_2O (17:3)] to give **14** (3 mg) and **17** (10 mg), and J_{18-4} was subjected to HPLC [CHA, MeCN–H_2O (19:1)] to give **9** (7 mg), respectively. Fraction J_{19} (0.64 g) was passed through a Lobar RP-8 column (H_2O) and HPLC [WBT, MeCN–H_2O (17:3)] to give **12** (4 mg). Fraction J_{22} (0.34 g) was passed through a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (19:1)] to give **19** (26 mg). Fraction J_{25} (0.75 g) was passed through a Lobar RP-8 column [MeCN–H_2O (1:99)], and the so-obtained glucide fraction was acetylated with Ac_2O and pyridine.

The acetylated fraction was subjected to Sephadex LH-20 (MeOH) and HPLC [ODS, MeCN–H_2O (1:1)] to give two fractions. These two fractions were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h. From the former fraction, **16** (95 mg) was isolated by HPLC [CHA, MeCN–H_2O (17:3)], and from the latter fraction, **18** (15 mg) was given by HPLC [CHA, MeCN-H_2O (17:3)].

The following compounds were identified by comparison with authentic compounds or published physical and spectral data: coniferin (**3**), syringin (**4**), benzyl

β-D-glucopyranoside (**5**), phenethyl β-D-glucopyranoside (**6**), 3-methyl β-D-glucopyranoside (**7**), uridine (**8**), glycerol 2-*O*-α-L-fucopyranoside (**9**), *meso*-erythritol (**10**), D-threitol (**11**), 1-deoxy-D-ribitol (**12**), (3*R*)-hydroxymethylbutane-1,2,3,4-tetrol (**13**), 1-deoxy-D-glucitol (**15**), D-glucitol (**16**), *meso*-galactitol (**17**), D-mannitol (**18**) and D-fructose (**19**).

3.3. 2-Methoxy-2-(4′-hydroxyphenyl)ethanol (*1*)

Amorphous powder, $[\alpha]_D^{21}$ −15° ($c = 0.2$, MeOH). Positive FAB–MS *m/z*: 207 $[M+K]^+$, 191.0675 $[M+Na]^+$ (base, calcd for $C_9H_{12}NaO_3$; 191.0683), 169 $[M+H]^+$. ^{1}H-NMR (Pyridine-d_5, 500 MHz) δ: 3.34 (3H, *s*, 2-OCH_3), 3.99 (1H, *dd*, $J = 4.0$, 11.5 Hz, H-1a), 4.16 (1H, *dd*, $J = 7.5$, 11.5 Hz, H-1b), 4.53 (1H, *dd*, $J = 4.0$, 7.5 Hz, H-2), 7.22 (2H, *d*, $J = 8.0$ Hz, H-3′ and H-5′), 7.44 (2H, *d*, $J = 8.0$ Hz, H-2′ and H-6′). ^{13}C-NMR (Pyridine-d_5, 125 MHz) δ: 56.66 (2-OCH_3), 67.68 (C-1), 85.94 (C-2), 116.24 (C-3′, C-5′), 129.05 (C-2′, C-6′), 130.88 (C-1), 158.74 (C-47). HMBC correlations: H-1b/C-2, C-1′; H-2/C-1′, C-2′, C-6′; 2-OCH_3/C-2; H-2′,6′/C-1′, C-3′, C-4′, C-5′, C-2; H-3′,5′/C-1′, C-2′, C-4′, C-6′.

3.4. Junipediol A 4-O-β-D-glucopyranoside (*2*)

Amorphous powder, $[\alpha]_D^{25}$ −34° ($c = 0.8$, MeOH). Positive FAB-MS *m/z*: 399 $[M+K]^+$, 383.1317 $[M+Na]^+$ (base, Calcd for $C_9H_{12}NaO_3$; 383.1318), 361 $[M+H]^+$, 181 $[M\text{-}C_6H_{12}O_6+H]^+$. ^{1}H NMR (Pyridine-$d_5$, 500 MHz) δ: 3.45 (1H, *quint*, $J = 6.0$ Hz, H-2′), 3.70 (3H, *s*, 3-OCH_3), 4.30, 4.42 (each 2H, *dd*, $J = 6.0$, 10.5 Hz, H_2-1′, H_2-3′), 5.62 (1H, *d*, $J = 7.5$, Glc H-1), 7.05 (1H, *dd*, $J = 2.0$, 8.5 Hz, H-6), 7.22 (1H, *d*, $J = 8.5$ Hz, H-2), 7.53 (1H, *d*, $J = 8.5$ Hz, H-5). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 51.70 (C-2′), 55.94 (3-OCH_3), 64.70 (C-1′, C-3′), 113.85 (C-2), 116.23 (C-5), 121.24 (C-6), 136.89 (C-4), 146.57 (C-5), 150.10 (C-6), Glc [62.30 (C-6), 71.23 (C-4), 74.86 (C-2), 78.39 (C-3), 78.69 (C-5)]. HMBC correlations: H-2/C-3, C-4, C-6, C-2′; H-5/C-1, C-3, C-4; H-6/C-2. C-4, C-5, C-2′; H-1′,3′/C-1, C-2′; H-2′/C-1, C-2, C-6, C-1′,3′; 3-OCH_3/C-3; Glc H-1/C-4.

3.5. Enzymatic hydrolysis of *2*

A mixture of **2** (11 mg) and hesperidinase (5 mg, ICN Biomedicals Inc., Lot. 72635) in water (5 ml) was shaken in a water bath at 37 °C for 20 days. The mixture was evaporated *in vacuo* to dryness and the residue was subjected to silica gel chromatography [eluted with $CHCl_3$-MeOH-H_2O (4:1:0.1 and 1:1:0.1)] to afford **2a** (3 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (waters), detector; JASCO RI-930 detector and JASCO OR-990 chiral detector, solv.; MeCN–H_2O (17:3), 2 ml/min; t_R 4.50 min (same location as that of D-glucose)] showed the presence of D-glucose. Compound **2a** was identified as junipediol by comparison with published NMR spectral data (Comte et al., 1997).

3.6. L-Fucitol (*14*)

Amorphous powder, $[\alpha]_D^{21}$ −5° ($c = 0.1$, H_2O). Positive FAB–MS (without matrix) *m/z*: 333 $[2M+H]^+$, 167.0937 $[M+H]^+$ (base, calcd for $C_6H_{15}O_5$; 167.0919). ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 1.60 (3H, *d*, $J = 6.5$ Hz, H_3-1), 4.26 (1H, *br d*, $J = 8.0$ Hz, H-3), 4.36, 4.38 (each 1H, *dd*, $J = 6.0$, 8.0 Hz, H_2-6), 4.59 (1H, *br d*, $J = 8.0$ Hz, H-4), 4.77 (1H, *br q*, $J = 6.5$ Hz, H-2), 4.85 (1H, *br t*, $J = 6.0$ Hz, H-5). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 20.76 (C-1), 65.17 (C-6), 67.35 (C-2), 72.15 (C-4), 72.40 (C-5), 74.97 (C-3).

3.7. $NaBH_4$ reduction of L-fucose

L-Fucose (13 mg, Kanto Chemical Co.) was dissolved in MeOH (7.5 ml) and stirred with $NaBH_4$ (10 mg) for 5 h at room temp, respectively. After the reaction mixture was condensed in vacuo, it was subjected to silica gel [$CHCl_3$–MeOH (1:1)] and Sephadex LH-20 (MeOH) CC to give L-fucitol [**14a**; $[\alpha]_D^{21}$ −4° ($c = 1.0$, H_2O)]. The ^{1}H and ^{13}C NMR spectroscopic data, and optical rotation value of **14** were identical with those of **14a**.

Acknowledgements

The authors thank Mr. Y. Takase and Dr. H. Suzuki of the Analytical Center of this University for NMR and MS measurements.

References

Comte, G., Allais, D.P., Chulia, A.J., Vercauterent, J., Pinaud, N., 1997. Three phenylpropanoids from *Juniperus phoenicea*. Phytochemistry 44, 1169–1173.

Kitajima, J., Ishikawa, T., Tanaka, Y., Ono, M., Ito, Y., Nohara, T., 1998. Water-soluble constituents of fennel. V. glycosides of aromatic compounds. Chem. Pharm. Bull. 46, 1587–1590.

Kitajima, J., Ishikawa, T., Tanaka, Y., 1998. Water-soluble constituents of fennel. I. Alkyl glycosides. Chem. Pharm. Bull. 46, 1643–1646.

Kitajima, J., Suzuki, N., Ishikawa, T., Tanaka, Y., 1998. New hemiterpenoid pentol and monoterpenoid glycoside of *Torillis japonica* fruit. Chem. Pharm. Bull. 46, 1583–1586.

Kitajima, J., Ishikawa, T., Tanaka, Y., Ida, Y., 1999. Water-soluble constituents of fennel. IX. Glucides and nucleosides. Chem. Pharm. Bull. 47, 988–992.

Kitajima, J., Ishikawa, T., Aoki, Y., 2001. Glucides of *Cnidium monnieri*. Phytochemistry 58, 641–644.

Matsumura, T., Ishikawa, T., Kitajima, J., 2001. New *p*-menthanetriols and their glucosides from the fruit of caraway. Tetrahedron 57, 8067–8074.

Matsumura, T., Ishikawa, T., Kitajima, J., 2002. Water-soluble constituents of caraway; carvone derivatives and their glucosides. Chem. Pharm. Bull. 50, 66–72.

Salveson, A., Svendsen, A.B., 1976. Gas liquid chromatographic separation and identification of the constituents of caraway seed oil. Plant Med. 30, 93–96.

Sugiyama, M., Nagayama, E., Kikuchi, M., 1993. Lignan and phenylpropanoid glycosides from *Osmanthus asiaticus*. Phytochemistry 33, 1215–1219.