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Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise

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Abstract

From the polar portion of the methanolic extract of the fruit of anise (*Pimpinella anisum* L.), which has been used as a spice and medicine since antiquity, four aromatic compound glucosides, an alkyl glucoside and a glucide were isolated together with 24 known compounds. The structures of the new compounds were clarified as (*E*)-3-hydroxyanethole β-D-glucopyranoside, (*E*)-1′-(2-hydroxy-5-methoxyphenyl)propane β-D-glucopyranoside, 3-hydroxyestragole β-D-glucopyranoside, methyl syringate 4-*O*-β-D-glucopyranoside, hexane-1,5-diol 1-*O*-β-D-glucopyranoside and 1-deoxy-L-erythritol 3-*O*-β-D-glucopyranoside by spectral investigation.

Keywords: Anise; *Pimpinella anisum* fruit; Umbelliferae; Aromatic compound glucoside; Alkyl glucoside; Glucide; Hexane-1,5-diol 1-*O*-β-D-glucopyranoside; 1-Deoxy-L-erythritol 3-*O*-β-D-glucopyranoside

1. Introduction

Anise (*Pimpinella anisum* L.; Umbelliferae) has been used as a popular aromatic herb and spice since antiquity, and has been cultivated throughout Europe. Its fruit has been used for medicine and in cooking, and is listed in British, German and European pharmacopoeia. For medicinal purposes, it is used to treat dyspeptic complaints and catarrh of the respiratory tract, and also as a mild expectorant. In previous papers (Kitajima et al., 2003; Ishikawa et al., 2002), we reported the isolation and characterization of eight 2-*C*-methyl-D-erythritol glycosides and 12 phenylpropanoid glucosides, from the water-soluble portion of anise. In continuation of our studies on the polar constituents of anise, we undertook further isolation and structure elucidation of aromatic compound glucosides, alkyl glucosides, monoterpenoid glucosides, norcarotenoid glucosides and glucides.

2. Results and discussion

Commercial anise was extracted with 70% aq. methanol, and the methanolic extract was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was subjected to Amberlite XAD-II chromatograph to give water and methanol eluate fractions. Each fraction was applied to Sephadex LH-20 column, and subjected to a combination of silica gel, Lobar RP-8 column chromatography and HPLC to isolate the compounds. Then, 20 anethole glycol glucosides and its related compounds (**A-1**–**A-20**; Ishikawa et al., 2002), two 2-*C*-methyl-D-erythritol glycosides (**E-7** and **E-8**; Kitajima et al., 2003), 17 aromatic compound glucosides (**1**–**17**), two alkyl glucosides (**20** and **21**), two monoterpenoid glucosides (**22** and **23**), two norcarotenoid glucoside (**24** and **25**) were obtained from the methanol eluate fraction. Furthermore, two alkyl glucosides (**18** and **19**) and five glucides (**26** to **30**) were isolated together with 2-*C*-methyl-D-erythritol and its glycosides (**E-1** to **E-7**; Kitajima et al., 2003) from the water eluate fraction. Among them, **1**–**3**, **9**, **21** and **28** are new, and **6** and **19** are newly isolated compounds from natural sources (Charts 1 and 2).

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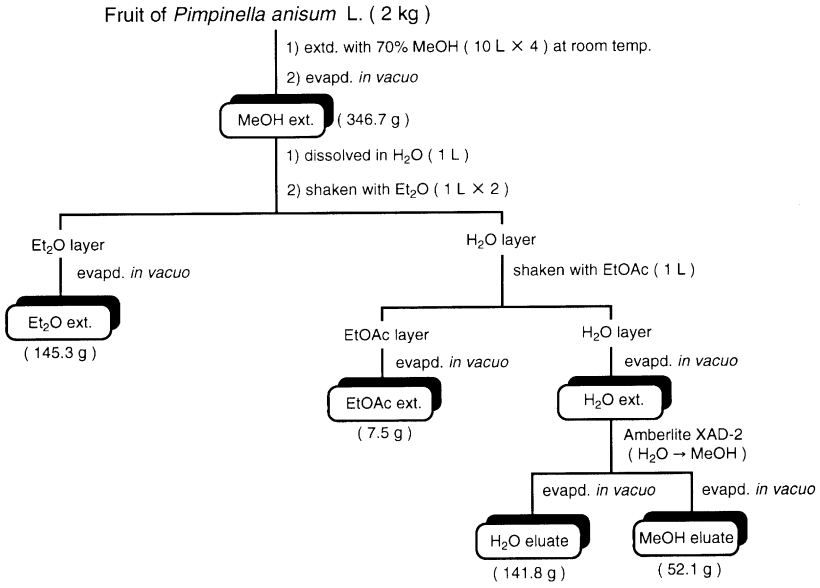

Chart 1. Extraction of polar constituents of anise.

All new glucosides described in this paper were β-D-glucopyranosides as shown by their ^{13}C NMR spectroscope data (Table 2), and this was confirmed by their $[\alpha]_D$ or $[M]_D$ values. Their molecular formulae were suggested from the accurate mass number of $[M+H]^+$ ion peaks in the high-resolution positive FAB-MS.

Aromatic compound glucosides **4**, **5**, **6**, **7**, **8**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17** were identified as 4-hydroxyphenylpropyl β-D-glucopyranoside (Higuchi et al., 1977), 1′-(4-hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-glucopyranoside (Kitajima et al., 1998a), 2-methoxyphenyl β-D-glucopyranoside (Yoshida et al., 1969; Kim et al., 1986), isotachioside (Ishimaru et al., 1987; Inoshiri et al., 1987), tachioside (Ishimaru et al., 1987; Inoshiri et al., 1987), vanilloloside (Ida et al., 1994), 4-hydroxy-3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (Kitajima et al., 1998b), benzyl β-D-glucopyranoside (Kitajima et al., 1998b), icariside F_2 (Kitajima et al., 1998b), 4-hydroxybenzyl β-D-glucopyranoside (Mao and Anderson, 1967; Vic and Thomas, 1992), icariside D_1 (Miyase et al., 1987a), icariside D_2 (Miyase et al., 1989), viridoside (Hiraoka, 1981), respectively, by comparison of physical and NMR spectra data with those of reported and/or spectral investigations.

Glucoside **1** ($C_{16}H_{22}O_7$, an amorphous powder, $[\alpha]_D^{25}$ −28°) and 2 ($C_{16}H_{22}O_7$, an amorphous powder, $[\alpha]_D^{25}$ −34°) showed $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m*/*z* 327 and 165 in the positive FAB-MS. Acid hydrolysis of **1** gave D-glucose as a sugar component. The ^1H and ^{13}C NMR chemical shifts (Tables 1 and 2) of **1** and **2** showed that both compounds were monoglucosides of (hydroxy-methoxyphenyl)propene. The aglycone of **1** was represented as 3-hydroxyanethole from the results of an HMBC experiment (see Experimental) and the observed NOE interactions between the signal of CH_3-*O*-/H-5 and Glc H-1/H-2 in its NOESY spectrum. The stereochemistry of the propenyl double bond was suggested to be *E* by its coupling constant between H-1′ and H-2′ ($J=16.0$ Hz). Then, **1** was characterized as (*E*)-3-hydroxyanethole β-D-glucopyranoside. On the other hand, the aglycone of 2 was suggested to be (*E*)-1′-(2-hydroxy-5-methoxyphenyl)propane from the analysis of the HMBC spectrum (see Experimental), the NOE interactions between the signal of CH_3-*O*-/H-4, H-6 and Glc H-1/H-3 in its NOESY spectrum, and the coupling constant value between H-1′ and H-2′ ($J=16.0$ Hz). So, 2 was characterized as (*E*)-1′-(2-hydroxy-5-methoxyphenyl)propane β-D-glucopyranoside.

Glucoside **3** ($C_{16}H_{22}O_7$, an amorphous powder, $[\alpha]_D^{23}$ −34°) revealed $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m*/*z* 327 and 165 in the positive FAB-MS. Its ^1H and ^{13}C NMR spectral data (Tables 1 and 2) and the results of HMBC experiment (see Experimental) showed that **3** was a β-glucopyranoside of 3-hydroxyesteragole, and the observed NOE interactions between CH_3-*O*-/H-5 and Glc H-1/H-2 in its NOESY spectrum supported this conclusion. From these facts, 3 was characterized as 3-hydroxyestragole β-D-glucopyranosides as described in Fig. 1.

Glucoside **9** ($C_{16}H_{22}O_{10}$, mp 91–93 °C, $[\alpha]_D^{24}$ −20°) showed $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m*/*z* 375 and 213 in the positive FAB-MS, and its NMR spectral data (Tables 1 and 2) showed the presence of one tetrasubstituted benzene ring with a symmetric axis between C-1 and C-4, two methoxyl, one hydroxyl and one methylcarbonate group, in addition to the β-D-glucopyranosyl moiety. The observed NOE interactions between the signal of CH_3-*O*-/H-2 (H-6) and Glc H-1/

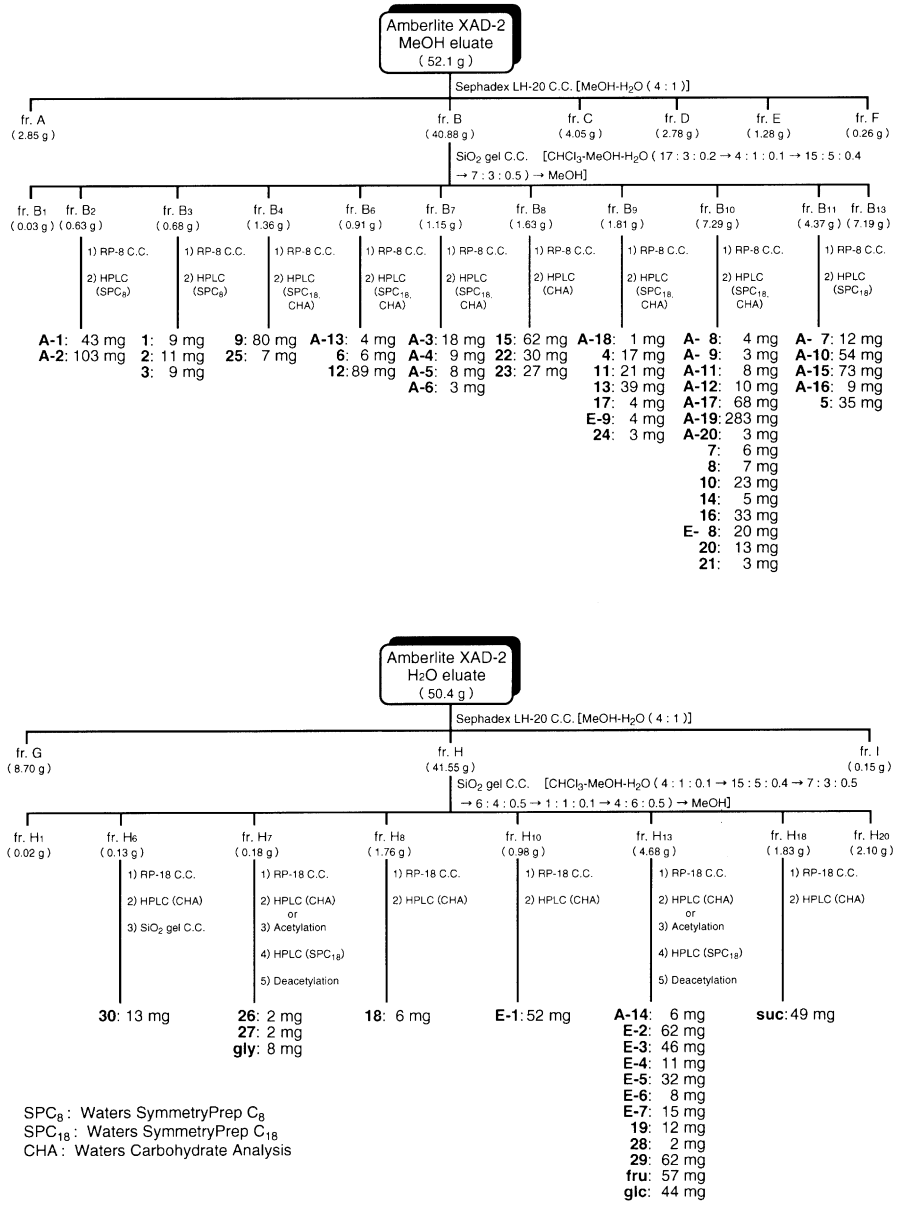

Chart 2. Separation of amberlite XAD-2 CC MeOH and H_2O eluate fractions. **A-1**: *erythro*-anethole glycol, **A-2**: *threo*-anethole glycol, **A-3**: (1′*R*,2′*S*)-anethole glycol 2′-*O*-β-D-glucopyranoside, **A-4**: (1′*S*,2′*R*)-anethole glycol 2′-*O*-β-D-glucopyranoside, **A-5**: (1′*R*,2′*R*)-anethole glycol 2-*O*-β-D-glucopyranoside, **A-6**: (1′*S*,2′*S*)-anethole glycol 2′-*O*-β-D-glucopyranoside, **A-7**: erythro-1′-(4-hydroxyphenyl)propane-1′,2′-diol 4-*O*-β-D-glucopyranoside, **A-8**: (1′*R*,2′*S*)-1′-(4-hydroxyphenyl)propane-1′,2′-diol 2′-*O*-β-D-glucopyranoside, **A-9**: (1′*S*,2′*R*)-1′-(4-hydroxyphenyl)propane-1′,2′-diol 2′-*O*-β-D-glucopyranoside, **A-10**: *threo*-1′-(4-hydroxyphenyl)propane 1′2′-diol 4-*O*-β-D-glucopyranoside, **A-11**: (1′*R*,2′*R*)-1′-(4-hydroxyphenyl)propane-1′,2′-diol 2′-*O*-D-glucopyranoside, **A-12**: (1′*S*,2′*S*)-l′-(4-hydroxyphenyl)propane-1′,2′-diol 2′-*O*-β-D-glucopyranoside, **A-13**: (1′*R*,2′*R*)-guaiacyl glycerol, **A-14**: (1′*R*,2′*R*)-guaiacyl glycerol 4-*O*-β-D-glucopyranoside, **A-15**: (1′*R*,2′*R*)-guaiacyl glycerol 3′-*O*-β-D-glucopyranoside, **A-16**: (1′*S*,2′*R*)-guaiacyl glycercol 3′-*O*-β-D-glucopyranoside, **A-17**: (1′*R*,2′*R*)-4-*O*-methylguaiacy glycerol 3′-*O*-β-D-glucopyranoside, **A-18**: 4-*O*-methytguaiacyl glycerol 2′-*O*-β-D-glucopyranoside, **A-19**: (*E*)-4-hydroxycinnamyl alcohol 4-*O*-β-D-glucopyranoside, **A-20**: (*Z*)-4-hydroxycinnamyl alcohol 4-*O*-β-D-glucopyranoside, **E-1**: 2-*C*-methyl-D-erythritol, **E-2**: 2-*C*-methyl-D-erythritol 1-*O*-D-glucopyranoside, **E-3**: 2-*C*-methyl-D-erythritol 3-*O*-β-D-glucopyranoside, **E-4**: 2-*C*-methyl-D-erythritol 4-*O*-β-D-glucopyranoside, **E-5**: 2-*C*-methyl-D-erythritol 1-*O*-D-fructofuranoside, **E-6**: 2-*C*-methyl-D-erythritol 3-*O*-β-D-fructofuranoside, **E-7**: 2-*C*-methyl-D-erythritol 4-*O*-β-D-fructofuranoside, **E-8**: 2-*C*-methyl-D-erythritol 1-*O*-β-D-(6-hydroxybenzoyl)glucopyranoside, **E-9**: 2-*C*-methyl-D-erythritol 1-*O*-β-D-(6-*O*-4-methoxybenzoyl)glucopyranoside, **1**: (*E*)-3-hydroxyanethole β-D-glucopyranoside, **2**: (*E*)-1′-(2-hydroxy-5-methoxyphenyl)propene β-*O*-D-glucopyranoside, **3**: 3-hydroxyestragole β-D-O-glucopyranoside, **4**: 4-hydroxyphenylpropyl β-D-glucopyranoside, **5**: 1′-(4-hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-glucopyranoside, **6**: 2-methoxyphenyl β-D-glucopyranoside, **7**: isotachioside, **8**: tachioside, **9**: methyl syringate 4-*O*-β-D-glucopyranoside, **10**: vannilloside, **11**: 4-hydroxy-3,5-dinethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside, **12**: benzyl β-D-glucopyranoside, **13**: icariside F_2, **14**: 4-hydroxybenzyl β-D-glucopyranoside, **15**: icariside D_1, **16**: icariside D_2, **17**: viridoside, **18**: ethyl-β-D-glucopyranoside, **19**: ethane-l,2-diol β-D-glucopyranoside, **20**: (2*S*)-2-butanol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, **21**: hexane-1,5-diol 1-*O*-β-D-glucopyranoside, **22**: betulalbuside, **23**: (3*R*,6*E*)-8-hydroxylinalool 3-*O*-β-D-glucopyranoside, **24**: icariside B_1, **25**: icanside B_2, **26**: 1-deoxythreitol, **27**: 1-deoxy-L-erythritol, **28**: 1-deoxy-L-erythritol 3-*O*-β-D-glucopyranoside, **29**: D-mannito, **30**: 2-deoxy-D-ribono-1,4-lactone, **gly**: glycerol, **fru**: D-fructose, **gle**: D-glucose, **suc**: sucrose.

Table 1
^{1}H NMR chemical shifts of **1**–**3** and **9**

	1	**2**	**3**	**9**
H-2	7.79 1H *d* (1.5)	–	7.50 1H *d* (2.0)	7.51 1H *s*
H-3	–	7.60 1H *d* (90)	–	–
H-4	–	6.82 1 H *dd* (9.0,3.0)	–	–
H-5	6.94 1H *d* (8.5)	–	6.95 1H *d* (8.0)	–
H-6	7.05 1H *dd* (8.5, 1.5)	7.28 1H d (3.0)	6.85 1H *dd* (8.0,2.0)	7.51 1H *s*
H-1′	6.38 1H *d* (16.0)	7.34 1H *dq* (16.0, 1.5)	3.30 2H d (7.0)	–
H-2′	625 1H *dq* (16.0, 6.5)	6.32 1H *dq* (16.0, 6.5)	5.97 1H *ddt* (17.0, 10.0, 7.0)	–
H-3′	1.70 3H *d* (6.5)	1.67 3H *dd* (6.5, 1.5)	5.02 1H *dd* (10.0,2.0)	–
	–	–	5.08 1H *dd* (17.0,2.0)	–
OCH_3	3.72 3H *s*	3.69 3H *s*	3.74 3H *s*	3.76 6H *s*
1′-OCH_3	–	–	–	3.86 3H *s*
Glc H-1	5.72 1H *d* (7.5)	5.52 1H *d* (7.0)	5.69 1H *d* (70)	6.04 1H *d* (7.0)

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

Table 2
^{13}C NMR chemical shifts of **1**–**3** and **9**

	1	**2**	**3**	**9**
C-1	131.94	129.52	133.27	125.61
C-2	114.02	149.59	117.11	108.27
C-3	148.19	118.44	147.93	153.36
C-4	149.34	113.63	148.53	140.03
C-S	113.08	155.49	113.27	153.36
C-6	120.58	111.39	122.31	108.27
C-1′	131.22	126.54	39.89	166.66
C-2′	123.98	126.45	138.23	–
C-3′	18.43	18.72	115.64	–
OCH_3	56.08	55.54	56.19	56.57
1′-OCH_3	–	–	–	52.11
Glc-1	102.55	103.69	102.27	104.00
Glc-2	74.99	75.14	74.95	76.01
Glc-3	78.98	78.72	78.83	78.48
Glc-4	71.44	71.39	71.30	71.58
Gtc-5	78.61	78.85	78.57	79.05
Glc-6	62.45	62.50	62.34	62.57

δ In ppm from TMS.

CH_3-*O*- in its NOESY spectrum suggested that the aglycone of **9** was methyl syringate and the position of the glucosyl unit was C-4. Then, **9** was characterized as methyl syringate 4-*O*-β-D-glucopyranoside as described in Fig. 1.

Alkyl glucoside **18**, **19** and **20** were identified as ethyl β-D-glucopyranoside (Kitajima et al., 1998c), ethane-1,2-diol β-D-glucopyranoside (Goodwin and Hodge, 1981), (2*S*)-2-butanol β-apiofuranosyl-(1→6)-glucopyranoside (Prawat et al., 1995), respectively, by comparison of physical and NMR data with those of reported and/or spectral investigations.

Glucoside **21** ($C_{12}H_{24}O_7$, an amorphous powder, $[\alpha]_D^{22}$ −33°) revealed $[M+H]^+$ and $[M-C_6H_{10}O_5+H]^+$ ion peaks at *m*/*z* 281 and 119 in the positive FAB-MS, and it was suggested to have one *sec*-methyl, three methylene, one hydroxylated methylene and one hydroxylated methine, in addition to the β-D-glucopyranosyl moiety, from the NMR spectral data. Further, the results of HMBC experiment (see Experimental) showed that **21** was a β-glucopyranoside of hexane-1,5-diol, and the position of the glucosyl unit was C-1. Then, **21** was concluded to be hexane-1,5-diol 1-*O*-β-D-glucopyranoside.

Monoterpenoid glucoside **22** and **23**, norcarotenoid **24** and **25**, glucide **26** ($[\alpha]_D^{21}$ +3°), **27** ($[\alpha]_D^{21}$ −7°), **29** and **30** were identified as betulabuside A (Ishikawa et al., 1998), (3*R*,6*E*)-8-hydroxylinalool 1-*O*-β-D-glucopyranoside (Kitajima and Tanaka, 1993), icariside B_1 (Miyase et al., 1987b) icariside B_2 (Miyase et al., 1987b), 1-deoxythreitol (Kitajima et al., 1999a,b), 1-deoxy-L-erythritol (Ishikawa et al., 2001), D-mannitol and 2-deoxy-D-ribono-1,4-lactone (Kitajima et al., 1999a), respectively.

Glucide **28** ($C_{10}H_{20}O_8$, an amorphous powder, $[\alpha]_D^{21}$ −29°) revealed $[M+H]^+$ and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 269 and 89 in the positive FAB-MS. Its ^{1}H and ^{13}C NMR spectral data (Table 3) showed, in addition to the β-D-glucopyranosyl group, to have one *sec*-methyl, one hydroxylated methylene and two hydroxylated methines. Then, **28** was suggested to be β-D-glucopyranoside of **26** or 1-deoxyerythritol (**27** or its enantiomer), and the NOE interaction between glucosyl H-1/H-3 suggested the position of the glucosyl unit was C-3. As the chemical shifts of the methyl group of β-D-glucopyranosides of propylene glycol derivative were considered to be shifted to up field from the corresponding aglycones (Kitajima et al., 1998a, 2003; Ishikawa et al., 2002), the stereochemistry of the triol moiety was supposed to be *erythro* as in **27** (chemical shift of H_3-1, **26**: δ 1.53, **27**: δ 1.65, **28**: δ 1.63). Further, comparison of its $[M]_D$ value with that of methyl β-D-glucopyranoside ($[M]_D$ value of **28**−$[M]_D$ value of methyl β-D-glucopyranoside = −16°) suggested that 1-deoxyerythritol was in the L-form, and the glucosylation shift values ($\Delta\delta$) of the α- and β-carbon [C-2 (β-pro-*S*);−1.5, C-3 (α); +10.6, C-4 (β-pro-*R*);−1.6] and

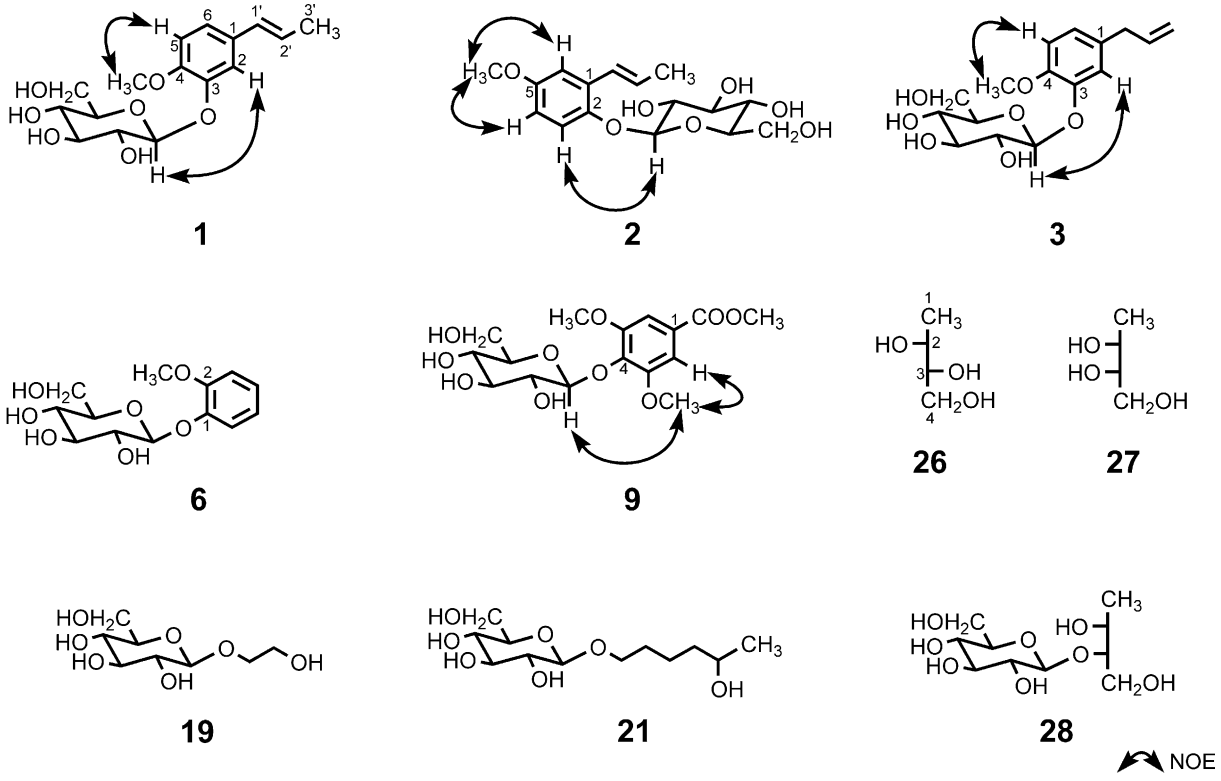

Fig. 1. Structures of **1**–**3**, **6**, **9**, **19**, **21** and **26**–**28**, and NOE interactions observed in the NOESY spectra of **1**–**3** and **9**.

Table 3
^{13}C NMR chemical shifts of **26**–**28**

	26	**27**	**28**
C-1	20.24	20.34	19.75
C-2	68.52	69.43	67.96 (−1.5)
C-3	77.02	77.17	87.74 (+10.6)
C-4	64.63	65.13	63.56 (−1.6)
Glc-1			105.72
GIc-2			75.58
Glc-3			78.56
Glc-4			71.75
Glc-5			78.56
GLc-6			62.71

δ In ppm from TMS. $\Delta\delta$(δ glucoside $-\delta$ aglycone) are given in parentheses.

the chemical shift of the anomeric carbon (δ 105.72) were in agreement with that of secondary *S*-alcohol (Kasai et al., 1977; Tori et al., 1977; Kitajima et al., 1999b). Thus, **28** was considered to be 1-deoxy-L-erythritol 3-*O*-β-D-glucopyranoside.

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 taken on Jeol JNM GX-270 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ values. 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 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 Symmetryprep C18 7 μm [Waters; column size, 7.8×300 mm; ODS], Carbohydrate analysis [Waters; column size, 3.9×300 mm; CHA] were used as columns. No acetoxyl group had been detected by the NMR spectral analysis of the materials prior to acetylation.

3.2. Extraction and isolation

Commercial anise (the fruit of *Pimpinella anisum* L.; purchased from Asaoka Spices Ltd., Lot. No. 99012001; 2.0 kg) was extracted with 70% aq. MeOH (5 l×4) at room temperature for 2 weeks. After evaporation of the solvent, the residue (346.7 g) was successively partitioned into diethyl ether–water, and then ethyl acetate–water. Removal of the solvent from each phase gave the

ether (145.3 g), ethyl acetate (7.5 g) and aqueous (193.9 g) extracts. The aqueous extract was subjected to Amberlite XAD-II chromatography (eluted with $H_2O \rightarrow$MeOH) with the methanol eluate (52.1 g) applied to Sephadex LH-20 column (MeOH) to give six fractions (frs. A→F). Fraction B (40.9 g) was subjected to a silica gel column [$CHCl_3$–MeOH–H_2O (17:3:0.2→4:1:0.1→15:5:0.4→7:3:0.5)→MeOH] to give 13 fractions (frs. $B_1 \rightarrow B_{13}$). Fraction B_3 (0.68 g) was applied to a Lobar RP-8 column [MeOH–H_2O (1:1)] and HPLC [ODS; MeOH–H_2O (9:11)] to give **2** (11 mg), **3** (9 mg) and **1** (9 mg). Fraction B_4 (1.36 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 11 fractions (frs. $B_{4-1} \rightarrow B_{4-11}$) and fr. B_{4-8} was subjected to HPLC [ODS; MeCN–H_2O (3:17) and CHA; MeCN–H_2O (24:1)] to give **25** (7 mg) and **9** (80 mg). Fraction B_6 (0.91 g) was subjected to a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [ODS; MeCN–H_2O (1:9) and CHA; MeCN–H_2O (24:1)] to give **12** (89 mg) and **6** (6 mg). Fraction B_8 (1.63 g) was also subjected to a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [CHA; MeCN–H_2O (24:1)] to give **22** (30 mg), **23** (27 mg) and **15** (62 mg). Fraction B_9 (1.81 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 18 fractions (frs. $B_{9-1} \rightarrow B_{9-18}$) and fr. B_{9-4} was subjected to Sephadex LH-20 (MeOH) and HPLC [CHA; MeCN-H_2O (14:1)] to give **11** (21 mg) and fr. B_{9-4c}. Fraction B_{9-4c} was passed through HPLC [ODS; MeCN–H_2O (3:37)] to give **17** (4 mg). Fraction B_{9-6} was passed through HPLC [ODS; MeCN–H_2O (3:37)] to give fr. B_{9-6a}, fr. B_{9-6b} and **13** (39 mg). Fraction B_{9-6a} and fr. B_{9-6b} was subjected to HPLC [CHA; MeCN–H_2O (19:1)] to give **4** (17 mg) and **24** (3 mg), respectively. Fraction B_{10} (7.29 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 10 fractions (frs. $B_{10-1} \rightarrow B_{10-10}$) and fr. B_{10-2} was subjected to a combination of HPLC [ODS; MeCN–H_2O (1:19) and CHA; MeCN–H_2O (14:1) to give **16** (33 mg), **14** (5 mg) and **10** (23 mg), respectively. Fraction B_{10-3} was passed through HPLC [ODS; MeCN–H_2O (3:37) and CHA; MeCN–H_2O (14:1)] to give **8** (7 mg), **7** (6 mg), **20** (13 mg) and fr. B_{10-3g}. Fraction B_{10-3g} was passed through HPLC [CHA; MeCN–H_2O (14:1)] to give **21** (3 mg). Fraction B_{11} (4.37 g) was passed through a Lobar RP-8 column [MeCN-H_2O (3:17)] to give 10 fractions (frs. $B_{11-1} \rightarrow B_{11-10}$) and fr. B_{10-3} was subjected to HPLC [ODS; MeCN–H_2O (3:197)] to give **5** (35 mg). A part of the water eluate fraction (50.4 g) was subjected to Sephadex LH-20 (MeOH) to give three fractions (frs. G→I). Fraction H (41.55 g) was applied to a silica gel column [$CHCl_3$–MeOH–H_2O (4:1:0.1→15:5:0.4→7:3:0.5→6:4:0.5→1:1:0.1)→MeOH] to give 20 fractions (frs. $H_1 \rightarrow H_{20}$). Fraction H_6 (0.13 g) was subjected to a Lobar RP-8 column (H_2O), HPLC [CHA, MeCN–H_2O (99:1)] and silica gel CC [$CHCl_3$–MeOH–H_2O (17:3:0.2)] to give **30** (52 mg). Fraction H_7 (0.18 g) was applied to a Lobar RP-8 column (H_2O) to give seven fractions (frs. $H_{7-1} \rightarrow H_{7-7}$). Fraction H_{7-2} was subjected to HPLC [CHA, MeCN–H_2O (99:1)] to give a glucide fraction. The resulting glucide fraction was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, MeCN–H_2O (2:3)] to give two components. These two components were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h. Then, **26** (8 mg) and **27** (11 mg) were obtained after being applied to a Sephadex LH-20 column (MeOH) CC. Fraction H_8 (1.76 g) was subjected to a Lobar RP-8 column (H_2O) and HPLC [CHA, MeCN–H_2O (19:1)] to give **18** (6 mg). Fraction H_{13} (4.68 g) was then applied to a Lobar RP-8 column (H_2O) to give six fractions (frs. $H_{13-1} \rightarrow H_{13-6}$). Fraction H_{13-3} was subjected to HPLC [CHA, MeCN–H_2O (14:1)] to give **29** (62 mg) and **19** (15 mg) and **28** (2 mg).

The following compounds were identified by comparison of physical and NMR data with those reported: 4-hydroxyphenylpropyl β-D-glucopyranoside (**4**), 1′-(4-hydroxyphenyl)propane-2′,3′-diol 4-*O*-β-D-glucopyranoside (**5**), isotachioside (**7**), tachioside (**8**), vanilloloside (**10**), 4-hydroxy-3,5-dimethoxybenzyl alcohol 4-*O*-β-D-glucopyranoside (**11**), benzyl β-D-glucopyranoside (**12**), icariside F_2 (**13**), 4-hydroxybenzyl β-D-glucopyranoside (**14**), icariside D_1 (**15**), icariside D_2 (**16**), viridoside (**17**), ethyl β-D-glucopyranoside (**18**), etane-1,2-diol β-D-glucopyranoside (**19**), (2S)-2-butanol β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside (**20**), betulabuside A (**22**), (3*R*,6*E*)-8-hydroxylinalool 1-*O*-β-D-glucopyranoside (**23**), icariside B_1 (**24**), icariside B_2 (**25**) 1-deoxythreitol (**26**), 1-deoxy-L-erythritol (**27**), D-mannitol (**29**) and 2-deoxy-D-ribono-1,4-lactone (**30**).

3.3. (*E*)-3-Hydroxyanethole β-D-glucopyranoside (*1*)

An amorphous powder, $[\alpha]_D^{23}$ −28° ($c = 0.4$, MeOH). Positive FAB-MS *m/z*: 349 $[M+Na]^+$, 327.1444 $[M+H]^+$ (calc. for $C_{16}H_{23}O_7$; 327.1443), 165 $[M-C_6H_{10}O_5+H]^+$ (base). ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-2/C-3, C-4, C-6, C-1′; H-5/C-1, C-3, C-4; H-6/C-2, C-4, C-1′; H-1′/C-2, C-6, C-2′, C-3′; H-2′/C-1, C-1′, C-3′; H_3-3′/C-1′, C-2′; O-CH_3/C-4; Glc H-1/C-3.

3.4. Acid hydrolysis of *1*

Glucoside **1** (5 mg) was dissolved in aq. 2 N H_2SO_4 and heated on a water bath for 3 h. The hydrolysate was neutralized with Na_2HCO_3. After addition of MeOH, the salt was removed by filtration, with the filtrate applied to a silica gel column [$CHCl_3$–MeOH–H_2O (7:3:0.5)]. The sugar fraction was subjected to HPLC [CHA, MeCN–H_2O (17:3); detector, Jasco RI-930

detector and Jasco OR-990 chiral detector; 2 ml/min, t_R 4.50 min (same location as that of D-glucose)] show the presence of D-glucose.

3.5. (*E*)-1′-(2-Hydroxy-5-methoxyphenyl)propane β-D-glucopyranoside (**2**)

An amorphous powder, $[\alpha]_D^{25}$ −34° (c = 0.2, MeOH). Positive FAB-MS m/z: 327.1436 $[M+H]^+$ (calc. for $C_{16}H_{23}O_7$; 327.1443), 165 $[M\text{-}C_6H_{10}O_5+H]^+$ (base). ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-3/C-1, C-2, C-5; H-4/C-2, C-5, C-6; H-6/C-2, C-4, C-5, C-1′; H-1′/C-2, C-6, C-3′; H-2′/C-1, C-3′; H_3-3′/C-1′, C-2′; O-CH_3/C-5; Glc H-1/C-2.

3.6. 3-Hydroxyestragole β-D-glucopyranoside (**3**)

An amorphous powder, $[\alpha]_D^{23}$ −34° (c = 0.4, MeOH). Positive FAB-MS m/z: 349 $[M+Na]^+$, 327.1428 $[M+H]^+$ (calc. for $C_{16}H_{23}O_7$; 327.1443), 165 $[M-C_6H_{10}O_5+H]^+$ (base). ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-2/C-1, C-3, C-4, C-6, C-1′; H-5/C-1, C-3, C-4, C-6; H-6/C-2, C-4, C-1′; H-1′/C-1, C-2, C-6, C-2′, C-3′; H-2′/C-1′; H_2-3′/C-1′; O-CH_3/C-4; Glc H-1/C-3.

3.7. 2-Methoxyphenyl β-D-glucopyranoside (**6**)

Colorless needles (MeOH), mp 65–68 °C, $[\alpha]_D^{23}$ −38° (c = 0.5, MeOH). Positive FAB-MS m/z: 287 $[M+H]^+$, 107 $[M-C_6H_{12}O_6+H]^+$ (base). ^{1}H NMR (pyridine-d_5, 500 MHz) δ: 6.97 (1H, *dd*, J = 8.0, 2.5 Hz, H-3), 7.00 (1H, *ddd*, J = 8.0, 7.0, 2.0 Hz, H-4), 6.94 (1H, *ddd*, J = 8.0, 7.0, 2.5 Hz, H-5), 7.60 (1H, dd, J = 8.0, 2.0 Hz, H-6), 3.71 (3H, *s*, O–CH_3), 5.67 (1H, *d*, J = 7.5 Hz. Glc-1). ^{13}C NMR (pyridine-d_5, 125 MHz) δ: 150.18 (C-1), 148.09 (C-2), 113.18 (C-3), 122.55 (C-4), 121.48 (C-5), 116.51 (C-6), 55.93 (O-CH_3), 102.24 (Glc-1), 74.90 (Glc-2), 78.54, 78.85 (Glc-3,-5), 71.26 (Glc-4), 62.35 (Glc-6).

3.8. Methyl syringate 4-O-β-D-glucopyranoside (**9**)

Colorless needles (MeOH), mp 91–93 °C, $[\alpha]_D^{24}$ −20° (c = 0.9, MeOH). Positive FAB-MS m/z: 397 $[M+Na]^+$, 375.1274 $[M+H]^+$ (calc. for $C_{16}H_{23}O_{10}$; 375.1291), 213 $[M-C_6H_{10}O_5+H]^+$ (base). ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.9. Ethane-1,2-diol 1-O-β-D-glucopyranoside (**19**)

A colorless syrup, $[\alpha]_D^{21}$ −17° (c = 0.6, MeOH). Positive FAB-MS m/z: 319 $[M+K]^+$, 303 $[M+Na]^+$, 225.0965 $[M+H]^+$ (calc. for $C_8H_{17}O_7$; 225.0974), 207 $[M\text{-}H_2O+H]^+$, 45 $[M\text{-}C_6H_{12}O_6+H]^+$ (base). ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 4.05, 4.32 (each 1H, *ddd*, J = 8.0, 5.0, 3.0 Hz, H_2-1), 4.04 (2H, *m*, H-2), 4.96 (1H, *d*, J = 8.0, Glc-1). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 72.38 (C-1), 61.92 (C-2), 105.00 (Glc-1), 75.30 (Glc-2), 78.55 (Glc-3), 71.70 (Glc-4), 78.55 (Glc-5), 62.74 (Glc-6).

3.10. Hexane-1,5-diol 1-O-β-D-glucopyranoside (**21**)

An amorphous powder, $[\alpha]_D^{21}$ −19° (c = 0.4, MeOH). Positive FAB-MS m/z: 319 $[M+K]^+$, 303 $[M+Na]^+$, 281.1599 $[M+H]^+$ (calc. for $C_{21}H_{25}O_7$; 281.1600), 119 $[M-C_6H_{10}O_5+H]^+$ (base). ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 3.70, 4.13 (each 1H, *ddd*, J = 9.5, 6.5, 6.5 Hz, H_2-1), 1.45→1.80 (6H, *m*, H_2-2, H_2-3, H_2-4), 3.94 (1H, *dq*, J = 6.5, 6.5 Hz, H-5), 1.29 (3H, *d*, J = 6.5 Hz, H_3-6), 4.85 (1H, *d*, J = 7.5, Glc-1). ^{13}C NMR (Pyridine-d_5, 125 MHz) δ: 69.89 (C-1), 22.97 (C-2), 30.44 (C-3), 39,89 (C-4), 66.99 (C-5), 24.25 (C-6), 104.75 (Glc-1), 75.25 (Glc-2), 78.51, 78.63 (Glc-3,-5), 71.75 (Glc-4), 62.85 (Glc-6). HMBC correlations: H_2-1/Glc C-1; H_3-6/C-5′; Glc H-1; C-1.

3.11. 1-Deoxy-L-erythritol 3-O-β-D-glucopyranoside (**28**)

An amorphous powder, $[\alpha]_D^{21}$ −29° (c = 0.1, MeOH). Positive FAB-MS m/z: 269.1230 $[M+H]^+$ (base, calc. for $C_{10}H_{21}O_8$; 269.1237). ^{1}H NMR (Pyridine-d_5, 500 MHz) δ: 1.62 (3H, *d*, J = 6.5 Hz, H_3-1), 4.41 (1H, *dq*, J = 6.5, 6.5 Hz, H-2), 4.25 (1H, *ddd*, J = 6.5, 6.0, 4.5 Hz, H-3), 4.28 (1H, *dd*, J = 11.0, 6.0 Hz, H-4a), 4.30 (1H, *dd*, J = 11.0, 4.5 Hz, H-4b), 5.16 (1H, *d*, J = 7.5, Glc-1). ^{13}C-NMR (pyridine-d_5, 125 MHz) δ: Table 3.

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