New Hemiterpenoid Pentol and Monoterpenoid Glycoside of *Torillis japonica* Fruit, and Consideration of the Origin of Apiose

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From the water-soluble portion of *Torillis japonica* D.C. fruit, a new hemiterpenoid pentol, (3*R*)-2-hydroxymethylbutane-1,2,3,4-tetrol (1) and a new monoterpenoid apiosyl-glucoside (4) have been isolated together with known (2*S*,3*R*)-2-methylbutane-1,2,3,4-tetrol (2) and hemiterpenoid glucoside (3).

As 2 is a commonly existing constituent in Umbelliferous plants, and considered to be the first putative precursor of isoprenoid derived from glucose, we assumed that apiose and these hemiterpenoids may be biosynthesized from glucose by a mevalonate-independent route of isoprenoid.

Key words hemiterpenoid pentol; hemiterpenoid tetrol; apiose; mevalonate-independent isoprenoid biosynthesis route; *Torillis japonica* fruit; Umbelliferae

We previously reported,[1] the isolation of two substances, namely, 3,7-dimethyloct-3(10)-ene-1,2,6,7-tetrol and (2*S*,3*R*)-2-methylbutane-1,2,3,4-tetrol (**2**; 2-*C*-methyl-D-erythritol)[2] from the herbal medicine She chuang zi [Japanese name "Jyashōshi," the fruit of *Cnidium monnieri* CUSSON (Umbelliferae)], which is a principal Chinese medicament used as a tonic, anti-uredo and to treat eczema.

The present study was aimed at the chemical investigation of the fresh fruit of *Torillis japonica* D.C. [Japanese name "Yabujirami" (Umbelliferae)], which is used as a substitute medicament of She chuang zi in Japan. We also examined the presence of **2** in some other Umbelliferous plants and discuss the relationship between apiose and isoprenoid.

The methanolic extract of the fresh fruit was suspended in water and then extracted successively with ether and ethyl acetate. The aqueous layer was chromatographed on Amberlite XAD-II to give water eluate and methanol eluate fractions. Each fraction was treated as described in the Experimental section to isolate **1**, **2** and betaine from the water eluate fraction, and **3**, **4** and adenosine from the methanol eluate fraction.

The new hemiterpenoid (**1**; $[\alpha]_D^{21}$ +4.3°) was obtained as a colorless syrup, and exhibited $[M+H]^+$ ion peak at *m/z* 153 with fragment peaks at *m/z* 135 $[M+H-H_2O]^+$ and *m/z* 117 $[M+H-2H_2O]^+$ in its chemical ionization mass spectrometry (CI-MS). Its positive FAB-MS showed an $[M+Na]^+$ ion peak at *m/z* 175 and the molecular formula was assigned as $C_5H_{12}O_5$ by HR FAB-MS. The ^{13}C-NMR spectrum of **1** showed five signals (Table 2) and its ^{1}H-NMR spectral data (Table 1) showed the presence of three hydroxymethyl groups and one hydroxymethine and hydroxylated quaternary carbon. Acetylation of **1** gave a tetraacetate (**5**), and **1** was concluded to be a hemiterpenoid built up with five hydroxylated carbons, 2-hydroxymethylbutane-1,2,3,4-tetrol. The configuration at C-3 was defined as *R* by comparison of optical rotation with the pentol (**6**) obtained by the $NaBH_4$ reduction of D-apiose[3] which exhibited an opposite value ($[\alpha]_D^{26}$ −4.0°) to **1**. Therefore, **1** was characterized as (3*R*)-2-hydroxymethylbutane-1,2,3,4-tetrol.

Hemiterpenoid glucoside (**3**; an amorphous powder, $[\alpha]_D^{23}$ −30.9°) showed $[M+H]^+$ ion peak at *m/z* 265 with fragment peak at *m/z* 85 $[M-C_6H_{12}O_6+H]^+$ in its positive FAB-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H correlation spectroscopy (COSY) NMR spectral data (Tables 1 and 2) for **3** revealed the presence of β-glucopyranosyl, one *tert*-methyl, two hydroxymethyl groups and one trisubstituted double bond, and **3** was suggested to be a β-glucopyranoside of 2-methyl-2-butene-1,4-diol. Thus, **3** was identified as (2*E*)-2-methyl-2-butene-1,4-diol 1-*O*-β-D-glucopyranoside, the constituent of *Ornithogalum montanum* (Liliaceae),[4] by comparison of their NMR data.

Monoterpenoid glycoside (**4**; $C_{21}H_{36}O_{11}$, an amorphous powder, $[\alpha]_D^{23}$ −24.1°) showed $[M+H]^+$ and $[M-(C_5H_{10}O_5)+H]^+$ ion peaks at *m/z* 465 and 315 in the positive FAB-MS. The ^{1}H- ^{13}C- and ^{13}C–^{1}H COSY NMR spectral data (Tables 1 and 2) for **4** revealed the presence of β-glucopyranosyl, β-apiofuranosyl, three *tert*-methyls, three methylenes, two methines (one of them oxygenated), and two oxygenated quaternary carbons. From the heteronuclear multiple-bond correlation (HMBC) experiment, the partial structure in **4** was obtained from the two or three bond correlations from the signals of three methyl protons and the H-2 proton (Fig. 1; shown in heavy lines). The unsaturation degree and two ^{13}C signals (C-1; δ 72.31, C-8; δ 73.29) indicated that the aglycone of **4** formed a cyclic ether linkage between C-1 and C-8. Thus, **4** was suggested to be a glycoside of 2-hydroxy-1,8-cineole. Partial hydrolysis of **4** with 0.5 N H_2SO_4 afforded (1*S*,2*S*,4*R*)-2-hydroxy-1,8-cineole β-D-glucopyranoside (**7**)[5] and apiose. By comparison of its ^{13}C-NMR spectral data (Table 2) and $[M]_D$ value ($[M]_D$ of **4**−$[M]_D$ of **7**=−130.1°)[6] with those of **7**, **4** was characterized as (1*S*,2*S*,4*R*)-2-hydroxy-1,8-cineole β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

D-Apiose, which is classified as a branched sugar, was first found in apiin, a glycoside isolated from *Apium petroselinum*,[7] and this sugar is now known to be widespread in the plant kingdom, especially in Umbelliferous plants. From the results of the experiments using ^{14}C-labeled compounds, apiose was proved to be biosynthesized from glucose-6-phosphate *via* glucuronic acid.[8,9] On the other hand, there is a hypothesis[8] that apiose is the compound of isoprenoid origin. This came from the facts that apiose has an isoprene skeleton and is found in a rubber tree. Supporting this hypothesis, we have now found that polyols of isoprenoid structure such as **3a** (the diol), **2** (the tetrol), and **1** (the pentol) coexist with apiose (**4s**) in a single plant (the fruit of *T. japonica*), sug-

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Table 1. ^{1}H-NMR Chemical Shifts of **1**—**5**

	1	**2**	**3**	**5**
H-1	4.46[a] 2H, d (11.5)	4.09 1H, d (11.0)	4.27 2H, d (6.5)	4.15, 4.21[a]
	—	4.20 1H, d (11.0)	—	each 1H, d (12.0)
H-3	4.64 1H, dd (4.0, 6.0)	4.46 1H, dd (4.0, 7.0)	6.17 1H, t (6.5)	5.29 1H, dd (3.0, 7.5)
H-4	4.48 1H, dd (6.0, 11.0)	4.28 1H, dd (7.0, 11.0)	4.48 2H, d (6.5)	4.21 1H, dd (7.5, 12.0)
	4.55 1H, dd (4.0, 11.0)	4.50 1H, dd (4.0, 11.0)	—	4.46 1H, dd (3.0, 12.0)
H-5	4.42[a] 2H, d (11.5)	1.61 3H, s	1.76 3H, s	4.20[a] 2H, br s
Glc-1	—	—	4.92 1H, d (7.5)	—
OAc	—	—	—	2.05 3H, s
				2.11 6H, s
				2.12 3H, s

	4
H-2*exo*	4.02 dd (3.0, 12.0)
H-3*endo*	2.03 ddd (3.0, 3.0, 14.5)
exo	2.85 dddd (3.0, 3.0, 12.0, 14.5)
H-4	1.37 br s
H-5*endo*	1.51 dddd (2.0, 6.0, 14.0, 14.0)
exo	1.83 dddd (3.0, 3.0, 14.0, 14.0)
H-6*endo*	1.49 m
exo	2.11 ddd (2.0, 12.5, 14.0)
H_3-7	1.43 s
H_3-9	1.25 s
H_3-10	1.23 s
Glc-1	4.86 d (7.5)
Api-1	5.89 d (2.5)

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. Solvent: pyridine-d_5 for **1**, **2**, **3** and **4**, $CDCl_3$ for **5**. *a*) Assignments are interchangeable in each column.

Table 2. ^{13}C-NMR Chemical Shifts of **1**—**5** and **7**

	1	**2**	**3**	**5**		**4**	**7**
C-1	64.57[a]	68.62	74.52	64.32[a]	C-1	72.31	72.37
C-2	76.46	74.54	133.60	73.73	C-2	80.15	80.09
C-3	75.05	75.73	128.89	71.29	C-3	34.35	34.28
C-4	63.64	63.60	58.59	62.08[a]	C-4	34.40	34.38
C-5	65.25[a]	20.24	14.25	64.47[a]	C-5	22.53	22.52
Glc-1			103.73		C-6	26.33	26.32
Glc-2			75.25		C-7	24.82	24.93
Glc-3			78.56		C-8	73.29	73.20
Glc-4			71.72		C-9	28.95	28.90
Glc-5			78.66		C-10	29.22	29.17
Glc-6			62.82		Glc-1	106.39	106.51
OAc				20.76 169.91	Glc-2	75.42	75.59
				20.76 170.61	Glc-3	78.52	78.62
				20.76 170.90	Glc-4	71.90	71.73
				20.81 170.90	Glc-5	77.31	78.38
					Glc-6	68.68	62.91
					Api-1	111.09	
					Api-2	77.85	
					Api-3	80.55	
					Api-4	75.07	
					Api-5	65.72	

δ in ppm from TMS. Solvent: pyridine-d_5 for **1**, **2**, **3**, **4** and **7**. $CDCl_3$ for **5**. *a*) Assignments are interchangeable in each column.

gesting their close biosynthetic relationship.

To confirm that such polyols of isoprenoid structure are common ingredients in Umbelliferous plants, we examined the presence of the tetrol **2** in the methanol extracts of the following plants: fruits of (A) *Angerica keiskei* KOIDE ("Ashitaba" in Japanese),[10] (B) *A. pubescens* MAXIM. ("Shishiudo" in Japanese),[11] (C) *Anethum graveolens* L. (Dill),[12] (D) *Carum ajowan* BENTH. *et* HOOK (Ajowan),[12] (E) *C. carvi* L. (Caraway),[12] (F) *Cnidium japonicum* MIQ. ("Hamazeri" in Japanese),[11] (G) *Coriendrum sativum* L. (Coriander),[12] (H) *Cuminum cymimum* L. (Cumin),[12] (I) *Foeniculum vulgare* MILLER (Fennel),[13] (J) *Pseuedavum japonicum* THUNB. ("Botanbōfu"),[11] (K) *Pimpinella anisum* L. (Anise),[12] and leaves of (L) *Apium petroselium* (Parsley).[14] Thirty grams of a sample was extracted with hot methanol and the methanol extract was fractionated accord-

ing to the procedure described in the Experimental section; the presence of **2** was detected from the carbohydrate fractions by TLC and NMR methods. A significant amount of **2** was found in the samples of D, G, H, K and L, and it is therefore believed that this tetrol **2** is a fairly common constituent in Umbelliferous plants as is apiose.

A mevalonate-independent route for bacterial isoprenoid biosynthesis was recently detected by experiments using ^{13}C-labeled acetate or glucose as a single carbon source (Chart 1).[15] This route was also found to be operating for biosynthesis of essential oils in some higher plants, in which tetrol **2** was identified as the first putative precursor of isoprenoid.[16] These facts, in combination of our finding that D-apiose and polyols **1**, **2**, and **3a** coexist, led us to assume that they are compounds of the mevalonate-independent route, though stereochemical change at C-3 from **1** to D-apiose still has to be explained. Oxidation of **1** at C-3 and isomerization through an enol would give D-apiose.[17] However, the possibility that these compounds are of isoprenoid origin (dotted arrows in Chart 1) cannot be ruled out.

Experimental

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. MS were recorded with a JEOL HX-110 spectrometer. ^1H- and ^{13}C-NMR spectra were taken on JEOL JNM FX-100 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. Column chromatography 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 carbohydrate analysis column [Waters; column size, 3.9×300 mm, solv.; CH_3CN–H_2O (92.5 : 7.5), flow rate; 5 ml/min: condition a] or ODS-3251-D [Senshu pak; column size, 8×250 mm, solv.; CH_3CN–H_2O (1 : 99), flow rate; 3 ml/min: condition b, solv.; MeOH–H_2O (1 : 9), flow rate; 3 ml/min: condition c].

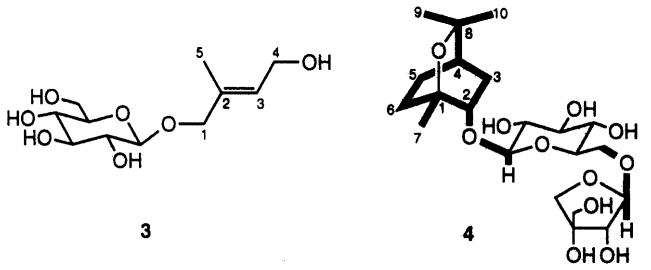

Fig. 1. Partial Structure of **4** Solved by HMBC Spectrum (Heavy Lines) and Structures of **3** and **4**

Extraction and Isolation of Water-Soluble Constituents *T. japonica* D. C. was collected at Machida City, Tokyo, Japan, in July 1992. The fresh fruit (1090 g) was extracted with methanol (6 l) at room temperature. After evaporation of the solvent, the residue (105.1 g) was suspended with water and successively extracted with ether and ethyl acetate. Removal of the solvent from each phase gave an ether-soluble (73.8 g), ethyl acetate-soluble (0.7 g) and an aqueous (30.6 g) residue. The aqueous residue was subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) to afford water eluate and methanol eluate fractions. Then the water eluate (19.5 g) was extracted with hot methanol (300 ml) and the methanol insoluble portion (5.2 g) was removed. The hot methanol soluble fraction (14.3 g) was subjected to column chromatography on a combination of Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] and Lobar RP-8 column (20% MeOH). Final purification was done by HPLC (condition a), and then, **1** (52 mg), **2** (320 mg) and betaine (20 mg) were obtained in the pure form. The hot methanol insoluble fraction was dissolved in water and subjected to column chromatography of Sephadex LH-20 (MeOH) to obtain a sugar mixture fraction [0.8 g; a component of this mixture was determined to be D-mannitol (t_R 9.8 min), D-fructose (t_R 6.3 min) and sucrose (t_R 25.0 min) about 4 : 1 : 1 by HPLC analysis; condition a]. Meanwhile, the methanol eluate fraction (11.1 g) was chromatographed on Sephadex LH-20 (MeOH) to give five fractions. Fraction 2 (9.3 g) was purified by repeated silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1 and 7 : 3 : 0.5)], Sephadex LH-20 (MeOH) and Lobar RP-8 column [MeOH–H_2O (3 : 7 and 1 : 4)] chromatography. Final purification was done by HPLC (**3**; condition b, **4**; condition c), and then **3** (10 mg) and **4** (16 mg) were obtained in the pure form. From the fr. 3 (1.39 g), adenosine (26 mg) was obtained by repeated silica gel

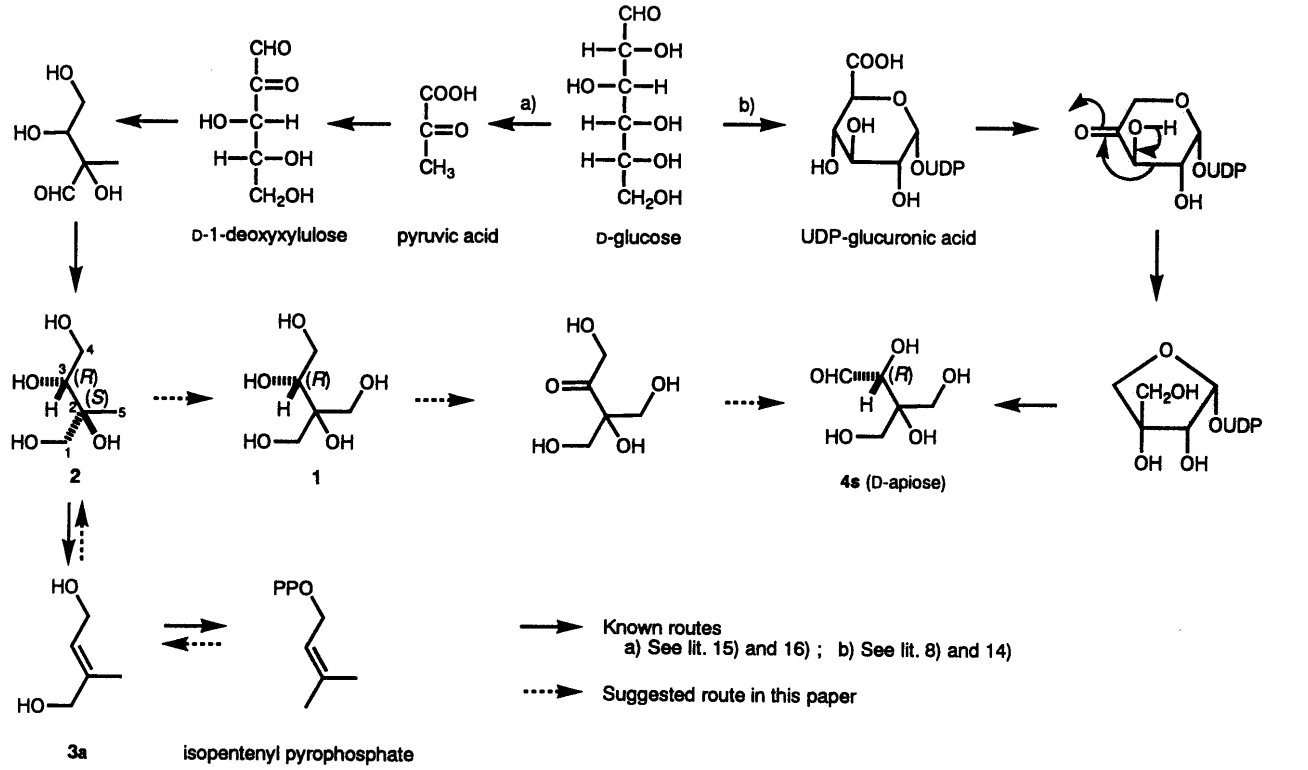

Chart 1. Possible Biosynthetic Routes to D-Apiose and Related Polyols

[$CHCl_3$–MeOH–H_2O (7:3:0.5)] and Sephadex LH-20 (MeOH) column chromatography.

(3*R*)-2-Hydroxymethylbutane-1,2,3,4-tetrol (1) A colorless syrup, $[\alpha]_D^{21}$ +4.3° (*c*=1.2, MeOH). CI-MS *m/z*: 153 $[M(C_5H_{12}O_5)+H]^+$, 135 $[M-H_2O+H]^+$, 117 $[M-2H_2O+H]^+$ (base). Positive FAB-MS *m/z*: 175.0526 $[M+Na]^+$ (Calcd for $C_5H_{12}O_5Na$: 175.0582).

1-Tetraacetate (5) A colorless oil, $[\alpha]_D^{21}$ +15.2° (*c*=0.7, $CHCl_3$).

$NaBH_4$ Reduction of D-Apiose D-Apiose (20 mg) which was obtained by the acid hydrolysis of apiin[14] was dissolved in methanol (5 ml) and stirred with $NaBH_4$ (5 mg) for 5 h at room temperature. The usual workup afforded the pentol **6** (10 mg). **6**: A colorless syrup, $[\alpha]_D^{26}$ −4.0° (*c*=0.8, MeOH). The results of ^{1}H- and ^{13}C-NMR were identical with those of **1**.

(2*S*,3*R*)-2-Methylbutane-1,2,3,4-tetrol (2) A colorless syrup, $[\alpha]_D^{25}$ +13.5° (*c*=1.2, MeOH). The results of ^{1}H- and ^{13}C-NMR were identical with those of authentic sample.

(2*E*)-2-Methyl-2-butene-1,4-diol 1-*O*-β-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{23}$ −30.9° (*c*=0.8, MeOH). Positive FAB-MS *m/z*: 287.1128 $[M+Na]^+$ (base, Calcd for $C_{11}H_{20}O_7Na$: 287.1107), 265 $[M+H]^+$, 85 $[M-C_6H_{12}O_6+H]^+$.

(1*S*,2*S*,4*R*)-2-Hydroxy-1,8-cineole β-D-Apiofuranosyl-(1→6)-β-D-glucopyranoside (4) An amorphous powder, $[\alpha]_D^{23}$ −24.1° (*c*=1.6, MeOH). Positive FAB-MS *m/z*: 487.2129 $[M+Na]^+$ (base, Calcd for $C_{21}H_{36}O_{11}Na$: 487.2155), 465 $[M+H]^+$, 315 $[M-C_5H_{10}O_5+H]^+$.

Partial Acid Hydrolysis of 4 **4** (8 mg) was dissolved in aq. 0.5 N H_2SO_4 and heated in a water bath for 2 h. The reaction mixture of hydrolysate was neutralized with $NaHCO_3$, the salt was filtered off, and the filtrate passed through Sephadex LH-20 (MeOH) to afford a monoglucoside fraction and a sugar fraction. The monoglucoside fraction was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (4:1:0.1)] to afford **7** (2 mg). The sugar fraction was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (7:3:0.5) to give a syrup, and silica gel TLC [*Rf*=0.29, $CHCl_3$–MeOH–H_2O (7:3:0.5)], showed the presence of apiose.

(1*S*,2*S*,4*R*)-2-Hydroxy-1,8-cineole β-D-Glucopyranoside (7) Colorless needles (MeOH), mp 83—84 °C, $[\alpha]_D^{23}$ +5.5° (*c*=0.2, MeOH), [lit.[5a]; 81—83 °C, $[\alpha]_D$ −9.1° (*c*=0.7, MeOH), lit.[5b]; $[\alpha]_D$ +1.5° (*c*=0.8, MeOH)]. ^{1}H-NMR (pyridine-d_5) δ: 4.01 (1H, ddd, *J*=1.5, 3.0, 10.0 Hz, H-2), 1.92 (1H, ddd, *J*=3.0, 3.0, 14.0 Hz, H-3*endo*), 2.61 (1H, dddd, *J*=3.0, 3.0, 10.0, 14.0 Hz, H-3*exo*), 1.33 (1H, br s, H-4), 1.52 (1H, dddd, *J*=1.5, 6.0, 13.0, 13.0 Hz, H-5*endo*), 1.84 (1H, dddd, *J*=3.0, 3.0, 13.0, 13.0 Hz, H-5*exo*), 2.13 (1H, ddd, *J*=1.5, 13.0, 13.0 Hz, H-6*endo*), 1.50 (1H, dddd, *J*=1.5, 6.0, 13.0, 13.0 Hz, H-6*exo*), 1.47, 1.24, 1.16 (each 3H, s, H_3-7, H_3-9 and H_3-10), 4.94 (1H, d, *J*=8.0 Hz, H-1 of glucose).

Detection Procedure of 2 in Umbelliferous Fruit and Leaf Each sample (30 g) was extracted with hot methanol (150 ml) for 2 h. After evaporation of the solvent, the residues were dissolved in water and extracted with ethyl acetate. The aqueous residues were subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) and the water eluates (carbohydrate fractions) were chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (7:3:0.5)]. Each fraction was performed on TLC using **2** as a standard [*Rf*=0.5; $CHCl_3$–MeOH–H_2O (7:3:0.5)] to detect the presence of **2**. Final detection was done by comparing ^{1}H- and ^{13}C-NMR spectra of detectable fractions with those of **2**.

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References and Notes

1) Kitajima J., Tanaka T., *Chem. Pharm. Bull.*, **41**, 1667—1669 (1993).
2) Anthonsen T., Hagen S., Kazi M. A., Shah S. W., Tagar S., *Acta. Chem. Scand., Ser. B*, **30**, 91—93 (1976); Anthonsen T., Hagen S., Sallam M. A. E., *Phytochemistry*, **19**, 2375—2377 (1980).
3) Obtained by the partial hydrolysis of apiin isolated from the leaves of *Apium petroselium.*
4) Nicoletti M., Tomassini L., Foddai S., *Planta Med.*, **58**, 472 (1992).
5) *a*) Sawabe A., Matsubara Y., Iizuka Y., *Nippon Nogeikagaku Kaishi*, **62**, 1475—1477 (1988); *b*) Yoshikawa Y., Kobayashi M., Arikawa S., *Natural Medicines*, **50**, 176—178 (1996).
6) Kitajima J., Okamura C., Ishikawa T., Tanaka Y., *Chem. Pharm. Bull.*, accepted; Hulyalkar R. K., Jones J. K. N., Perry M. B., *Can. J. Chem.*, **43**, 2085—2091 (1965).
7) Vongerichten E., *Liebigs Ann.*, **318**, 121 (1901).
8) Watson R. R., Orenstein N. S., *Adv. Carbohydr. Chem. Biochem.*, **31**, 135—184 (1975).
9) Vickery M. L., Vickery B., "Secondary Plant Metabolism," The Macmillan Press Ltd., London, 1981, pp. 26—27.
10) The material was collected at Miura Peninsula in Kanagawa Prefecture, Japan, in November 1993.
11) The materials were collected at Miura Peninsula in Kanagawa Prefecture, Japan, in October 1993.
12) The materials were purchased from Asaoka Spices Commercial, Ltd. (Tokyo).
13) The material was purchased from Kinokuniya Chinese Medicine Pharmacy, Ltd. (Gunma Prefecture, Japan).
14) The material was purchased in a market in Tokyo in December 1994.
15) Rohmer M., Seemann M., Horbach S., Bringer-Meyer S., Sahm H., *J. Am. Chem. Soc.*, **118**, 2564—2566 (1996).
16) Eisenreich W., Sagner S., Zenk M. H., Bacher A., *Tetrahedron Lett.*, **38**, 3888—3892 (1997).
17) Mann J., Davidson R. S., Hobbs J. B., Banthorpe D. V., Harborne J. B., "Natural Products: Their Chemistry and Biological Significance," Addison Wesley Longmann, Ltd., Essex, 1994, pp. 15—17.