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New *p*-menthanetriols and their glucosides from the fruit of caraway☆

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Abstract—Ten new *p*-menthanetriols, including eight stereoisomers of *p*-menthane-2,8,9-triol, and five new glucosides were isolated from the water-soluble portion of the methanol extract of the fruit of caraway (*Carum carvi* L.), which has been used as a spice and medicine. Their structures were clarified by spectral investigation.

1. Introduction

Caraway [*Carum carvi* L.; Umbelliferae] has been used as a popular aromatic herb and spice since antiquity and has been cultivated in Europe since the Middle Ages.[1,2] Its fruit has been used for medicine and in cooking, and is listed in British, German and European pharmacopoeia.[2,3] For medicinal purpose, it is used to relieve flatulent indigestion, colic and bronchitis.[1,2] Studies on the fruit were made on the essential oil, and many monoterpenoids [*d*-carvone (main; 50–60%), *l*-limonene, carvacrol, *trans*-carveol, *d*-dihydrocarveol, *l*-dihydrocarveol, etc.] were reported as the constituents.[4] However, no report has been published on the water-soluble portion of this fruit. In continuation of our studies on the water-soluble constituents of spices, and to reveal the relationship between the essential oil and the water-soluble constituent, we undertook a detailed investigation on the constituents of this fruit. In this paper, we discuss the isolation and the characterization of 10 new monoterpenoid triols and five new monoterpenoid triol glucosides.

2. Results and discussion

Commercial caraway was extracted with 70% methanol, and the methanolic extract 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 eluate fractions. The methanol eluate fraction was chromatographed on Sephadex LH-20, and subjected to a combination of silica gel, Lobar RP-8 column chromatography and HPLC to isolate monoterpenoid triols (**1**–**10**) and their glucosides (**11**–**15**). All glucosides described in this paper were β-D-glucopyranosides as shown by their ^{13}C NMR data (Table 2), and this was confirmed by hydrolysis to yield D-glucose or by a comparison of the $[\alpha]_D$ or $[M]_D$ values with those of their aglycones except **15**.[5] Their molecular formulae were suggested from the accurate mass number of $[M+H]^+$ or $[M+Na]^+$ or $[M+K]^+$ ion peaks in the high-resolution positive FAB-MS.

Triol **1** ($C_{10}H_{20}O_3$, an amorphous powder, $[\alpha]^{21}_D+14°$) and **2** ($C_{10}H_{20}O_3$, an amorphous powder, $[\alpha]^{21}_D+8°$) showed $[M+H]^+$ ion peaks at *m/z* 189 in the positive FAB-MS. They revealed quite similar ^1H and ^{13}C NMR spectral features (Tables 1 and 2), and have one *tert*-methyl, one *sec*-methyl, four methylenes (one of them was oxygenated), three methines (one of them was oxygenated) and one oxygenated quaternary carbon. From the analysis of HMBC spectral data of **1**, they were suggested to be *p*-menthane-2,8,9-triol. Hirai et al. isolated two monoterpenoidtriols, (1*R*,2*R*,4*R*,8*S*)- and (1*R*,2*R*,4*R*,8*R*)-*p*-menthane-2,8,9-triol (**1a** and **2a**) from the fruiting body of *Flammulina velutipes* (Tricholomataceae),[6] and **1** and **2** showed the identical ^1H and ^{13}C NMR spectral data with those of **1a** and **2a**. Furthermore, **1a** and **2a** had the opposite optical rotation values to that of **1** and **2** (**1a**; $[\alpha]^{24}_D-17.0°$, **2a**; $[\alpha]^{24}_D-5.3°$). Therefore, **1** and **2** were concluded to be (1*S*,2*S*,4*S*,8*R*)- and (1*S*,2*S*,4*S*,8*S*)-*p*-menthane-2,8,9-triol, respectively.

Triol **3** ($C_{10}H_{20}O_3$, mp 119–122°C, $[\alpha]^{25}_D-31°$) and **4** ($C_{10}H_{20}O_3$, mp 115–117°C, $[\alpha]^{21}_D-35°$) were indicated to be *p*-menthane-2,8,9-triol by the ^1H and ^{13}C NMR spectral data (Tables 1 and 2) and the result of HMBC experiment of **3**. The observed NOE interactions between

☆ This is the first report of the isolation of all eight stereoisomeric terpenoids having four asymmeric carbons in the molecule, from the plant.

Keywords: caraway; *Carum carvi* fruit; *p*-menthanetriol; *p*-menthanetriol glucoside; *p*-menthane-2,8,9-triol; stereoisomers.

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Table 1. ^{1}H NMR chemical shifts of **1**–**15** (in pyridine-d_5, 500 MHz)

	1	**2**	**3**	**4**
H-1_{ax}	1.56 m	1.56 m	–	–
eq	–	–	2.28 m	2.28 m
H-2_{ax}	3.44 ddd (3.0, 10.0, 11.0)	3.45 ddd (3.5, 10.0, 11.0)	4.10 ddd (3.5, 3.5, 11.0)	4.10 ddd (4.0, 4.0, 11.0)
H-3_{ax}	1.64 ddd (11.0, 12.0, 12.0)	1.73 ddd (11.0, 12.0, 12.0)	1.84 ddd (11.0, 12.0, 12.0)	1.94 ddd (11.0, 12.0, 12.0)
eq	2.58 dddd (3.0, 3.0, 3.0, 12.0)	2.83 dddd (3.5, 3.5, 3.5, 12.0)	2.24 ddd (3.5, 3.5, 12.0)	2.48 ddd (3.5, 4.0, 12.0)
H-4_{ax}	2.09 dddd (3.0, 3.0, 12.0, 12.0)	2.10 dddd (3.5, 3.5, 12.5, 12.0)	2.05 dddd (3.5, 3.5, 12.0, 12.0)	2.06 dddd (3.5, 3.5, 12.0, 12.0)
H-5_{ax}	1.43 dddd (3.0, 12.0, 12.0, 12.0)	1.30 dddd (3.5, 12.0, 12.0, 12.0)	1.64 dddd (3.5, 12.0, 12.0, 12.0)	1.58 dddd (4.0, 12.0, 12.0, 12.0)
eq	2.20 dddd (3.0, 3.0, 3.0, 12.0)	1.92 dddd (3.5, 3.5, 3.5, 12.0)	1.92 dddd (3.5, 3.5, 3.5, 12.0)	1.65 m
H-6_{ax}	1.09 dddd (3.0, 12.0, 12.0, 12.0)	1.09 dddd (3.5, 12.0, 12.0, 12.0)	1.58 dddd (3.5, 3.5, 12.0, 12.0)	1.58 dddd (4.0, 4.0, 12.0, 12.0)
eq	1.81 dddd (3.0, 3.0, 3.0, 12.0)	1.79 dddd (3.5, 3.5, 3.5, 12.0)	1.72 dddd (3.5, 3.5, 3.5, 12.0)	1.67 dddd (4.0, 6.0, 12.0, 12.0)
H_3-7	1.24 d (6.5)	1.25 d (6.5)	1.22 d (7.0)	1.22 d (7.0)
H_2-9	3.91 d (10.5)	3.91 d (11.0)	3.92 d (11.0)	3.92 d (11.0)
	3.96 d (10.5)	3.94 d (11.0)	3.96 d (11.0)	3.95 d (11.0)
H_3-10	1.42 s	1.41 s	1.45 s	1.44 s
	5	**6**	**7**	**8**
H-1_{ax}	1.55 m	1.55 m	–	–
eq	–	–	2.13 m	2.11 m
H-2_{eq}	4.13 br dd (3.0, 3.0)	4.16 br dd (3.0, 3.0)	4.11 br dd (3.0, 3.0)	4.12 br dd (3.0, 3.0)
H-3_{ax}	1.60 ddd (3.0, 13.0, 13.0)	1.68 ddd (3.0, 13.0, 13.0)	1.83 ddd (3.0, 13.0, 13.0)	1.88 ddd (3.0, 13.0, 13.0)
eq	2.37 ddd (3.0, 3.0, 13.0)	2.62 ddd (3.0, 3.0, 13.0)	2.13 ddd (3.0, 3.0, 13.0)	2.34 ddd (3.0, 3.0, 13.0)
H-4_{ax}	2.64 dddd (3.0, 3.0, 13.0, 13.0)	2.66 dddd (3.0, 3.0, 13.0, 13.0)	2.67 dddd (3.0, 3.0, 13.0, 13.0)	2.66 dddd (3.0, 3.0, 13.0, 13.0)
H-5_{ax}	1.49 dddd (3.0, 13.0, 13.0, 13.0)	1.35 dddd (3.0, 13.0, 13.0, 13.0)	1.71 dddd (3.0, 13.0, 13.0, 13.0)	1.54 dddd (3.0, 13.0, 13.0, 13.0)
eq	2.30 dddd (3.0, 3.0, 3.0, 13.0)	2.03 dddd (3.0, 3.0, 3.0, 13.0)	2.01 dddd (3.0, 3.0, 3.0, 13.0)	1.73 dddd (3.0, 3.0, 3.0, 13.0)
H-6_{ax}	1.85 dddd (3.0,13.0, 13.0, 13.0)	1.84 dddd (3.0, 13.0, 13.0, 13.0)	2.35 dddd (3.0, 3.0, 13.0, 13.0)	2.31 dddd (3.0, 3.0, 13.0, 13.0)
eq	1.54 dddd (3.0, 3.0, 3.0, 13.0)	1.51 dddd (3.0, 3.0, 3.0, 13.0)	1.49 br ddd (3.0, 3.0, 13.0)	1.43 br ddd (3.0, 3.0, 13.0)
H_3-7	1.17 d (7.0)	1.17 d (7.0)	1.02 d (7.0)	0.99 d (7.0)
H_2-9	3.91 d (10.5)	3.89 d (10.5)	3.92 d (10.5)	3.88 d (10.5)
	3.95 d (10.5)	3.96 d (10.5)	3.96 d (10.5)	3.93 d (10.5)
H_3-10	1.44 s	1.42 s	1.46 s	1.42 s
	9	**10**	**11**	**12**
H-1_{eq}	–	–	2.46 m	2.25 m
H-2_{ax}	–	–	4.30 br d (12.0)	4.03 ddd (4.5, 4.5, 12.0)
eq	4.26 dd (3.0, 3.0)	4.18 dd (3.5, 3.5)	–	–
H-3_{ax}	2.43 ddd (3.0, 12.5, 12.5)	2.33 ddd (3.5, 13.0, 13.0)	1.81 ddd (12.0, 13.0, 13.0)	1.73 ddd (12.0, 12.0, 12.0)
eq	2.36 ddd (3.0, 3.0, 12.5)	2.04 ddd (3.5, 3.5, 13.0)	2.29 br d (13.0)	2.24 br d (12.0)
H-4_{ax}	2.48 dddd (3.0, 3.0, 12.5, 12.5)	1.86 m	1.93 dddd (3.0, 3.0, 13.0, 13.0)	2.03 br dd (12.0, 12.0)
H-5_{ax}	2.13 dddd (3.0, 12.5, 12.5, 12.5)	2.05 dddd (3.5, 13.0, 13.0, 13.0)	1.57 dddd (3.0, 13.0, 13.0, 13.0)	1.56 dddd (3.0, 12.0, 12.0, 12.0)
eq	2.02 ddd (3.0, 3.0, 12.5)	1.71 dddd (3.5, 3.5, 3.5, 13.0)	1.85 m	1.84 br d (12.0)
H-6_{ax}	2.27 ddd (3.0, 12.5, 12.5)	2.22 ddd (3.5, 13.0, 13.0)	1.43 dddd (3.0, 3.0, 13.0, 13.0)	1.53 dddd (3.0, 3.0, 12.0, 12.0)
eq	1.93 ddd (3.0, 3.0, 12.5)	1.85 ddd (3.5, 3.5, 13.0)	1.6 br dd (3.0, 13.0)3	1.67 br d (12.0)
H_3-7	1.69 s	1.69 s	1.20 d (7.0)	1.18 d (7.0)
H-8	–	2.42 m	–	–
H_3-9	1.44 s	1.46 s	–	–
H_2-9	–	3.78 dd (7.0, 10.5)	3.87 d (11.0)	3.83 d (10.0)
	–	3.99 dd (5.5, 10.5)	3.90 d (11.0)	4.30 d (10.0)
H_3-10	1.44 s	1.17 d (7.0)	1.38 s	1.35 s
Glc-1	–	–	5.03 d (7.5)	4.95 d (7.5)
	13	14	15	
H-2_{eq}	4.18 dd (3.0, 3.0)	4.16 dd (3.0, 3.0)	4.20 dd (3.0, 3.0)	
H-3_{ax}	2.36 ddd (3.0, 12.0, 12.0)	2.22 ddd (3.0, 13.0, 13.0)	2.49 ddd (3.0, 13.0, 13.0)	
eq	2.42 ddd (3.0, 3.0, 12.0)	2.33 ddd (3.0, 3.0, 13.0)	2.50 m	
H-4_{ax}	2.54 dddd (3.0, 3.0, 12.0, 12.0)	1.80 m	3.00 dddd (3.0, 3.0, 13.0, 13.0)	
H-5_{ax}	2.00 dddd (3.0, 12.0, 12.0, 12.0)	2.00 dddd (3.0, 13.0, 13.0, 13.0)	2.14 dddd (3.0, 13.0, 13.0, 13.0)	
eq	1.95 ddd (3.0, 3.0, 12.0)	1.54 dddd (3.0, 3.0, 3.0, 13.0)	1.75 dddd (3.0, 3.0, 3.0, 13.0)	
H-6_{ax}	2.20 ddd (3.0, 12.0, 12.0)	2.06 ddd (3.0, 13.0, 13.0)	2.07 ddd (3.0, 13.0, 13.0)	
eq	1.85 ddd (3.0, 3.0, 12.0)	1.79 ddd (3.0, 3.0, 13.0)	1.78 ddd (3.0, 3.0, 13.0)	
H_3-7	1.69 s	1.81 s	1.82 s	
H-8	–	2.41 m	–	
H_3-9	1.46 s	–	–	
H-9a	–	3.73 dd (7.0, 10.0)	5.10 br s	
b	–	3.93 dd (6.0, 10.0)	5.41 br s	
H_3-10	1.46 s	1.07 d (7.0)	–	
H_2-10	–	–	4.48 br s	
Glc-1	5.10 d (7.5)	5.03 d (8.0)	5.03 d (7.5)	

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

Table 2. ^{13}C NMR chemical shifts of **1**–**15** and **18** (in pyridine-d_5, 125 MHz)

	1	2	3	4	5	6	7	8	9	10
C-1	41.11	41.11	34.90	34.91	37.46	37.41	35.06	35.07	70.82	71.09
C-2	76.28	76.37	72.53	72.58	70.16	70.20	71.44	71.48	74.47	74.24
C-3	38.18	37.30	31.22	30.29	36.01	35.21	29.95	29.10	31.48	32.73
C-4	44.31	44.20	44.13	44.01	38.04	37.94	38.55	38.49	42.31	41.40
C-5	26.74	27.64	20.14	21.12	27.21	28.05	20.94	21.88	23.26	26.45
C-6	34.01	34.05	31.20	31.27	29.09	29.07	27.06	27.06	34.97	34.91
C-7	19.37	19.34	11.55	11.58	19.29	19.31	17.04	17.07	28.87	28.59
C-8	74.01	73.92	74.08	74.02	74.16	74.10	74.25	74.18	71.63	32.65
C-9	69.05	69.13	69.00	69.15	69.03	69.14	68.91	69.00	27.77[a]	65.75
C-10	21.75	21.55	21.96	21.74	21.86	21.50	21.93	21.67	27.93[a]	14.52

	11	12	13	14	15	18
C-1	30.32 (−4.6)	34.73	70.78	70.73 (−0.4)	70.41	30.29 (−4.6)
C-2	78.61 (+6.1)	72.43	74.45	84.72 (+10.5)	84.14	78.44 (+5.9)
C-3	29.15 (−2.1)	31.08	31.10	30.38 (−2.3)	33.94	28.21 (−2.1)
C-4	44.02	43.99	40.94 (−1.4)	40.91	34.35	44.09
C-5	19.99	19.91	23.17	26.13	27.61	21.00
C-6	30.78	31.08	35.03	35.40	35.30	30.88
C-7	11.41	11.41	28.71	28.26	28.27	11.41
C-8	74.06	73.55 (−0.5)	79.77 (+8.1)	32.43	156.13	73.93
C-9	68.90	77.43 (+8.4)	24.28[a] (−3.5)	65.60	107.01	69.00
C-10	21.75	21.63	24.41[a] (−3.5)	13.95	64.75	21.63
Glc-1	101.35	106.00	98.63	106.47	106.31	101.28
Glc-2	75.42	75.30	75.52	75.73	75.70	75.45
Glc-3	78.63	78.65	78.95	78.73	78.74	78.71
Glc-4	71.75	71.64	71.88	71.61	71.74	71.73
Glc-5	78.39	78.55	78.10	78.30	78.34	78.52
Glc-6	62.75	62.73	63.02	62.86	62.90	62.75

δ in ppm from TMS. $\Delta\delta$ (δ glucoside−δ aglycone) are given in parentheses.
[a] Assignments may be interchanged in each column.

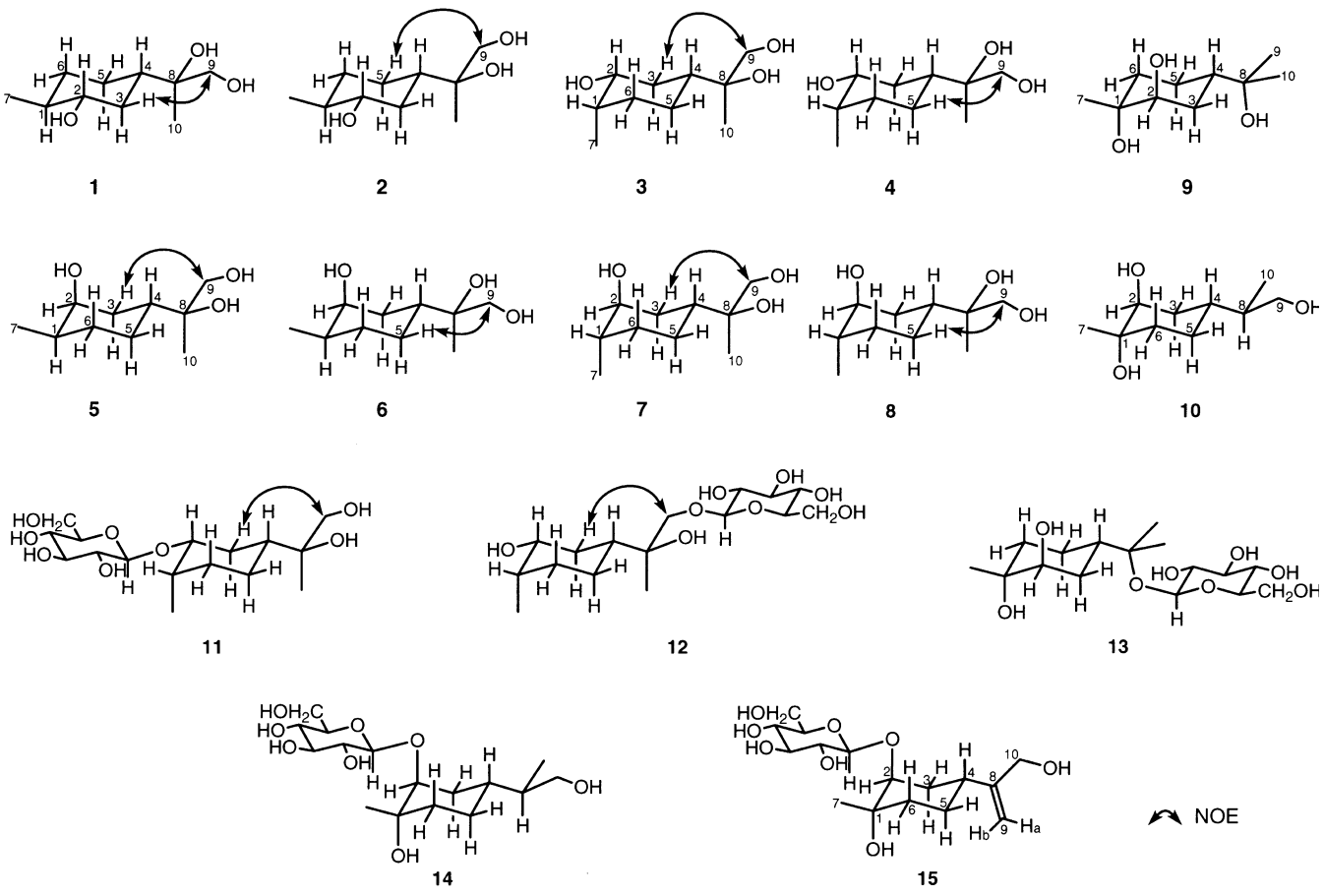

Figure 1. Structures of **1**–**15**, and NOE interactions between H_2-9 and H_2-3 or H_2-5 observed in the NOESY spectra of **1**–**8**, **11** and **12**.

H_3-7/H-3_{ax}, H-5_{ax} and between H-2/H-4 of both triols suggested that the configuration of C-1 methyls was axial and that of C-2 hydroxyls was equatorial, and the conformation of the cyclohexane rings were chair-form with 7–8 *cis* relation (Fig. 1). So, **3** and **4** were concluded to be stereoisomers of *cis*-*p*-menthane-2_{eq},8,9-triol at C-8. The stereochemical relationship between **3** and **4** was established by comparison of their ^{13}C NMR spectra with those of epimeric pairs of **1** and **2**, and (4*R*,8*R*)- and (4*R*,8*S*)-*p*-menth-8,9-diol (**16** and **17**),[7] where C-3 signals in (4*R**,8*S**)-forms (**1**; δ 38.18, **16**; δ 26.93) appeared significantly downfield to those in the (4*R**,8*R**)-forms (**2**; δ 37.30, **17**; δ 25.81), and C-5 in (4*R**,8*S**)-forms (**1**; δ 26.74, **16**; δ 23.05) appeared significantly upfield to those in the (4*R**,8*R**)-forms (**2**; δ 27.64, **17**; δ 24.30). For **3** and **4**, the ^{13}C chemical shift at C-3 of **3** (δ 31.22) was downfield to that of **4** (δ 30.29), whereas C-5 of **3** (δ 20.14) was upfield to that of **4** (δ 21.12). Thus, the stereochemical relationship between C-4 and C-8 was considered to be 4*R**,8*S** in **3** and 4*R**,8*R** in **4** as in the pair of **1** and **2**. This was also supported by the results of the NOESY spectrum which showed interactions between H_3-10/H_2-3, H_2-5, and between H_2-9/H-3_{eq} in **1** and **3**, and interactions between H_3-10/H_2-3, H_2-5, and between H_2-9/H-5_{eq} in **2** and **4**. Glucoside **11** ($C_{16}H_{30}O_8$, an amorphous powder, $[\alpha]^{25}_D$−46°) and **12** ($C_{16}H_{30}O_8$, an amorphous powder, $[\alpha]^{21}_D$−35°) showed $[M+H]^+$ ion peaks at *m*/*z* 351 in the positive FAB-MS. Both glucosides were hydrolyzed with hesperidinase and, from the hydrolyzed mixtures, **3** and D-glucose were obtained. Consequently, **11** and **12** were monoglucosides of **3**, respectively. The position of the β-glucosyl unit of **11** was proved to be C-2 from the HMBC correlation of glucosyl H-1/C-2, and from the observed NOE interaction between the glucosyl H-1/H-2 in the NOESY spectrum. The absolute configuration at C-2 of **3** was indicated as *R* by the values of the glycosylation shift of the α- and β-carbon and the chemical shift of the glucosyl anomeric carbon as shown in Table 2.[8] Thus, **3** and **11** were characterized as (1*S*,2*R*,4*R*,8*S*)-*p*-menthane-2,8,9-triol and (1*S*,2*R*,4*R*,8*S*)-*p*-menthane-2,8,9-triol 2-*O*-β-D-glucopyranoside, respectively. The position of the β-glucosyl unit of **12** was proved to be C-9 by the downfield shift of the C-9 (by 8.4 ppm) signal and upfield shift of C-8 (by 0.5 ppm) signal, and from the observed NOE interaction between the glucosyl H-1/H_2-9 in the NOESY spectrum. So, **12** was characterized as (1*S*,2*R*,4*R*,8*S*)-*p*-menthane-2,8,9-triol 9-*O*-β-D-glucopyranoside. On the other hand, the absolute configuration of **4** was indicated to be 1*S*,2*R*,4*R*,8*R* as (1*S*,2*R*,4*R*,8*R*)-*p*-menthane-2,8,9-triol 2-*O*-β-D-glucopyranoside (**18**), which was isolated from the fruit of *Anethum gravealens* (Dill) by us,[9] gave an aglycone identical to **4**. Therefore, **4** was suggested to be (1*S*,2*R*,4*R*,8*R*)-*p*-menthane-2,8,9-triol.

Triol **5** ($C_{10}H_{20}O_3$, mp 123–126°C, $[\alpha]^{21}_D$−22°) and **6** ($C_{10}H_{20}O_3$, an amorphous powder, $[\alpha]^{21}_D$−30°) showed $[M+K]^+$ ion peaks at *m*/*z* 227 and were indicated to be *p*-menthane-2,8,9-triol by the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) and the result of HMBC experiment of **5**. The configuration of C-2 hydroxyls was suggested to be axial by the H-2 signals which found double doublets with half bandwidths of 3 Hz in their ^{1}H NMR spectra. The conformation of **5** and **6** was found to be 7,8-*trans* form from the observed NOE interactions between H-1/H-3_{ax}, between H_3-7/H-2_{eq}, H_2-6, and between H_3-10/H-3_{ax}, H-5_{ax} in their NOESY spectra. So, **5** and **6** were revealed to be stereoisomers of *trans*-*p*-menthane-2_{ax},8,9-triol at C-8, respectively. Further, the ^{13}C chemical shift of C-3 of **5** (δ 36.01) showed downfield to that of **6** (δ 35.21) and C-5 of **5** (δ 27.21) appeared upfield to that of **6** (δ 28.05); the relationship between **5** and **6** was indicated to be the same as that between **3** and **4**. This conclusion was supported by the results of the NOESY spectra of **5** and **6**, which showed the same interactions as **3** (between H_3-10/H_2-3, H_2-5, and between H_2-9/H-3_{eq}) and **4** (between H_3-10/H_2-3, H_2-5, and between H_2-9/H-5_{eq}). Then, **5** and **6** were concluded to be rel-(1*R*,2*S*,4*R*,8*S*)-*p*-menthane-2,8,9-triol and rel-(1*R*,2*S*,4*R*,8*R*)-*p*-menthane-2,8,9-triol, respectively.

Triol **7** ($C_{10}H_{20}O_3$, mp 130–133°C, $[\alpha]^{21}_D$−7°) and **8** ($C_{10}H_{20}O_3$, an amorphous powder, $[\alpha]^{21}_D$−13°) were indicated to be *p*-menthane-2,8,9-triol by the same way described for **1**–**6**, and the configuration of C-2 hydroxyls was concluded to be axial by the equatorial H-2 signal patterns of their ^{1}H NMR spectra (Table 1). The stereochemical relationship between C-7 and C-8 was suggested to be *cis* from the observed NOE interactions between H_3-7/H-2_{eq}, H-3_{ax}, H-5_{ax}, H-6_{eq}, and between H_3-10/H-3_{ax}, H-5_{ax} in their NOESY spectra. So, **7** and **8** were concluded to be stereoisomers of *cis*-*p*-menthane-2_{ax},8,9-triol at C-8, respectively. The ^{13}C chemical shifts of C-3 (**7**; δ 29.95, **8**; δ 29.10) and C-5 (**7**; δ 20.94, **8**; δ 21.88) indicated the relative configuration at C-4 and C-8 was 4*R**,8*S** for **7** and 4*R**,8*R** for **8**. In addition, the NOESY spectrum of **7** showed NOE interactions between H_3-10/H_2-3, H_2-5 and between H_2-9/H-3_{eq} which were also shown in the NOESY spectra of **1**, **3** and **5**, and the NOESY or 1D-NOE spectra of **8** showed the NOE interactions between H_3-10/H_2-3, H_2-5 and between H_2-9/H-5_{eq} which were also observed in the NOESY spectra of **2**, **4** and **6**. Then, **7** and **8** were concluded to be rel-(1*S*,2*S*,4*R*,8*S*)-*p*-menthane-2,8,9-triol and rel-(1*S*,2*S*,4*R*,8*R*)-*p*-menthane-2,8,9-triol, respectively.

Triol **9** ($C_{10}H_{20}O_3$, mp 134–135°C, $[\alpha]^{23}_D$−25°) showed $[M+H]^+$ ion peaks at *m*/*z* 189 in the positive FAB-MS, and the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) revealed the presence of three *tert*-methyls, three methylenes, two methines (one of them was oxygenated) and two oxygenated quaternary carbons. From the analysis of the HMBC spectral data, **9** was suggested to be *p*-menthane-1,2,8-triol. By comparison of NMR data with those of (4*R*)-*p*-menthane-1,2,8-triols, which were synthesized by Carman et al.,[10] **9** was indicated to be (1*S**,2*S**,4*R**)-form. Furthermore, (1*S*,2*S*,4*R*)-*p*-menthane-1,2,8-triol showed a positive optical rotation ($[\alpha]^{24}_D$+44°($CHCl_3$)) contrary to that of **9** ($[\alpha]^{21}_D$−47°($CHCl_3$)), and **9** was characterized as (1*R*,2*R*,4*S*)-*p*-menthane-1,2,8-triol. Glucoside **13** ($C_{16}H_{30}O_8$, an amorphous powder, $[\alpha]^{23}_D$−31°) showed $[M+H]^+$ ion peaks at *m*/*z* 351 and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m*/*z* 171 in the positive FAB-MS, and gave an aglycone, which was identical to **9**, and D-glucose by enzymatic hydrolysis. The position of attachment of the glucosyl unit was revealed to be C-8 of **9** from the H-C long-range correlation between the glucosyl anomeric proton signal and the C-8 carbon in the HMBC spectrum. Therefore, **13** was determined to be (1*R*,2*R*,4*S*)-*p*-menthane-1,2,8-triol 8-*O*-β-D-glucopyranoside.

Triol **10** ($C_{10}H_{20}O_3$, an amorphous powder, $[\alpha]^{24}_D+25°$) was also indicated to be monoterpenoidtriol by ^1H and ^{13}C NMR spectral data (Tables 1 and 2), and its planar structure was revealed to be *p*-menthane-1,2,9-triol by the HMBC experiment. The configuration of C-1 methyl, C-4 isopropyl was suggested to be equatorial, and the conformation of the cyclohexane ring was found to be chair-form from the NOE interactions between H_3-7/H-2, H_2-6, and between H-4_{ax}/H-6_{ax} observed in the NOESY spectrum. The configuration of C-2 hydroxyl was indicated to be axial by the narrow equatorial H-2 signal of its ^1H NMR spectrum (Table 1). The stereochemical relationship between C-4 and C-8 was revealed to be 4*R**,8*R** from the observed NOE interactions between H_3-10/H_2-3, and between H-3_{ax}/H-8 in the NOESY spectrum. Then, the relative configuration was concluded to be 1*S**,2*S**,4*R**,8*R**, respectively. Glucoside **14** ($C_{16}H_{30}O_8$, mp 174–178°C, $[\alpha]^{24}_D+21°$) showed $[M+H]^+$ ion peaks at *m/z* 351 and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 171 in the positive FAB-MS. **14** was hydrolyzed with β-glucosidase and, from the hydrolyzed mixtures, **10** and D-glucose were obtained. So, **14** was a monoglucoside of **10**. The position of the β-glucosyl unit was suggested to be C-2 from the HMBC correlation of glucosyl H-1/C-2, and from the observed NOE interaction between the glucosyl H-1/H-2 in the NOESY spectrum. The absolute configuration at C-2 of **10** was indicated as *S* by the values of the glycosylation shift of the α- and β-carbon and the chemical shift of the glucosyl anomeric carbon as shown in Table 2.[8] Thus, **10** and **14** were characterized as (1*S*,2*S*,4*R*,8*R*)-*p*-mentane-1,2,9-triol and (1*S*,2*S*,4*R*,8*R*)-*p*-menthane-1,2,9-triol 2-*O*-β-D-glucopyranoside, respectively.

Glucoside **15** ($C_{16}H_{28}O_8$, an amorphous powder, $[\alpha]^{23}_D+7°$) showed $[M+H]^+$ ion peaks at *m/z* 349 and $[M-C_6H_{12}O_6+H]^+$ ion peaks at *m/z* 169 in the positive FAB-MS. The ^1H, ^{13}C and ^{13}C–^1H COSY NMR spectral data (Tables 1 and 2) showed the presence of one β-glucopyranosyl, one *tert*-methyl, four methylenes (one of them was oxygenated), two methines (one of them was oxygenated), one oxygenated quaternary carbon and one terminal-methylene group. By comparison of its ^{13}C NMR data with that of **14**, **15** was suggested to be a dehydro-derivative of **14** having a double bond at C-8. The NOE interactions between H-2_{eq}/glucosyl H-1, between H_3-7/H-2_{eq}, H_2-6, glucosyl H-1, between H-9b/H-5_{ax}, and between H_2-10/H_2-3 also supported the suggested structure, and the configuration of the C-2 hydroxyl group was confirmed to be axial by the coupling constant (dd, *J*=3.0, 3.0 Hz) of the H-2 signal proton. Since this glucose was considered to be D-form the same as other glucosides, the absolute configuration at C-2 should be *S*. So, **15** was concluded to be (1*S*,2*S*,4*R*)-*p*-menth-8-ene-1,2,10-triol 2-*O*-β-D-glucopyranoside.

This is the first report of the isolation of all eight stereoisomeric monoterpenoids, except the optical isomer, having four asymmetric carbons in the molecule (**1**–**8**). Furthermore, the relationship between the essential oil and the water-soluble constituent was confirmed by the isolation of these monoterpenoids and glucosides which showed a biosynthetic relation to *d*-carbone.

3. Experimental

3.1. General procedures

Melting points were determined on a Yanagimoto micro-melting 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. ^1H 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 value. ^1H–^{13}C COSY, HMBC and NOESY spectra were obtained with the usual pulse sequence, and data processing was performed with standard JEOL software. Column chromatography (C. C.) 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. Acetylation was done in the usual way using Ac_2O and pyridine, and no acetoxyl group was detected by NMR spectral data for those acetylated fractions.

3.2. Plant material, extraction and isolation

Commercial caraway (the fruit of *Carum carvi* L.; purchased from Asaoka Spices Ltd., Lot. No. 93010; 2.0 kg) was extracted with 70% methanol (4**L×2) at room temperature for two weeks. After evaporation of the solvent, the residue (402.3 g) was partitioned into ether–water, ethyl acetate–water. Removal of the solvent from each phase gave the ether (200.9 g), ethyl acetate (3.6 g) and aqueous (40.6 g) extracts, The aqueous extract was chromatographed over Amberlite XAD-II (H_2O–MeOH). The methanol eluate (27.6 g) was subjected to Sephadex LH-20 (MeOH) to give eight fractions (frs. A–H). Fraction B (18.9 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 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. $B_{3\text{-}1}$–$B_{3\text{-}9}$), and fr. $B_{3\text{-}5}$ was subjected to HPLC [ODS, MeCN–H_2O (3:37)]. The main 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 fractions. These two fractions were deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h, and passed through Sephadex LH-20 (MeOH) to give **9** (6 mg) and **10** (2 mg). Fraction $B_{3\text{-}7}$ was subjected to HPLC [ODS, MeCN–H_2O (1:9)] to give **3** (4 mg), **1** (10 mg), **4** (43 mg) and **2** (9 mg). Fraction $B_{3\text{-}7}$ was subjected to HPLC [ODS, MeCN–H_2O (1:9)] to give **7** (8 mg), **8** (1 mg), **5** (9 mg) and a mixture of **6** and an alkyl glucoside. From this mixture, **6** (3 mg) was isolated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (9:1:0.1)]. Fraction B_9 (0.83 g) was subjected to a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [CHA,

MeCN–H_2O (9:1)] to give **12** (5 mg). Fraction B_{11} (0.71 g) was also subjected to a Lobar RP-8 column [MeCN–H_2O (3:17)] and HPLC [CHA, MeCN–H_2O (9:1)] to give **13** (6 mg). Fraction B_{10} (1.38 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give eight fractions (frs. $B_{10\text{-}1}$–$B_{10\text{-}8}$). Fraction $B_{10\text{-}4}$, fr. $B_{10\text{-}5}$ and $B_{10\text{-}7}$ were subjected to HPLC [CHA, MeCN–H_2O (9:1)]. to give **15** (2 mg), **14** (11 mg) and **11** (610 mg), respectively.

3.2.1. (1*S*,2*S*,4*S*,8*R*)-*p*-Menthane-2,8,9-triol (1). An amorphous powder, $[\alpha]^{21}_D$+14° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 211 $[M+Na]^+$, 189.1486 $[M+H]^+$ (base, Calcd for $C_{10}H_{21}O_3$; 189.1490), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$, 135 $[M-3H_2O+H]^+$. ^{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-1_{ax}/C-2, C-3, C-5, C-6, C-7; H-3_{ax}/C-1, C-2, C-4, C-5, C-8; H-3_{eq}/C-1, C-2, C-4, C-5, C-8; H-4_{ax}/C-2, C-3, C-5, C-6, C-8, C-9, C-10; H-5_{ax}/C-1, C-3, C-4, C-6, C-8; H-5_{eq}/C-1, C-3, C-4, C-6; H-6_{ax}/C-1, C-2, C-4, C-5, C-7; H-6_{eq}/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/C-4, C-8, C-9.

3.2.2. (1*S*,2*S*,4*S*,8*S*)-*p*-Menthane-2,8,9-triol (2). An amorphous powder, $[\alpha]^{21}_D$+8° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 211 $[M+Na]^+$, 189.1476 $[M+H]^+$ (base, Calcd for $C_{10}H_{21}O_3$; 189.1490), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$, 135 $[M-3H_2O+H]^+$. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.2.3. (1*S*,2*R*,4*R*,8*S*)-*p*-Menthane-2,8,9-triol (3). Colorless needles (MeOH), mp 119–122°C, $[\alpha]^{25}_D$−31° (*c*=3.1, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 227 $[M+K]^+$, 211 $[M+Na]^+$, 189.1502 $[M+H]^+$ (base, Calcd for $C_{10}H_{21}O_3$; 189.1490), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$, 135 $[M-3H_2O+H]^+$. ^{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-1_{eq}/C-2, C-3, C-5, C-6, C-7; H-2_{ax}/C-1, C-3, C-4, C-6, C-7; H-3_{ax}/C-1, C-2, C-4, C-5, C-8; H-3_{eq}/C-1, C-2, C-4, C-5; H-4_{ax}/C-2, C-3, C-5, C-6, C-8, C-9, C-10; H-5_{ax}/C-1, C-3, C-4, C-6, C-8; H-5_{eq}/C-1, C-3, C-4, C-6, C-8; H-6_{ax}/C-1, C-2, C-4, C-5, C-7; H-6_{eq}/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/C-4, C-8, C-9.

3.2.4. (1*S*,2*R*,4*R*,8*R*)-*p*-Menthane-2,8,9-triol (4). Colorless needles (MeOH), mp 115–117°C, $[\alpha]^{21}_D$−35° (*c*=0.4, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 211 $[M+Na]^+$, 189.1493 $[M+H]^+$ (base, Calcd for $C_{10}H_{21}O_3$; 189.1490), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$, 135 $[M-3H_2O+H]^+$. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.2.5. Rel-(1*R*,2*S*,4*R*,8*S*)-*p*-menthane-2,8,9-triol (5). Colorless needles (MeOH), mp 123–126°C, $[\alpha]^{21}_D$−22° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 227.1040 $[M+K]^+$ (Calcd for $C_{10}H_{20}KO_3$; 227.1050), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{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-1_{ax}/C-2, C-5, C-7; H-3_{ax}/C-4, C-5; H-3_{eq}/C-2, C-4, C-5; H-4_{ax}/C-3, C-5, C-6, C-8, C-10; H-5_{ax}/C-1, C-3, C-4, C-6; H-5_{eq}/C-3, C-4, C-6; H-6_{ax}/C-2, C-4, C-5, C-7; H-6_{eq}/C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/C-4, C-8, C-9.

3.2.6. Rel-(1*R*,2*S*,4*R*,8*R*)-*p*-menthane-2,8,9-triol (6). An amorphous powder, $[\alpha]^{21}_D$−30° (*c*=0.4, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 227.1051 $[M+K]^+$ (Calcd for $C_{10}H_{20}KO_3$; 227.1050), 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.2.7. Rel-(1*S*,2*S*,4*R*,8*S*)-*p*-menthane-2,8,9-triol (7). Colorless needles (MeOH), mp 130–133°C, $[\alpha]^{21}_D$−7° (*c*=0.3, MeOH). Positive FAB-MS *m/z*: 399 $[2M+Na]^+$, 377 $[2M+H]^+$, 227.1032 $[M+K]^+$ (Calcd for $C_{10}H_{20}KO_3$; 227.1050), 189 $[M+H]^+$, 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{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_{eq}/C-3; H-3_{ax}/C-4, C-8; H-4_{ax}/C-3, C-8, C-10; H-5_{ax}/C-4, C-8; H-6_{ax}/C-1, C-5, C-7; H-6_{eq}/C-1; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/C-4, C-8, C-9.

3.2.8. Rel-(1*S*,2*S*,4*R*,8*R*)-*p*-menthane-2,8,9-triol (8). An amorphous powder, $[\alpha]^{21}_D$−13° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 399 $[2M+Na]^+$, 377 $[2M+H]^+$, 227.1047 $[M+K]^+$ (Calcd for $C_{10}H_{20}KO_3$; 227.1050), 189 $[M+H]^+$, 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{1}H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.2.9. (1*R*,2*R*,4*S*)-*p*-Menthane-1,2,8-triol (9). Colorless needles (MeOH), mp 134–135°C, $[\alpha]^{23}_D$−25° (*c*=0.2, MeOH), $[\alpha]^{21}_D$−47° (*c*=0.1, $CHCl_3$). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 211.1305 $[M+Na]^+$ (Calcd for $C_{10}H_{20}NaO_3$; 211.1310), 189 $[M+H]^+$, 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{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_{ax}/C-1, C-2, C-5; H-3_{eq}/C-1, C-2, C-5; H-5_{ax}/C-3, C-4, C-6; H-6_{ax}/C-4, C-5; H-6_{eq}/C-1, C-2, C-4, C-5; H_3-7/C-1, C-2, C-6; H_3-9/ C-4, C-8, C-10; H_3-10/ C-4, C-8, C-9.

3.2.10. (1*S*,2*S*,4*R*,8*R*)-*p*-Menthane-1,2,8-triol (10). An amorphous powder, $[\alpha]^{24}_D$+25° (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 377 $[2M+H]^+$, 211.1323 $[M+Na]^+$ (Calcd for $C_{10}H_{20}NaO$; 211.1310), 189 $[M+H]^+$, 171 $[M-H_2O+H]^+$, 153 $[M-2H_2O+H]^+$ (base), 135 $[M-3H_2O+H]^+$. ^{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_{eq}/C-1, C-3, C-6; H-3_{ax}/C-2, C-4, C-5, C-8; H-3_{eq}/C-1, C-2, C-4, C-5, C-8; H-4_{ax}/C-2, C-3, C-5, C-6, C-8, C-9, C-10; H-5_{ax}/C-1, C-4, C-6; H-5_{eq}/C-1, C-3, C-6, C-8; H-6_{ax}/C-1, C-5, C-7; H-6_{eq}/C-1, C-2, C-5, C-7; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/ C-4, C-8, C-9.

3.2.11. (1*S*,2*R*,4*R*,8*S*)-*p*-Menthane-2,8,9-triol 4-*O*-β-D-glucopyranoside (11). An amorphous powder, $[\alpha]^{25}_D$−46° (*c*=1.8, MeOH). Positive FAB-MS *m/z*: 373 $[M+Na]^+$, 351.2048 $[M+H]^+$ (base, Calcd for $C_{16}H_{31}O_8$;

351.2019), 189 $[M-C_6H_{10}O_5+H]^+$. ^1H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-1_{eq}/C-2; H-2_{ax}/C-3, C-4, C-7, Glc C-1; H-3_{ax}/C-1, C-2, C-4, C-5; H-3_{eq}/C-1, C-2, C-4, C-5; H-4_{ax}/C-3, C-8, C-10; H-5_{ax}/C-4, C-6; H-5_{eq}/C-1, C-3, C-4, C-6; H-6_{ax}/C-1, C-4, C-5, C-7; H-6_{eq}/C-2, C-4, C-5; H_3-7/C-1, C-2, C-6; H_2-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Clc H-1/C-2.

3.3. Enzymatic hydrolysis of 11

A mixture of **11** (12 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 chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (4:1:0.1 and 1:1:0.1)] to afford **3** (6 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^{-1}; *t R* 4.50 min (same location as that of D-glucose)] show the presence of D-glucose.

3.3.1. (1*S*,2*R*,4*R*,8*S*)-*p*-Menthane-2,8,9-triol 9-*O*-β-D-glucopyranoside (12). An amorphous powder, $[\alpha]^{21}_D-35°$ (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 723 $[2M+Na]^+$, 701 $[2M+H]^+$, 389 $[M+K]^+$, 373 $[M+Na]^+$, 351.2018 $[M+H]^+$ (Calcd for $C_{16}H_{31}O_8$; 351.2019), 171 $[M-C_6H_{12}O_6+H]^+$ (base). ^1H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

3.4. Enzymatic hydrolysis of 12

A mixture of **12** (9 mg) and hesperidinase (5 mg) in water (5 ml) was shaken in a water bath at 37°C for 20 days. The mixture was treated in the same way described for **11** to afford **3** (2 mg) and a sugar fraction. From the sugar fraction, the presence of D-glucose was revealed as **11**.

3.4.1. (1*S*,2*R*,4*S*)-*p*-Menthane-1,2,8-triol 8-*O*-β-D-glucopyranoside (13). An amorphous powder, $[\alpha]^{23}_D-31°$ (*c*=0.4, MeOH). Positive FAB-MS *m/z*: 701 $[2M+H]^+$, 389 $[M+K]^+$, 373.1847 $[M+Na]^+$ (base, Calcd for $C_{16}H_{30}NaO_8$; 373.1838), 351 $[M+H]^+$, 171 $[M-C_6H_{12}O_6+H]^+$. ^1H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-2_{eq}/C-4; H-3_{ax}/C-4, C-5; H-3_{eq}/C-1, C-2, C-4, C-5; H-4_{ax}/C-5, C-8, C-9, C-10; H-5_{ax}/C-1, C-3, C-4, C-6; H-5_{eq}/C-1, C-6; H-6_{ax}/C-1, C-4, C-5; H-6_{eq}/C-1, C-2, C-4; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/ C-4, C-8, C-9; Clc H-1/C-8.

3.5. Enzymatic hydrolysis of 13

A mixture of **13** (5 mg) and β-glucosidase (5 mg, TOYOBO Co. Ltd Lot. 52275) in water (5 ml) was shaken in a water bath at 37°C for 7 days. The mixture was treated in the same way described for **11** to afford **9** (2 mg) and a sugar fraction. From the sugar fraction, the presence of D-glucose was revealed as **11**.

3.5.1. (1*S*,2*S*,4*R*,8*R*)-*p*-Menthane-1,2,9-triol 2-*O*-β-D-glucopyranoside (14). Colorless needles (MeOH), mp 174–178°C, $[\alpha]^{24}_D+21°$ (*c*=0.2, MeOH). Positive FAB-MS *m/z*: 723 $[2M+Na]^+$, 701 $[2M+H]^+$, 389 $[M+K]^+$, 373 $[M+Na]^+$, 351.2018 $[M+H]^+$ (Calcd for $C_{16}H_{31}O_8$; 351.2019), 171 $[M-C_6H_{12}O_6+H]^+$ (base). ^1H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2. HMBC correlations: H-2_{eq}/C-1, C-6, C-7, Glc C-1; H-3_{ax}/C-1, C-2, C-4, C-5, C-8; H-3_{eq}/C-1, C-2, C-4, C-5, C-8; H-4_{ax}/C-8, C-9, C-10; H-5_{ax}/C-1, C-4, C-6; H-5_{eq}/C-1, C-3, C-4, C-8; H-6_{ax}/C-1, C-5, C-7; H-6_{eq}/C-1, C-3, C-5; H_3-7/C-1, C-2, C-6; H_2-9/ C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Clc H-1/C-2.

3.6. Enzymatic hydrolysis of 14

A mixture of **14** (8 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37°C for 7 days. The mixture was treated in the same way described for **11** to afford **10** (4 mg) and a sugar fraction. From the sugar fraction, the presence of D-glucose was revealed as **11**.

3.6.1. (1*S*,2*S*,4*R*)-*p*-Menthane-1,2,10-triol 2-*O*-β-D-glucopyranoside (15). An amorphous powder, $[\alpha]^{23}_D+7°$ (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 371.1681 $[M+Na]^+$ (Calcd for $C_{16}H_{30}NaO_8$; 371.1681), 349 $[M+H]^+$, 169 $[M-C_6H_{12}O_6+H]^+$ (base). ^1H NMR (pyridine-d_5, 500 MHz) δ: Table 1. ^{13}C NMR (pyridine-d_5, 125 MHz) δ: Table 2.

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