Water-Soluble Constituents of Fennel. VI.[1] 1,8-Cineole Type Glycosides

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Four stereoisomers of 2-hydroxy-1,8-cineole β-D-glucopyranoside were isolated together with five new 1,8-cineole glycosides and five new dihydroxy-1,8-cineoles from the water-soluble portion of fennel. Their structures were clarified by spectral methods.

Key words fennel; *Foeniculum vulgare* fruit; 1,8-cineole glycoside; Umbelliferae; stereoisomeric glycoside; ^{13}C-NMR

In continuation of our studies[1—3] on the water-soluble constituents of fennel, the fruit of *Foeniculum vulgare* MILLER (Umbelliferae), we describe 1,8-cineole derivatives in this paper. In a previous paper,[3a] we reported the isolation and characterization of foeniculosides V—IX (**6—10**), which are glycosides of dihydroxy-1,8-cineole derivatives, from the water-soluble portion of the methanol extract of commercial fennel. Detailed examination of the same extract has now resulted in the isolation of a further nine glycosides (**1—5**, **11—14**) and five diols (**1a—5a**) which are derivatives of this monoterpenoid.

All glycosides obtained in this paper were β-D-glucopyranosides as evidenced from their ^{13}C-NMR data (Table 2). This was confirmed by acid hydrolysis to yield D-glucose. They were also classified into two groups, glycosides of monools, $C_{16}H_{28}O_7$ and glycosides of diols, $C_{16}H_{28}O_8$. These molecular formulae were established from the accurate mass number of $[M+H]^+$ ion peaks in high-resolution positive FAB-MS.

Glycoside **1** (an amorphous powder, $[\alpha]_D^{21}$ −46.3°), **2** (mp 94—95 °C, $[\alpha]_D^{21}$ −7.2°), **3** (mp 83—84 °C, $[\alpha]_D^{23}$ +5.5°), and **4** (mp 85—86 °C, $[\alpha]_D^{23}$ −66.3°) were stereoisomers. In addition to the glucopyranoside moiety, three *tert*-methyls, three methylenes, two methines (oxygenated and non-oxygenated), and two oxygenated quaternary carbons, were observed in the ^{1}H- and ^{13}C-NMR data (Tables 1, 2). These data and the observed H–C long-range correlations between H_3-7 and the C-2 oxygenated carbon, and between the anomeric proton and C-2 in the heteronuclear multiple-bond correlation (HMBC) spectra suggested that they are β-D-glucopyranosides of 2-hydroxy-1,8-cineole. In glycosides **1** and **2**, nuclear Overhauser effect (NOE) interaction between H-2 and H-6*endo* in their nuclear Overhauser and exchange spectroscopy (NOESY) spectra (Fig. 1), indicates that the configuration at H-2 should be *endo*. Thus, **1** and **2** were concluded to be 2-*exo*-hydroxy-1,8-cineole β-D-glucopyranosides. The results of $[M]_D$ value calculations using methyl β-D-glucopyranoside (Me β-D-Glc; −62°) [$[M]_D$ of **1**: −154°, Δ $[M]_D$ (**1**−Me β-D-Glc) −92°; $[M]_D$ of **2**: −24°, Δ $[M]_D$ +38°)] suggested that the aglycones of **1** and **2** should be (−)- and (+)-forms, respectively.[4,5] In glycosides **3** and **4**, the observed long-range W type coupling (1.5 Hz) between H-2 and H-6*exo* in their ^{1}H-NMR spectra suggested that configuration at H-2 was *exo*. Thus, **3** and **4** were concluded to be 2-*endo*-hydroxy-1,8-cineole β-D-glucopyranosides. The $[M]_D$ values of both glycosides indicated that the aglycones of **3** and **4** ($[M]_D$ of **3**: +18°, Δ $[M]_D$ +80°; $[M]_D$ of **4**: −220°, Δ $[M]_D$ −158°) were suggested to be (+)- and (−)-forms, respectively.[6]

The absolute configurations at C-2 of **1** to **4** were established by analysis of ^{13}C-NMR glycosylation shifts.[7] In the case of β-D-glucopyranosides of secondary alcohols, the values of the glycosylation shift (*R*-alcohols, $\Delta\ \delta$ +6 to +8; *S*-alcohols, $\Delta\ \delta$ +10 to +11) and the chemical shifts of the anomeric carbon (*R*-alcohols, about δ 102; *S*-alcohols, about δ 106) are dependent on the absolute configuration of the alcohols. When this empirical rule was applied for these isomers [Table 2; $\Delta\ \delta$ C-2 (**2**−**1**)=+5.25, $\Delta\ \delta$ C-2 (**3**−**4**)= +3.95, δ glucosyl C-1 (**1**: 101.27, **2**: 107.16, **3**: 106.49, **4**: 102.13)], the absolute configuration at C-2 was confirmed to be *R* for **1** and **4**, and *S* for **2** and **3**. Therefore, **1**, **2**, **3** and **4** were characterized as (1*S*,2*R*,4*R*)-, (1*R*,2*S*,4*S*)-, (1*S*,2*S*,4*R*)- and (1*R*,2*R*,4*S*)-2-hydroxy-1,8-cineole β-D-glucopyranosides, respectively.

Glycoside **2** is a new compound as natural product. Glycoside **1** has previously been isolated from the leaves of *Cunila spicata* (Lamiaceae), which has been used in traditional Brazilian medicine,[5] and **3** was isolated from the peel and flower buds of *Citrus unshiu* (Rutaceae).[8] Glycosides **3** and **4** were also obtained as biotransformation products from a cell suspension culture of *Eucalyptus perriniana* (Myrtaceae) following administration of 1,8-cineole.[6] Co-occurrence of these four stereoisomers means that the racemic forms of each aglycone exist in the plant and they are glycosylated.

Glycoside **5** (an amorphous powder, $[\alpha]_D^{21}$ −18.0°) showed the presence of three *tert*-methyls, four methylenes and three oxygenated quaternary carbons, in the ^{1}H- and ^{13}C-NMR data (Tables 1, 2). The aglycone of **5** was concluded to be 4-hydroxy-1,8-cineole, and the location of the glucosyl group was confirmed to be C-4 by the HMBC experiment. Therefore, **5** was characterized as 4-hydroxy-1,8-cineole β-D-glucopyranoside.

Glycosides **11** (an amorphous powder, $[\alpha]_D^{23}$ +5.0°), **12** (an amorphous powder, $[\alpha]_D^{23}$ −23.5°), **13** (an amorphous powder, $[\alpha]_D^{23}$ −24.7°) and **14** (an amorphous powder, $[\alpha]_D^{23}$ −18.0°) were revealed to be β-D-glucopyranosides of dihydroxy-1,8-cineole derivatives by comparison of ^{1}H- and ^{13}C-NMR data (Tables 1, 2) with those of **1** to **10**, and from their

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Table 1. ^{1}H-NMR Chemical Shifts of **1**—**14** and **1a**—**5a** (in Pyridine-d_5, 500 MHz)

	1	**2**	**3**	**4**
H-2 *endo*	4.08 dd (3.0, 10.0)	3.70 dd (3.0, 10.0)	—	—
exo	—	—	4.01 ddd (1.5, 3.0, 10.0)	4.25 ddd (1.5, 3.0, 9.5)
H-3 *endo*	1.85 br dd (10.0, 14.0)	2.07 ddd (3.0, 10.0, 14.0)	1.92 ddd (3.0, 3.0, 14.0)	1.87 ddd (3.0, 3.0, 14.0)
exo	2.41 br dd (3.0, 14.0)	2.43 ddd (3.0, 10.0, 14.0)	2.61 dddd (3.0, 3.0, 10.0, 14.0)	2.49 dddd (3.0, 3.0, 9.5, 14.0)
H-4	1.37 br s	1.29 br s	1.33 br s	1.42 br s
H-5 *endo*	1.19 m	1.26 m	1.52 dddd (1.5, 6.0, 13.0, 13.0)	1.55 dddd (1.5, 6.0, 12.0, 12.0)
exo	1.86 m	1.87 ddd (3.0, 12.5, 12.5)	1.84 dddd (3.0, 3.0, 13.0, 13.0)	1.83 m
H-6 *endo*	1.30 ddd (3.0, 13.0, 13.0)	1.37 ddd (3.0, 12.5, 12.5)	2.13 ddd (3.0, 13.0, 13.0)	2.05 ddd (3.0, 12.0, 12.0)
exo	1.67 ddd (6.0, 13.0, 13.0)	1.70 ddd (6.0, 12.5, 12.5)	1.50 dddd (1.5, 6.0, 13.0, 13.0)	1.51 dddd (1.5, 6.0, 12.0, 12.0)
H_3-7	1.30 s	1.43 s	1.47 s	1.35 s
H_3-9	1.23 s	1.24 s	1.24 s	1.25 s
H_3-10	1.42 s	1.36 s	1.16 s	1.15 s
Glc-1	5.00 d (7.5)	4.84 d (8.0)	4.94 d (8.0)	4.98 d (8.0)

	5	**6**	**7**	**8**
H-2 *endo*	1.69[a] ddd (4.0, 12.5, 12.5)	4.34 dd (3.0, 10.0)	—	—
exo	1.85[b] m	—	4.42 dd (1.5, 3.0, 9.5)	4.15 br d (10.0)
H-3 *endo*	2.16 ddd (5.5, 12.5, 12.5)	2.25 dd (10.0, 13.0)	2.31 dd (3.0, 13.0)	2.42 dd (3.5, 14.0)
exo	2.37 dddd (4.0, 4.0, 12.5, 12.5)	2.85 ddd (3.0, 3.0, 13.0)	2.88 ddd (3.0, 9.5, 13.0)	3.08 ddd (3.5, 10.0, 14.0)
H-4	—	—	—	—
H-5 *endo*	2.11 ddd (5.5, 12.5, 12.5)	1.68 ddd (5.5, 12.5, 12.5)	1.97 ddd (6.5, 12.0, 12.0)	2.40 br dd (5.5, 13.0)
exo	2.24 dddd (4.0, 4.0, 12.5, 12.5)	2.23 m	2.17 dddd (3.0, 3.0, 12.0, 12.0)	2.27 dddd (3.5, 3.5, 13.0, 13.0)
H-6 *endo*	1.63[a] ddd (4.0, 12.5, 12.5)	1.60 ddd (3.0, 12.5, 12.5)	2.33 ddd (3.0, 12.0, 12.0)	2.53 ddd (3.5, 13.0, 13.0)
exo	1.79[b] m	1.93 ddd (5.5, 12.5, 12.5)	1.78 dddd (1.5, 6.5, 12.0, 12.0)	1.83 dddd (1.5, 5.5, 13.0, 13.0)
H_3-7	1.06 s	1.37 s	1.41 s	1.38 s
H_3-9	1.50 [c] s	1.55 s	1.55 s	1.61 s
H_3-10	1.54 [c] s	1.76 s	1.48 s	1.50 s
Glc-1	5.05 d (7.5)	5.04 d (8.0)	5.04 d (7.5)	5.14 d (8.0)

	9	**10**	**11**	**12**
H-2 *endo*	4.15 dd (3.0, 10.0)	4.11 dd (3.5, 10.0)	—	3.74 dd (3.0, 10.5)
exo	—	—	4.21 dd (1.5, 3.0, 10.0)	—
H-3 *endo*	1.79 ddd (3.0, 10.0, 14.0)	1.90 ddd (3.5, 10.0, 14.0)	2.39 dd (3.0, 13.5)	2.03 ddd (3.0, 10.0, 14.5)
exo	2.49 dddd (3.0, 3.0, 3.0, 14.0)	2.44 ddd (3.5, 3.5, 14.0)	3.03 ddd (3.0, 10.0, 13.5)	2.52 dddd (3.0, 3.0, 3.0, 14.5)
H-4	1.49 dddd (3.0, 3.0, 3.0, 3.0)	1.81 ddd (3.5, 3.5, 3.5)	—	1.40 dddd (3.0, 3.0, 3.0, 3.0)
H-5 *endo*	1.91 ddd (3.0, 10.0, 14.0)	4.25 ddd (3.5, 6.0, 8.5)	1.91 ddd (6.0, 12.5, 12.5)	1.93 ddd (3.0, 10.5, 14.0)
exo	2.23 dddd (3.0, 3.0, 3.0, 14.0)	—	2.19 dddd (3.0, 3.0, 12.5, 12.5)	2.22 dddd (3.0, 3.0, 3.0, 14.0)
H-6 *endo*	3.69 dd (3.0, 10.0)	2.05 dd (8.5, 13.5)	2.42 ddd (3.0, 12.5, 12.5)	3.77 dd (3.0, 10.5)
exo	—	2.10 dd (6.0, 13.5)	1.79 dddd (1.5, 6.0, 12.5, 12.5)	—
H_3-7	1.61 s	1.38 s	1.54 s	1.73 s
H_3-9	1.53 s	1.83 s	1.55 s	1.56 s
H_3-10	1.59 s	1.51 s	1.48 s	1.48 s
Glc-1	5.03 d (8.0)	4.98 d (8.0)	5.00 d (7.5)	4.90 d (7.5)

	13	**14**	**1a**	**2a**
H-2 *endo*	—	3.79 dd (3.0, 10.5)	4.03 dd (3.5, 10.5)	—
exo	3.98 dd (3.0, 9.5)	—	—	4.22 br d (9.5)
H-3 *endo*	1.94 ddd (3.0, 3.0, 14.5)	2.13 ddd (3.0, 10.5, 14.0)	2.40 dd (10.5, 13.5)	2.08 dd (3.5, 13.5)
exo	2.69 ddd (3.0, 9.5, 14.5)	2.71 br dd (3.0, 14.0)	2.59 ddd (3.5, 3.5, 13.5)	2.97 ddd (3.5, 9.5, 13.5)
H-4	1.80 ddd (3.0, 3.0, 3.0)	1.97 br s	—	—
H-5 *endo*	4.56 br d (10.5)	1.31 m	1.73 ddd (5.0, 12.0, 12.0)	2.04 ddd (6.0, 12.5, 13.5)
exo	—	1.96 br dd (13.0, 13.0)	2.26 dddd (3.5, 3.5, 12.0, 12.0)	2.30 dddd (3.5, 3.5, 12.5, 13.5)
H-6 *endo*	2.80 dd (10.5, 14.0)	1.43 ddd (3.0, 13.0, 13.0)	1.69 ddd (3.5, 12.0, 12.0)	2.54 ddd (3.5, 12.5, 13.5)
exo	2.01 ddd (1.5, 6.0, 14.0)	1.81 ddd (6.0, 13.0, 13.0)	2.00 ddd (5.0, 12.0, 12.0)	1.86 dddd (1.5, 6.0, 12.5, 13.5)
H_3-7	1.58 s	—	1.38 s	1.43 s
H_2-7	—	3.82 d (11.0)	—	—
	—	4.43 d (11.0)	—	—
H_3-9	1.83 s	1.55 s	1.59 s	1.62 s
H_3-10	1.25 s	1.48 s	1.75 s	1.56 s
Glc-1	4.96 d (8.0)	4.87 d (7.5)	—	—

	3a	**4a**	**5a**
H-2 *endo*	3.82 dd (3.0, 10.0)	3.78 dd (3.5, 10.0)	—
exo	—	—	4.00 br d (9.0)
H-3 *endo*	1.99 ddd (3.0, 10.0, 14.5)	2.08 ddd (3.5, 10.0, 14.0)	1.65 ddd (3.0, 3.0, 14.0)
exo	2.28 dddd (3.0, 3.0, 3.0, 14.5)	2.24 ddd (3.5, 3.5, 14.0)	2.69 ddd (3.0, 9.0, 14.0)
H-4	1.51 dddd (3.0, 3.0, 3.0, 3.0)	1.82 ddd (3.5, 3.5, 3.5)	1.93 ddd (3.0, 3.0, 3.0)
H-5 *endo*	1.99 ddd (3.0, 10.0, 14.5)	4.36 ddd (3.5, 7.0, 9.5)	4.71 ddd (3.0, 6.0, 10.0)
exo	2.28 dddd (3.0, 3.0, 3.0, 14.5)	—	—
H-6 *endo*	3.82 dd (3.0, 10.0)	2.16 dd (9.5, 14.0)	2.92 dd (10.0, 14.0)
exo	—	2.20 dd (7.0, 14.0)	2.09 ddd (3.0, 6.0, 14.0)
H_3-7	1.38 s	1.40 s	1.47 s
H_3-9	1.59 s	1.88 s	1.91 s
H_3-10	1.59 s	1.52 s	1.34 s
Glc-1	—	—	—

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. *a*—*c*) Assignments may be interchanged.

Table 2. ^{13}C-NMR Chemical Shifts of **1**—**14** and **1a**—**5a** (in Pyridine-d_5, 125 MHz)

	1	2	3	4	5
C-1	71.90	72.41	72.37	71.85	69.28
C-2	74.74	79.99	80.08	76.13	33.85[a]
C-3	31.63	34.61	34.26	32.07	28.67
C-4	33.70	33.86	34.37	34.21	76.63
C-5	22.31	22.28	22.51	22.37	26.30
C-6	30.70	30.68	26.30	26.44	33.99[a]
C-7	23.90	23.48	24.92	25.13	27.00
C-8	73.54	73.62	73.21	73.20	76.93
C-9	29.34	29.29	28.90	28.88	25.99[b]
C-10	28.60	28.61	29.17	29.13	25.87[b]
Glc-1	101.27	107.16	106.49	102.13	98.58
Glc-2	74.66	75.37	75.55	75.14	75.31
Glc-3	78.74	78.44	78.60	78.72	78.91
Glc-4	71.96	71.89	71.72	71.81	71.82
Glc-5	78.69	78.30	78.34	78.54	78.24
Glc-6	63.09	63.15	62.90	62.90	63.20

	6	7	8	9	10
C-1	71.62	71.60	73.03	75.75	72.66
C-2	76.76	78.09	72.20	73.64	74.24
C-3	39.57	40.08	41.31	30.81	30.20
C-4	69.51	69.88	77.18	33.68	42.17
C-5	30.48	30.54	26.29	34.75	68.98
C-6	32.20	29.28	28.31	68.86	42.67
C-7	23.40	24.55	24.33	19.52	23.67
C-8	77.52	76.87	76.51	73.69	73.95
C-9	25.94	25.62	25.97	28.91	31.59
C-10	25.00	25.72	26.26	28.87	30.57
Glc-1	101.51	102.29	98.63	101.45	101.55
Glc-2	74.78	75.16	75.31	74.83	74.75
Glc-3	78.78	78.71	78.89	78.85	78.74
Glc-4	71.95	71.82	71.74	71.99	71.94
Glc-5	78.69	78.54	78.17	78.77	78.69
Glc-6	63.11	62.91	62.93	63.08	63.10

	11	12	13	14
C-1	72.14	76.21	72.70	76.57
C-2	81.75	79.22	79.82	80.03
C-3	42.12	33.95	33.61	34.40
C-4	69.64	33.83	42.81	29.58
C-5	30.71	34.66	68.97	22.18
C-6	29.08	68.93	39.03	31.31
C-7	24.39	19.21	24.58	68.47
C-8	76.89	73.76	73.42	72.48
C-9	25.65	28.91	30.95	25.09
C-10	25.76	28.83	31.06	23.48
Glc-1	106.48	107.58	106.62	107.02
Glc-2	75.54	75.47	75.54	75.43
Glc-3	78.62	78.55	78.62	78.52
Glc-4	71.70	71.92	71.70	71.96
Glc-5	78.39	78.39	78.40	78.35
Glc-6	62.85	63.21	62.91	63.18

	1a	2a	3a	4a	5a
C-1	72.73	73.14	76.70	73.63	73.36
C-2	72.06	72.35	68.98	69.64	70.07
C-3	43.36	43.47	34.91	34.17	34.91
C-4	69.53	69.94	34.03	42.70	43.05
C-5	30.68	30.97	34.91	69.26	69.21
C-6	31.85	28.61	68.98	41.98	38.91
C-7	23.11	24.37	19.40	23.46	24.71
C-8	77.48	76.83	73.64	73.91	73.80
C-9	25.99	25.69	28.94	31.66	31.18
C-10	25.14	25.96	28.94	30.60	31.19

δ in ppm from TMS. *a*, *b*) Assignments may be interchanged.

molecular formulae $C_{16}H_{28}O_8$. Since H–C long-range correlations between H_3-7 (or H_2-7) and C-2 oxygenated carbon, and between the anomeric proton and C-2 were observed in HMBC spectra, the position of the glycosyl units were indicated to be C-2. These comparisons also indicated that the glycosides **11**, **12** and **13** were stereoisomers of **7**, **9** and **10**, respectively. In glycoside **11**, the observed NOE interaction between H-2 and H_3-10 in the NOESY spectrum suggested that the configuration of the C-2 hydroxy group was *endo* as in **7**. The absolute configuration at C-2 in **11** was established as *S* by comparison of C-2 and glucosyl C-1 chemical shifts with those of **7** [δ C-2 (**7**: 78.09, **11**: 81.75), δ glucosyl C-1 (**7**: 102.29, **11**: 106.48)]. Therefore, **11** was characterized as (1*S*,2*S*,4*S*)-2,4-dihydroxy-1,8-cineole 2-*O*-β-D-glucopyranoside. In glycoside **12**, the configuration of C-2 and C-6 hydroxyl groups were clarified to be *exo* as in **9** by the observed NOE interaction between H-2 and H-6*endo* in the NOESY spectrum. The absolute configuration at C-2 in **12** was established as *S* by the same method applied to **11** [δ C-2 (**9**: 73.64, **12**: 79.22), δ glucosyl C-1 (**9**: 101.45, **12**: 107.58)]. Therefore, **12** was characterized as (1*R*,2*S*,4*S*,6*R*)-2,6-dihydroxy-1,8-cineole 2-*O*-β-D-glucopyranoside. In glycoside **13**, the observed NOE interactions between H-2 and H_3-10, and between H-5 and H-3*endo*, suggested that the configuration of the C-2 and C-5 hydroxyl group was *endo* and *exo*, respectively. The absolute configuration at C-2 in **13** was established by comparison of the chemical shift values of C-2 and the anomeric carbon with those of **3** and **4**, [C-2 (**13**: δ 79.82, **3**: δ 80.08, **4**: δ 76.13), glucosyl C-1 (**13**: δ 106.62, **3**: δ 106.49, **4**: δ 102.13)]. Thus, the absolute configuration at C-2 in **13** was *S*. Therefore, **13** was characterized as (1*S*,2*S*,4*S*,5*R*)-2,5-dihydroxy-1,8-cineole 2-*O*-β-D-glucopyranoside. Glycoside **14** was concluded to be 2,7-dihydroxy-1,8-cineole 2-*O*-β-D-glucopyranoside by the result of the HMBC experiment, and the observed NOE interactions between H-2 and H-6*endo* in the NOESY spectrum suggested that the configuration of the C-2 hydroxyl group was *exo*. The ^{13}C chemical shifts of C-2 (δ 80.03) and the anomeric carbon (δ 107.02) indicated that the absolute configuration at C-2 was *S*. From these results, **14** was characterized as (1*S*,2*S*,4*S*)-2,7-dihydroxy-1,8-cineole 2-*O*-β-D-glucopyranoside.

Diols **1a** to **4a** were identified as the aglycones of **6**, **7**, **9** and **10**, respectively.[3] Diol **5a** (an amorphous powder, $[\alpha]_D^{23}$ −13.0°) was characterized as the aglycone of **13** by comparison of the ^{13}C-NMR data with that of **13** and the observed NOE interactions between H-2 and H_3-10, and between H-5 and H-3*endo* in the NOESY spectrum.

Experimental

The instruments and experimental conditions for obtaining spectral data and for chromatography were the same as in the preceeding paper.[2]

Extraction and Isolation of 1—14 and 1a—5a Derivatives The methanol extract of fennel (2.0 kg) was treated as described in part I, and seven fractions (frs. A—G) were obtained from the aqueous portion by Amberlite XAD-II and Sephadex LH-20 chromatographies.[2] Fraction C (16.9 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1)→MeOH] to give fifteen fractions (frs. C_1—C_{15}). Fraction C_3 (1.3 g) was subjected to a Lobar RP-8 column [CH_3CN–H_2O (3 : 17)] to give ten fractions (frs. $C_{3\text{-}1}$—$C_{3\text{-}10}$). Fraction $C_{3\text{-}2}$ was subjected to HPLC [octadecyl silica (ODS), MeOH–H_2O (3 : 37)] to give two fractions, and then each fraction was chromatographed over silica gel [$CHCl_3$–MeOH (20 : 1)] to afford **4a** (25 mg) and **1a** (5 mg). Fraction $C_{3\text{-}3}$ was subjected to HPLC [ODS, MeOH–H_2O (3 : 37)] to give four fractions, and then each fraction was chromatographed over silica gel [$CHCl_3$–MeOH (20 : 1)] to afford **5a** (5 mg), **2a**

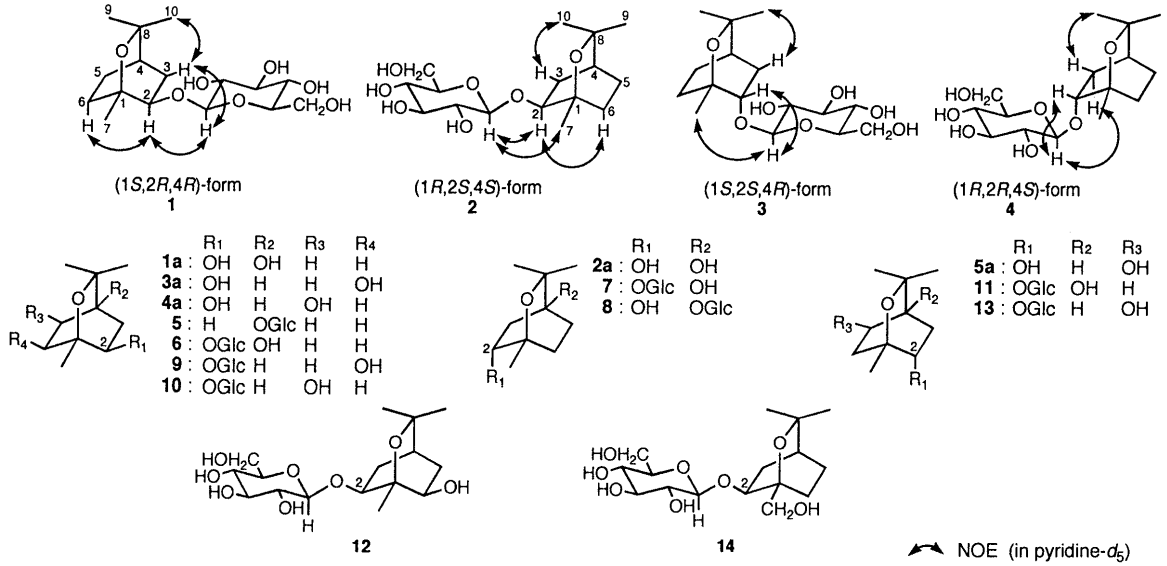

Fig. 1. Structures of **1**—**14** and **1a**—**5a**, and NOE Interactions Observed in the NOESY Spectra of **1**—**4**

(11 mg) and **3a** (7 mg). Fraction C_5 (1.7 g) was subjected to a Lobar RP-8 column [CH_3CN-H_2O (3 : 17)] to give twelve fractions (frs. $C_{5\text{-}1}$—$C_{5\text{-}12}$). Fraction $C_{5\text{-}5}$ was subjected to HPLC [ODS, CH_3CN–H_2O (1 : 9)] to afford **5** (35 mg). Fraction $C_{5\text{-}7}$ was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, CH_3CN–H_2O (1 : 1)] to give four fractions (frs. $C_{5\text{-}7\text{-}1}$–$C_{5\text{-}7\text{-}4}$). Fraction $C_{5\text{-}7\text{-}3}$ was deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h to afford a mixture of **3** and **4** (50 mg). This mixture was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (24 : 1)], to obtain **4** (18 mg) and **3** (24 mg) in the pure form. Fraction $C_{5\text{-}8}$ was subjected to HPLC [ODS, CH_3CN–H_2O (3 : 17)] to afford **1** (90 mg). Fraction $C_{5\text{-}9}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (24 : 1)] to give **2** (20 mg). Fraction C_7 (0.7 g) was subjected to a Lobar RP-8 column [CH_3CN–H_2O (1 : 9→3 : 17)] to give nine fractions (frs. $C_{7\text{-}1}$—$C_{7\text{-}9}$). Fraction $C_{7\text{-}7}$ was subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (14 : 1)] to give **14** (3 mg). Fraction C_9 (1.3 g) was subjected to a Lobar RP-8 column [MeOH–H_2O (3 : 17→1 : 4)] to give eleven fractions (frs. $C_{9\text{-}1}$—$C_{9\text{-}11}$). Fraction $C_{9\text{-}4}$ was subjected to HPLC [ODS, MeOH–H_2O (1 : 9)] to afford **10** (48 mg). Fraction $C_{9\text{-}5}$ was acetylated with Ac_2O and pyridine, and the acetylated fraction was subjected to HPLC [ODS, CH_3CN–H_2O (1 : 1)] to give seven fractions (frs. $C_{9\text{-}5\text{-}1}$—$C_{9\text{-}5\text{-}7}$). Fraction $C_{9\text{-}5\text{-}1}$ was deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h to afford **6** (24 mg). Fraction $C_{9\text{-}5\text{-}2}$ was chromatographed over silica gel [hexane–EtOAc (11 : 9)] to give two fractions and each fraction was deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 2 h to afford **11** (13 mg) and **7** (8 mg). Fraction $C_{9\text{-}5\text{-}4}$ was deacetylated by heating in a water bath with 15% NH_4OH–MeOH for 4 h, and then subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (9 : 1)] to afford **9** (35 mg). Fraction $C_{9\text{-}5\text{-}5}$ was deacetylated as for fr. $C_{9\text{-}5\text{-}4}$, and then subjected to HPLC [carbohydrate analysis, CH_3CN–H_2O (24 : 1)] to afford **12** (3 mg). Fraction $C_{9\text{-}5\text{-}7}$ was deacetylated as for fr. $C_{9\text{-}5\text{-}4}$, and then subjected to HPLC [ODS, MeOH–H_2O (3 : 97)] to afford **8** (5 mg) and **13** (15 mg).

(1*S*,2*R*,4*R*)-2-Hydroxy-1,8-cineole β-D-Glucopyranoside (1) An amorphous powder, $[\alpha]_D^{21}$ −46.3° (*c*=1.5, MeOH), [lit.[5]; $[\alpha]_D^{21}$ −53° (*c*=0.4, MeOH)]. Positive FAB-MS *m/z*: 665 $[2M+H]^+$, 371 $[M+K]^+$, 355 $[M+Na]^+$, 333.1924 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1913), 153 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*R*,2*S*,4*S*)-2-Hydroxy-1,8-cineole β-D-Glucopyranoside (2) Colorless needles (MeOH), mp 94—95 °C, $[\alpha]_D^{21}$ −7.2° (*c*=1.0, MeOH). Positive FAB-MS *m/z*: 665 $[2M+H]^+$, 355 $[M+Na]^+$, 333.1909 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1913), 153 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*S*,2*S*,4*R*)-2-Hydroxy-1,8-cineole β-D-Glucopyranoside (3) Colorless needles (MeOH), mp 83—84 °C, $[\alpha]_D^{23}$ +5.5° (*c*=1.6, MeOH), [lit.[6]; $[\alpha]_D$ +7.2° (*c*=3.1, MeOH)]. Positive FAB-MS *m/z*: 665 $[2M+H]^+$, 355 $[M+Na]^+$, 333.1890 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1913), 153 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*R*,2*R*,4*S*)-2-Hydroxy-1,8-cineole β-D-Glucopyranoside (4) Colorless needles (MeOH), mp 85—86 °C, $[\alpha]_D^{23}$ −66.3° (*c*=1.0, MeOH), [lit.[6]; $[\alpha]_D$ −66° (*c*=1.3, MeOH)]. Positive FAB-MS *m/z*: 665 $[2M+H]^+$, 355 $[M+Na]^+$, 333.1937 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1913), 153 $[M-C_6H_{12}O_6+H]^+$ (base).

4-Hydroxy-1,8-cineole β-D-Glucopyranoside (5) An amorphous powder, $[\alpha]_D^{21}$ −18.0° (*c*=1.3, MeOH). Positive FAB-MS *m/z*: 333.1904 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1913), 153 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*S*,2*S*,4*S*)-2,4-Dihydroxy-1,8-cineole 2-*O*-β-D-Glucopyranoside (11) An amorphous powder, $[\alpha]_D^{23}$ +5.0° (*c*=0.5, MeOH). Positive FAB-MS *m/z*: 441 $[M+H+glycerol]^+$, 349.1852 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_8$; 349.1862), 169 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*R*,2*S*,4*S*,6*R*)-2,6-Dihydroxy-1,8-cineole 2-*O*-β-D-Glucopyranoside (12) An amorphous powder, $[\alpha]_D^{23}$ −23.5° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 697 $[2M+H]^+$, 371 $[M+Na]^+$, 349.1853 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_8$; 349.1862), 331 $[M-H_2O+H]^+$, 187 $[M-C_6H_{10}O_5+H]^+$ (base).

(1*S*,2*S*,4*S*,5*R*)-2,5-Dihydroxy-1,8-cineole 2-*O*-β-D-Glucopyranoside (13) An amorphous powder, $[\alpha]_D^{23}$ −24.7° (*c*=0.3, MeOH). Positive FAB-MS *m/z*: 441 $[M+H+glycerol]^+$, 371 $[M+Na]^+$, 349.1856 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_8$; 349.1862), 169 $[M-C_6H_{12}O_6+H]^+$ (base).

(1*S*,2*S*,4*S*)-2,7-Dihydroxy-1,8-cineole 2-*O*-β-D-Glucopyranoside (14) An amorphous powder, $[\alpha]_D^{23}$ −18.0° (*c*=0.1, MeOH). Positive FAB-MS *m/z*: 697 $[2M+H]^+$, 371 $[M+Na]^+$, 349.1871 $[M+H]^+$ (Calcd for $C_{16}H_{29}O_8$; 349.1862), 331 $[M-H_2O+H]^+$, 169 $[M-C_6H_{12}O_6+H]^+$ (base).

Acid Hydrolysis of 1—5, 11 and 13 Each compound, **1**—**5**, **11** and **13** (5 mg) was dissolved in aq. 2 N H_2SO_4 and heated on a water bath for 3 h, respectively. The hydrolysate was the neutralized with $NaHCO_3$ and the salt filtered off, and the filtrate was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)]. The sugar fraction was subjected to HPLC analysis [column, carbohydrate analysis (3.9×300 mm); detector, JASCO RI-930 detector and JASCO OR-990 chiral detector; solv., CH_3CN–H_2O (17 : 3), 2 ml/min. A peak of t_R 4.53 min (same retention time as that of D-glucose)] which showed the presence of D-glucose.

(1*S*,2*R*,4*S*)-2,4-Dihydroxy-1,8-cineole (1a) An amorphous powder, $[\alpha]_D^{23}$ −30.5° (*c*=0.2, MeOH), [lit.[3a]; $[\alpha]_D^{26}$ −31.2° (*c*=0.2, MeOH)] Positive FAB-MS *m/z*: 187.1352 $[M+H]^+$ (base, Calcd for $C_{10}H_{19}O_3$: 187.1335), 169 $[M-H_2O+H]^+$, 151 $[M-2H_2O+H]^+$.

(1*R*,2*R*,4*R*)-2,4-Dihydroxy-1,8-cineole (2a) Colorless needles (MeOH), mp 158—159 °C, $[\alpha]_D^{23}$ −20.0° (*c*=0.3, MeOH), [lit.[3a]; $[\alpha]_D^{26}$ −23.0° (*c*=0.2, MeOH)]. Positive FAB-MS *m/z*: 187.1338 $[M+H]^+$ (Calcd for $C_{10}H_{19}O_3$: 187.1335), 169 $[M-H_2O+H]^+$ (base), 151 $[M-2H_2O+H]^+$.

2,6-Diexohydroxy-1,8-cineole (3a) Colorless needles (MeOH), mp 164—165 °C. Positive FAB-MS *m/z*: 209 $[M+Na]^+$, 187.1346 $[M+H]^+$ (base, Calcd for $C_{10}H_{19}O_3$: 187.1335), 169 $[M+H_2O+H]^+$, 151 $[M-2H_2O+H]^+$.

(1*S*,2*R*,4*S*,5*R*)-2,5-Dihydroxy-1,8-cineole (4a) Colorless needles (MeOH), mp 151—153 °C, $[\alpha]_D^{23}$ −51.2° (*c*=0.7, MeOH), [lit.[3a]; $[\alpha]_D^{26}$ −62.2° (*c*=0.5, MeOH)]. Positive FAB-MS *m/z*: 187.1319 $[M+H]^+$ (base, Calcd for $C_{10}H_{19}O_3$; 187.1335), 169 $[M-H_2O+H]^+$, 151 $[M-2H_2O+H]^+$.

(1*S*,2*S*,4*S*,5*R*)-2,5-Dihydroxy-1,8-cineole (5a) An amorphous powder, $[\alpha]_D^{23}$ −13.0° (*c*=0.3, MeOH). Positive FAB-MS *m/z*: 373 $[2M+H]^+$, 187.1337 $[M+H]^+$ (base, Calcd for $C_{10}H_{19}O_3$; 187.1335), 169 $[M-$

$H_2O+H]^+$, 151 $[M-2H_2O+H]^+$.

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