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Monoterpenoids and their glycosides from the leaf of thyme

Junichi Kitajima *, Toru Ishikawa, Atushi Urabe, Mitsuru Satoh

Showa Pharmaceutical University, Higashi-Tamagawagakuen 3, Machida, Tokyo 194-8543, Japan

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Abstract

From the polar portion of the methanol extract of thyme (leaf of *Thymus vulgaris*; Labiatae), which has been used as an important stomachic, carminative, a component of prepared cough tea, and a spice, seven monoterpenoid glycosides were isolated together with two known monoterpenoids and three known monoterpenoid glucosides. Structures of the seven monoterpenoid glycosides were determined by spectral analysis.

Keywords: Thyme; Thymus vulgaris; Labiatae; Monoterpenoid; *p*-Menthane; Monoterpenoid glucoside; Thymuside A

1. Introduction

Thyme [*Thymus vulgaris*; Labiatae] is indigenous to central and southern Europe, and is now widely cultivated as a tea, spice, and herbal medicine. Its leaf is listed in the German and British Herbal Pharmacopoeia, and has been used as a stomachic, carminative, diuretic, urinary disinfectant, and vermifuge. Thyme leaf tea is said to promote rest and sleep (Wichtl, 1994). As the constituents of this leaf, an essential oil (1.0–2.5%) containing mainly the isomeric monoterpenoid thymol (30–70%) and carvacrol (3–15%), and other monoterpenoids such as methylcarvacrol, *p*-cymene, α-phellandrene, limonene, α-terpineol, terpinen-4-ol, 1,8-cineole, borneol, bornyl acetate, etc., are known (Reddy et al., 1998; Rustaiyan et al., 2000). However, no terpenoid glycosides have been reported from the polar portion of this leaf. In continuation of our studies on the polar constituents of spices and herbal medicines (Kitajima et al., 2003), we undertook an investigation of thyme leaves, and this paper discuss the isolation and characterization of compounds **1**–**12**, of which six were new.

2. Results and discussion

The commercial thyme leaf tissue was extracted with methanol, and the methanolic extract was suspended in water and successively extracted with ether and ethyl acetate. The aqueous layer was applied to an Amberlite XAD-II column to give water and methanol eluate fractions, respectively. The ethyl acetate extract was subjected to Sephadex LH-20 chromatography, and then to a combination of silica gel, Lobar RP-8 column chromatography and HPLC to isolate four monoterpenoid glycosides (**2, 3, 4** and **10**). The methanol eluate fraction was applied to a Sephadex LH-20 column, and then subjected to a combination of silica gel, Lobar RP-8 column chromatography and HPLC to isolate two monoterpenoids (**1** and **9**), and eight monoterpenoid glycosides (**2, 5**–**8**, and **10**–**12**). Among them, **2**–**6** and **11** are new, and **10** is a newly isolated compound. All new glucosides described in this paper are β-D-glucopyranosides as shown by their ^{1}H and ^{13}C NMR spectral data (Tables

* Corresponding author. Tel.: +81 427 21 1577; fax: +81 427 21 1588.
E-mail address: kitajima@ac.shoyaku.ac.jp (J. Kitajima).

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1–3), and this was confirmed by hydrolysis to yield D-glucose and comparison of the $[M]_D$ values with those of their aglycones (Klyne, 1950). Their molecular formulae were suggested from the accurate mass number of the $[M + H]^+$, $[M + Na]^+$ or $[M + K]^+$ ion peak in the high-resolution positive FAB-MS.

Monoterpenoid **1** ($C_{10}H_{18}O_2$, an amorphous powder, $[\alpha]_D^{24} = -22^\circ$) was suggested to be *p*-menth-4(5)-ene-1,2-

Table 1
^{1}H NMR chemical shifts of **1-6** and **6a** (in pyridine-d_5, 500 MHz)[a]

	1	**2**	**3**	**4**
H-2ax	4.21 *dd* (5.5, 9.0)	4.32 *dd* (5.0, 10.0)	4.32 *dd* (5.0, 10.0)	4.13 *dd* (5.0, 9.0)
H-3ax	2.31 *dd* (9.0, 17.0)	2.25 *dd* (10.0, 17.0)	2.25 *dd* (10.0, 17.0)	2.19 *dd* (9.0, 17.0)
eq	2.69 *dd* (5.5, 17.0)	2.58 *dd* (5.0, 17.0)	2.62 *dd* (5.0, 17.0)	2.64 *dd* (5.0, 17.0)
H-5	5.38 *dd* (2.5, 4.5)	5.23 *dd* (2.5, 5.0)	5.32 *dd* (2.5, 5.0)	5.29 *dd* (2.5, 4.0)
H-6ax	2.52 *br d* (17.5)	2.74 *br d* (17.5)	2.70 *br d* (17.0)	2.44 *br d* (17.0)
eq	2.48 *dd* (4.5, 17.5)	2.35 *dd* (5.0, 17.5)	2.42 *dd* (5.0, 17.0)	2.39 *dd* (4.0, 17.0)
H_3-7	1.54 *s*	1.54 *s*	1.52 *s*	1.57 *s*
H-8	2.19 *septet* (7.0)	2.13 *septet* (6.5)	2.16 *septet* (7.0)	2.16 *septet* (7.0)
H_3-9	0.99 *d* (7.0)	0.95 *d* (6.5)	0.97 *d* (7.0)	0.98 *d* (7.0)
H_3-10	0.99 *d* (7.0)	0.95 *d* (6.5)	0.97 *d* (7.0)	0.98 *d* (7.0)
Glc H-1		5.19 *d* (8.0)	5.15 *d* (7.5)	5.24 *d* (8.0)
Glc H-2		4.06 *dd* (8.0, 8.0)	4.03 *dd* (7.5, 8.0)	5.54 *dd* (8.0, 8.0)
Glc H-6a		4.36 *dd* (6.0, 12.0)	4.75 *dd* (6.5, 11.0)	4.28 *dd* (6.0, 11.5)
b		4.56 *dd* (2.5, 12.0)	4.95 *dd* (2.0, 11.0)	4.55 *dd* (2.0, 11.5)
OAc		2.02 *s*	2.13 *s*	

	5		**6**	**6a**
H-2ax	4.93 *dd* (7.5, 10.0)	H-2ax	4.83 *br s*	4.59 *br s*
H-3ax	2.06 *m*	H-3	5.60 *br s*	5.77 *br s*
eq	2.06 *m*	H-5ax	2.03 *m*	2.10 *ddd* (3.0, 8.0, 17.0)
H-5	5.16 *br d* (5.0)	eq	2.03 *m*	2.23 *ddd* (5.0, 5.5, 17.0)
H-6ax	2.45 *br d* (16.5)	H-6ax	2.02 *m*	1.98 *ddd* (5.5, 8.0, 13.0)
eq	2.21 *dd* (5.0, 16.5)	eq	2.02 *m*	2.05 *ddd* (3.0, 5.0, 13.0)
H_3-7	1.64 *s*	H_3-7	1.58 *s*	1.62 *s*
H-8	2.08 *septet* (6.5)	H-8	2.11 *septet* (7.0)	2.18 *septet* (7.0)
H_3-9	0.87 *d* (6.5)	H_3-9	0.93 *d* (7.0)	0.99 *d* (7.0)
H_3-10	0.85 *d* (6.5)	H_3-10	0.93 *d* (7.0)	0.99 *d* (7.0)
sug H-1	5.32 *s*	Glc H-1	5.29 *d* (7.5)	–
sug H-3	4.27 *d* (9.5)			
sug H-4	4.46 *dd* (9.5, 9.5)			
sug H-5	3.89 *ddd* (2.5, 6.0, 9.5)			
sug H-6a	4.42 *dd* (6.0, 11.5)			
b	4.60 *dd* (2.5, 11.5)			

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

Table 2
^{1}H NMR chemical shifts of **10** and **11** (in pyridine-d_5, 500 MHz)[a]

	10	**11**
H-2exo	4.41*ddd* (2.0, 3.0, 9.0)	4.63 *ddd* (2.0, 3.0, 9.0)
H-3endo	1.48 *br dd* (3.0, 13.0)	1.63 *br dd* (3.0, 13.0)
exo	2.25 *ddd* (4.5, 9.0, 13.0)	2.52 *ddd* (4.5, 9.0, 13.0)
H-4	1.58 *br dd* (4.5, 4.5)	2.20 *br dd* (4.5, 4.5)
H-5endo	1.29 *br ddd* (4.0, 9.0, 13.0)	1.40 *br ddd* (4.5, 9.0, 13.0)
exo	1.65 *dddd* (4.5, 9.0, 13.0, 13.0)	1.74 *dddd* (4.5, 9.0, 13.0, 13.0)
H-6endo	2.40 *ddd* (4.5, 9.0, 13.0)	2.49 *ddd* (4.5, 9.0, 13.0)
exo	1.20 *dddd* (2.0, 4.0, 13.0, 13.0)	1.31 *dddd* (2.0, 4.5, 13.0, 13.0)
H_3-8	0.79 *s*	1.21 *s*
H_3-9	0.77 *s*	–
H_2-9	–	3.66 *d* (11.0)
	–	3.92 *d* (11.0)
H_3-10	1.01 *s*	1.17 *s*
Glc H-1	4.88 *d* (7.5)	4.90 *d* (8.0)

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

Table 3
^{13}C NMR chemical shifts of **1**–**6**, **6a**, **10** and **11** (in pyridine-d_5, 125 MHz)[a]

	1	**2** (δ **2**–**1**)	**3** (δ **3**–**2**)	**4** (δ **4**–**2**)	**5**	**6** (δ **6**–**6a**)	**6a**	**10**	**11**
C-1	71.61	80.02 (+8.4)	80.14	79.66	73.18	79.96 (+8.1)	71.83	49.44	54.47
C-2	73.86	69.68 (−4.2)	69.82	71.91	70.68	72.95 (−1.8)	74.78	83.79	83.97
C-3	34.33	33.71	33.61	33.36	30.46	123.64	123.55	36.42	36.33
C-4	141.58	141.44	141.51	141.30	142.22	143.45	144.25	45.25	42.27
C-5	117.35	117.30	117.17	116.06	117.16	25.68	25.45	28.54	28.67
C-6	39.99	36.83 (−3.2)	36.71	36.59	39.77	33.23 (−1.6)	34.87	27.14	27.99
C-7	21.50	18.62 (−2.9)	18.49	18.60	20.42	17.02 (−5.6)	22.65	48.23	49.96
C-8	34.84	34.65	34.67	34.75	34.92	34.28	34.60	19.32	15.11
C-9	21.38[b]	21.23[b]	21.23[b]	21.28	21.05	21.35[b]	21.54[b]	18.97	64.38
C-10	21.66[b]	21.66[b]	21.63[b]	21.28	21.62	21.53[b]	21.65[b]	13.96	14.80
Glc C-1		97.06	97.15	96.11 (−1.0)		98.12		103.70	103.71
Glc C-2		75.52	75.31	75.46 (−0.1)		75.56		75.32	75.30
Glc C-3		78.84	78.57	76.42 (−2.4)		78.85		78.68	78.66
Glc C-4		71.71	71.61	71.91		72.13		71.79	71.74
Glc C-5		78.61	75.08 (−3.5)	78.47		78.42		78.37	78.31
Glc C-6		62.73	64.81 (+2.1)	62.47		62.96		62.91	62.84
OAc			20.75	21.32					
			170.81	169.93					
Sug C-1					97.85				
Sug C-2					94.08				
Sug C-3					80.31				
Sug C-4					69.74				
Sug C-5					78.51				
Sug C-6					63.13				

[a] δ in ppm TMS.
[b] Assignments may be interchanged in each column.

diol from its ^{1}H, ^{13}C NMR spectral data (Tables 1 and 3), and the results of heteronuclear multiple bond connectivity (HMBC) spectral data (see Section 3). The observed NOE interactions between H-2/H-6$_{ax}$, and between H$_3$-7/H-3$_{ax}$ in its nuclear Overhauser and exchange spectroscopy (NOESY) suggested that the configuration of C-1 methyl was axial, and C-1, C-2 hydroxyls were equatorial (Fig. 1).

Consequently, **1** was found to be *p*-menth-4(5)-ene-1β,2α-diol, which was reported as a constituent of the

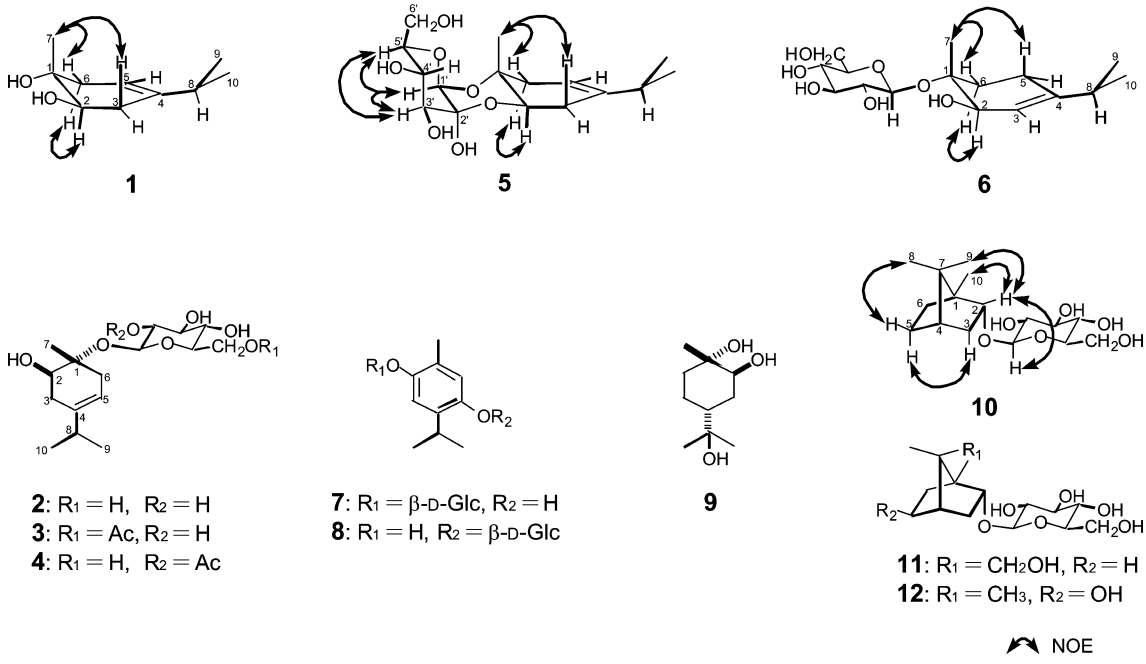

Fig. 1. Structures of **1**–**12**, and NOE interactions observed in the NOESY spectra of **1**, **5**, **6** and **10**.

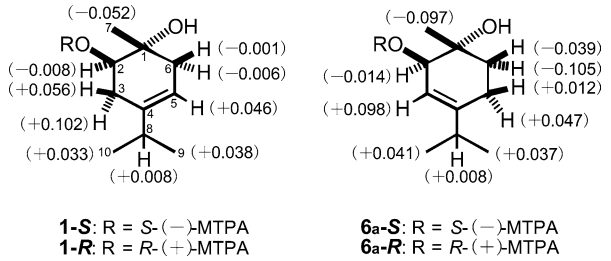

Fig. 2. $\Delta\delta$ Values in Hz [$=\delta S$-(−) − δR-(+)] of **1**-MTPA and **6a**-MTPA ester.

steam distilled oil of *Ferula jaeschkeana* rhizome (Garg and Agarwal, 1988). Since the absolute structure of **1** was not previously defined, its absolute configuration at C-2 was investigated by means of the modified Mosher's method (Ohtani et al., 1991; Kitajima et al., 2002). Treatment of **1** with *S*-(−)- and *R*-(+)-2-methoxy-2-trifluoromethyl acetic acid (MTPA) chloride gave the 1-*O*-ester of *S*-(−)- MTPA (**1-S**) and *R*-(+)-MTPA (**1-R**), respectively. Signals due to protons on C-3, C-8, C-9 and C-10 of **1-S** appeared at lower field than those of **1-R**, while the proton signals due to C-6 and C-7 of **1-S** were observed at higher field than those of **1-R** (Fig. 2). Therefore, the absolute configuration at C-2 of **1** is assigned as *R*, and the absolute structure of **1** was described as shown in Fig. 1 [(1*R*,2*R*)-*p*-menth-4(5)-ene-1,2-diol].

Glucoside **2** ($C_{16}H_{28}O_7$, m.p. 137–139 °C, $[\alpha]_D^{24} = -42°$) showed $[M + Na]^+$ and $[M + H]^+$ ion peaks at *m/z* 355 and 333, and an $[M - C_6H_{12}O_6 + H]^+$ ion peak at *m/z* 153 in the positive FAB-MS. Enzymatic hydrolysis of **2** with β-glucosidase gave **1** and D-glucose, and **2** was suggested to be a monoglucoside of **1**. The position of the glucosyl unit was found to be C-1 by the observed cross-peak between Glc H-1/C-1 in the HMBC spectrum. Therefore, **2** was characterized as (1*R*,2*R*)-*p*-menth-4(5)-ene-1,2-diol 1-*O*-β-D-glucopyranoside. The glycosylation shift values of the α- and the β-pro-*S*-side carbons and the chemical shift of glucosyl C-1 are shown in Table 3.

Glucoside **3** ($C_{18}H_{30}O_8$, an amorphous powder, $[\alpha]_D^{24} = -38°$) showed $[M + Na]^+$, $[M + H]^+$ and $[M - C_8H_{14}O_7 + H]^+$ ion peaks at *m/z* 397, 375 and 153 in the positive FAB-MS. It displayed similar NMR spectral features to those of **2** except for the presence of an acetoxyl group (Tables 1 and 3), and the IR spectrum of **2** showed an absorption due to an ester carbonyl functionaly (1720 cm^{-1}). Glycoside **3** was deacylated to **2** by heating in a water bath with 5% NH_4OH–MeOH for 2 h. From comparison of the NMR spectral data with those of **2**, **3** was the monoacetate of **2**, and the position of the acetoxyl group was revealed to be C-6 of the glucose from the analysis of the HMBC spectrum, which showed a cross-peak between the glucosyl H_2-6 and the carbonyl carbon of the acetoxyl group. So, **3** was determined to be (1*R*,2*R*)-*p*-menth-4(5)-ene-1,2-diol 1-*O*-β-D-(6-*O*-acetyl)-glucopyranoside.

Glucoside **4** ($C_{18}H_{30}O_8$, an amorphous powder, $[\alpha]_D^{22} = -32°$) showed $[M + Na]^+$ and $[M + H]^+$ ion peaks at *m/z* 397 and 375, and an $[M - C_8H_{14}O_7 + H]^+$ ion peak at *m/z* 153 in the positive FAB-MS. Since **4** was deacetylated to **2** by alkaline hydrolysis, it was also a monoacetate of **2**. The position of the acetoxyl group was revealed to be C-2 of the glucose moiety by the downfield shift of H-2 (by 1.48 ppm) and the upfield shift of C-1 (by 1.0 ppm) and C-3 (by 2.4 ppm) signals of the glucosyl moiety (Yamazaki et al., 1977; Konishi et al., 1978), and the HMBC correlation between the glucosyl H-2 and the carbonyl carbon of the acetoxyl group. Then, **4** was characterized as (1*R*,2*R*)-*p*-menth-4(5)-ene-1,2-diol 1-*O*-β-D-(2-*O*-acetyl)-glucopyranoside.

Thymuside A (**5**, $C_{16}H_{26}O_7$, an amorphous powder, $[\alpha]_D^{22} = +2°$) showed $[M + Na]^+$, $[M - H_2O - H]^+$ and $[M - C_6H_{12}O_7 + H]^+$ ion peaks at *m/z* 353, 313 and 135 in the positive FAB-MS, and $[M - H]^-$ and $[M - H_2O + H]^-$ ion peaks at *m/z* 329 and 311 in the negative FAB-MS. The ^1H, ^{13}C and ^{13}C–^1H COSY NMR spectral data of **5** (Tables 1 and 3) showed the presence of a monoterpenoid which was characterized to be *p*-menth-4(5)-ene-1,2-diol, and a sugar moiety which corresponded to hydroxyhexose. From comparison of the NMR spectral data with those of **1** and **2**, and analysis of its HMBC spectral data (see Section 3), **5** was concluded to be composed of *p*-menth-4(5)-ene-1,2-diol and 2-hydroxyhexose, which were linked with two ether linkages. As the HMBC correlations between H′-1 of the sugar moiety and C-1 of the monoterpenoid, and between H-2 of the monoterpenoid and C′-2 of the sugar moiety were observed, the monoterpenoid and 2-hydroxyhexose should be linked between C-1 and C′-1, and between C-2 and C′-2, respectively. The NOE interaction between H3-7/H-3_{ax}, and between H-2/H-6_{ax} confirmed that the configuration of C-1 and C-2 hydroxyl was equatorial, the same as that of **1**, and the NOE interactions between H′-1/H′-3, H′-5 of the sugar moiety in its NOESY spectrum suggested that the configuration of H′-1, H′-3 and H′-5 was axial, the same as that of methyl β-D-glucopyranoside. Furthermore, the downfield shift of the H-2_{ax} (by 0.60 ppm) signal to that in **2** suggested that the H-2_{ax} and the free hydroxyl group of C′-2 were in 1,3-diaxial relationship. Therefore, **5** was concluded to be as shown in Fig. 1.

Glucoside **6** ($C_{16}H_{28}O_7$, an amorphous powder, $[\alpha]_D^{22} = -23°$) showed $[M + K]^+$, $[M + Na]^+$ and $[M - C_6H_{12}O_6 + H]^+$ ion peaks at *m/z* 371, 355 and 153 in the positive FAB-MS. Enzymatic hydrolysis of **6** with β-glucosidase gave an aglycone (**6a**; $C_{10}H_{18}O_2$, an amorphous powder, $[\alpha]_D^{22} = -38°$) and D-glucose. It showed similar ^1H and ^{13}C NMR spectral data (Tables 1 and 3) to those of **2**, except for the chemical shifts

and coupling constants of the H-2_{ax} and methylene protons. From the results of HMBC spectral data (see Section 3), the structure of the aglycone moiety was concluded to be *p*-menth-3-ene-1,2-diol, and the position of the glucosyl unit was C-1. As the NOE interactions between H-2/H-6_{ax}, and between H_3-7/H-6_{eq} were observed in its NOESY spectrum in CD_3OD, the configuration of C-1 methyl and C-2 hydroxyl was suggested to be axial and equatorial, respectively (Fig. 1). The absolute structure of **6a** was defined by the same way described for **1**. Treatment of **6a** with *S*-(−)- and *R*-(+)-MTPA chloride gave the 1-*O*-ester of *S*-(−)-MTPA (**6a-S**) and *R*-(+)-MTPA (**6a-R**), respectively, and the signals due to protons on C-3, C-9 and C-10 of **6a-S** appeared at lower field than those of **6a-R**, while the proton signals due to C-6 and C-7 of **6a-S** were observed at higher field than those of **6a-R** (Fig. 2). Therefore, the absolute configuration at C-2 of **6a** is assigned as *R*, and the absolute structure of **6a** was characterized as (1*R*,2*R*)-*p*-menth-3-ene-1,2-diol. Therefore, **6** was characterized as (1*R*,2*R*)-*p*-menth-3-ene-1,2-diol 1-*O*-β-D-glucopyranoside.

Glucosides **7** ($C_{16}H_{24}O_7$, m.p. 178–180 °C, $[\alpha]_D^{25} = -45°$), and **8** ($C_{16}H_{24}O_7$, an amorphous powder, $[\alpha]_D^{25} = -39°$) were identified as 6-hydroxythymol 6-*O*-β-D-glucopyranoside and 6-hydroxythymol 3-*O*-β-D-glucopyranoside, respectively, which were isolated from the fruit of ajowan (Ishikawa et al., 2001b). Monoterpenoid **9** ($C_{10}H_{20}O_3$, m.p. 134–135 °C, $[\alpha]_D^{23} = -31°$) was identified as (1*S*,2*S*,4*R*)-*p*-menthane-1,2,8-triol which was isolated from the fruit of *Glehenia littoralis* (Ishikawa et al., 2001a).

Glucoside **10** ($C_{16}H_{28}O_6$, m.p. 126–130 °C, $[\alpha]_D^{24} = -53°$) showed $[M + Na]^+$, $[M + H]^+$ and $[M - C_6H_{12}O_6 + H]^+$ ion peaks at *m/z* 339, 317 and 137 in the positive FAB-MS. Its ^1H and ^{13}C NMR spectral data (Tables 2 and 3) revealed the presence of three *tert*-methyls, three methylenes, one methine, two quaternary carbons, and one hydroxylated methine, in addition to the β-D-glucopyranosyl moiety. From analysis of the HMBC spectrum (see Section 3), the aglycone of **10** was clarified as borneol and the location of the glucosyl group was C-2. The observed NOE interactions between H-2/H_3-9 suggested the configuration of C-2 hydroxyl was endo (Fig. 1). Enzymatic hydrolysis of **10** gave an aglycone **10a** ($C_{10}H_{18}O_2$, m.p. 205–209 °C, $[\alpha]_D^{24} = -33°$) and D-glucose, and the absolute configuration at C-2 of **10a** was indicated to be *R* by the empirical rule of ^{13}C NMR glycosylation shift (Kasai et al., 1977; Tori et al., 1977), [C-1 (β-pro-*R*), Δδ −0.5; C-2 (α-carbon), Δδ +7.7; C-4′ (β-pro-*S*), Δδ −3.1, glucosyl C-1, δ 103.70]. Further, glucoside **10** was identified as (1*S*,2*R*,4*S*)-borneol β-D-glucopyranoside, which was reported as a biotransformation product from a cell suspension culture of *Eucalyptus perriniana* following administration of (−)-borneol, by comparison of its physical and NMR spectral data with those reported (Orihara and Furuya, 1993).

Glucoside **11** ($C_{16}H_{28}O_7$, an amorphous powder, $[\alpha]_D^{23} = -48°$) showed $[M + K]^+$, $[M + Na]^+$, $[M + H]^+$ and $[M - C_6H_{12}O_6 + H]^+$ ion peaks at *m/z* 371, 355, 333 and 153 in the positive FAB-MS. It showed similar NMR spectral features with those of **10** except the absence of one *tert*-methyl and the presence of a hydroxylated methylene group (Tables 2 and 3). Enzymatic hydrolysis of **11** gave an aglycone **11a** ($C_{10}H_{18}O_2$, an amorphous powder, $[\alpha]_D^{23} = -16°$) and D-glucose, and the NMR spectra of **11a** were identical with those of vicodiol first isolated from *Vicoa indica* (Vasanth et al., 1990). The position of the glucosyl unit was confirmed to be C-2 by the HMBC analysis (see Section 3). As the vicodiol, which has the 1*S*, 2*R*, 4*S*, 7*R* configuration, showed a minus $[\alpha]_D$ value (−16°) the same as **11a**, **11** was concluded to have the 1*S*, 2*R*, 4*S*, 7*R* configuration. It was also supported by comparison of the ^{13}C NMR glycosylation shift values [Δδ(δ glucoside − δ aglycone)] of the α- and β-carbon and the chemical shift of Glc C-1 [C-1 (β-pro-*R*), Δδ +0.2; C-2 (α-carbon), Δδ +7.7; C-4′ (β-pro-*S*), Δδ-3.2, glucusyl C-1, δ 103.71] with those of **10**. So, **11** was characterized as (1*S*, 2*R*, 4*S*, 7*R*)-vicodiol 2-*O*-β-D-glucopyranoside.

Glucoside **12** ($C_{16}H_{28}O_7$, an amorphous powder, $[\alpha]_D^{22} = -32°$) was identified as (1*S*, 2*R*, 4*S*, 5*R*)-angelicoidenol 2-*O*-β-D-glucopyranoside which was isolated from the root and rhizoma of *Glehenia littoralis* (Kitajima et al., 1998).

The ingredient relationship between the essential oil and the polar portion was confirmed by the isolation of these glycosides which showed the biosynthetic relation to thymol, carvacrol, α-terpineol and borneol. It is worthy of note that, a novel glycoside thymuside A, which is linked a monoterpenoid and a hydroxyhexose with two ether linkages, was isolated from the leaf of thyme.

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. IR spectra were obtained with a JASCO A-103 IR spectrophotometer. FAB-MS were recorded with a JEOL HX-110 spectrometer using glycerol as matrix. ^1H and ^{13}C NMR spectra were taken on JEOL A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. 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), respectively. TLC was performed on silica gel (Merck 5721) and spots were detected with *p*-anisaldehyde–H_2SO_4 reagent. HPLC separation was carried out with Symmetryprep C_{18} 7 μm [Waters; column size, 7.8 × 300 mm; ODS] and carbohydrate analysis [Waters; column size, 3.9 × 300 mm; CHA] columns.

3.2. Extraction and separation

Commercial thyme leaves (from *Thymus vulgaris* L.; purchased from Asaoka Spices Ltd., Lot. No. 00011801; 2.0 kg) were extracted with MeOH (5 L × 2) for two weeks at room temperature, and the extract (419.1 g) was partitioned into Et_2O–H_2O and EtOAc–H_2O extracts, respectively. Removal of the solvents from each phase gave Et_2O (140.3 g), EtOAc (20.9 g) and aqueous (257.9 g) fractions.

The EtOAc fraction was subjected to Sephadex LH-20 (MeOH) chromatography to give seven fractions (frs. A–G). Fraction C (2.48 g) was applied to a silica gel column [$CHCl_3$–MeOH–H_2O (17:3:0.2) → MeOH] to give nine fractions (frs. C_1–C_9). Fraction C_4 (0.40 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:2)] to give eight fractions (frs. C_{4-1}–C_{4-8}). Fraction C_{4-2} was subjected to silica gel CC [$CHCl_3$–MeOH (19:1)] to give four fractions (frs. C_{4-2-1}–C_{4-2-4}), and fr. C_{4-2-1} was applied to HPLC [CHA, MeCN–H_2O (49:1)] to give **3** (40 mg) and **4** (11 mg). Fraction C_6 (0.30 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:2)] to give 23 fractions (frs. C_{6-1}–C_{6-23}), and fr. C_{6-20} was subjected to HPLC [ODS, MeOH–H_2O (3:2) to give **10** (120 mg). Fraction C_7 (0.11 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:7)] to give **2** (75 mg).

The aqueous fraction was applied to an Amberlite XAD-II column (H_2O → MeOH) to give a water (106.6 g) and MeOH eluate (143.4 g) fractions. The MeOH eluate fraction was subjected to Sephadex LH-20 (MeOH) chromatography to give five fractions (frs. H–L). Fraction I (124.31 g) was applied to silica gel CC [$CHCl_3$–MeOH–H_2O (17:3:0.2 → 4:1:0.1 → 7:3:0.5) → MeOH] to give 13 fractions (frs. I_1–I_{13}). Fraction I_3 (6.74 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17 → 3:7)] to give 25 fractions (frs. I_{3-1}–I_{3-25}), and fr. I_{3-4} was subjected to HPLC [ODS, MeOH–H_2O (3:37)] to give **9** (9 mg). Fraction I_{3-20} was applied to HPLC [CHA, MeCN–H_2O (19:1)] to give **5** (20 mg), and fr.I_{3-22} was subjected to HPLC [ODS, MeOH–H_2O (3:2)] to give **1** (9 mg) and **10** (105 mg). Fraction I_4 (5.51 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17 → 3:7)] to give 21 fractions (frs. I_{4-1}–I_{4-21}), and fr. I_{4-6} was subjected to HPLC [ODS, MeCN–H_2O (3:17)] to give **8** (2 mg) and **7** (123 mg). Fraction I_5 (4.30 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17 → 3:7)] to give 20 fractions (frs. I_{5-1}–I_{5-20}). Fraction I_{5-12} was subjected to HPLC [ODS, MeCN–H_2O (1:1)] to give **6** (20 mg), and fr. I_{5-15} was further purified by HPLC [CHA, MeCN–H_2O (19:1)] to give **2** (440 mg). Fraction I_6 (5.79 g) was passed through a Lobar RP-8 column [MeCN–H_2O (3:17)] to give 15 fractions (frs. I_{6-1}–I_{6-15}), and fr. I_{6-5} was subjected to HPLC [CHA, MeCN–H_2O (19:1)] to give **11** (117 mg) and **12** (7 mg).

The following compounds were identified by comparison with authentic compounds: 6-hydroxythymol 6-*O*-β-D-glucopyranoside (**7**), 6-hydroxythymol 3-*O*-β-D-glucopyranoside (**8**), (1*S*,2*S*,4*R*)-*p*-menthane-1,2,8-triol (**9**) and (1*S*,2*R*,4*S*,5*R*)-angelicoidenol 2-*O*-β-D-glucopyranoside (**12**).

3.3. (1R,2R)-p-menth-4(5)-ene-1,2-diol (1)

Amorphous powder, $[\alpha]_D^{24} = -22°$ ($c = 0.6$, MeOH). Positive FAB-MS *m/z*: 209.0935 $[M + K]^+$ (Calcd for $C_{10}H_{18}KO_2$; 209.0944), 153 $[M - H_2O + H]^+$ (base), 135 $[M - 2H_2O + H]^+$ (base). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 3. HMBC correlations: H-2ax/C-1, C-3, C-4, C-6, C-7; H-3ax/C-1, C-2, C-4, C-5, C-8; H-3eq/C-1, C-2, C-4, C-5, C-8; H-5/C-1, C-3, C-4, C-6, C-8; H-6ax/C-1, C-2, C-4, C-5, C-7; H-6eq/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9. NOE correlations: H-2ax/H-6ax; H-3ax/H_3-7, H_3-10; H-3eq/H_3-10; H-5/H-8, H_3-9; H-6eq/H_3-7.

3.4. 2-O-MTPA ester of 1

A solution of **1** (2 mg) in a mixture of $CHCl_3$ and pyridine (each 0.5 ml) was treated with *R*-(−)-MTPA-chloride (5 drops) and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with EtOAc, and the EtOAc extract was subjected to silica gel column chromatography (hexane–EtOAc = 4:1) to give 1-*O*-*S*-(−)-MTPA ester of **1-S** (**1a**-*S*-(−)-MTPA) In a similar procedure, **1-R** (**1a**-*R*-(+)-MTPA) was obtained with *S*-(+)-MTPA-chloride.

3.5. (1R,2R)-p-menth-4(5)-ene-1,2-diol 1-O-β-D-glucopyranoside (2)

Colorless needles (MeOH), m.p. 137–139 °C, $[\alpha]_D^{24} = -42°$ ($c = 2.0$, MeOH). Positive FAB-MS *m/z*: 355 $[M + Na]^+$, 333.1918 $[M + H]^+$ (Calcd for $C_{16}H_{29}O_7$; 333.1914), 315 $[M - H_2O + H]^+$, 153 $[M - C_6H_{12}O_6 + H]^+$ (base). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 3. HMBC correlations: H-2ax/C-1, C-3, C-4, C-6, C-7; H-3ax/ C-1, C-2, C-4, C-5, C-8; H-3eq/C-1, C-2, C-4, C-5, C-8; H-5/C-1, C-3, C-4, C-6, C-8; H-6ax/C-1, C-2, C-4, C-5, C-7; H-6eq/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6;

H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Glc H-1/C-1.

3.6. Enzymatic hydrolysis of **2**

A mixture of **2** (100 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 15 d. The mixture was concentrated in vacuo to dryness and the residue was subjected to silica gel CC [$CHCl_3$–MeOH (9:1 → 1:1)] to afford **1** (45 mg) and a sugar fraction. The sugar fraction was passed through Sephadex LH-20 (MeOH) to give a syrup, and HPLC [carbohydrate analysis (Waters), detector; JASCO RI-930 detector and JASCO OR-990 chiral detector, solv.; MeCN–H_2O (17:3), 2 ml/min; *t R* 4.50 min (same location as that of D-glucose)] established the presence of D-glucose.

3.7. (1R,2R)-p-menth-4(5)-ene-1,2-diol 1-O-β-D-(6-O-acetyl) glucopyranoside (**3**)

Amorphous powder, $[\alpha]_D^{24} = -38°$ ($c = 0.4$, MeOH). IRν_{max} nujol cm^{-1}: 3350 (OH), 1720 (acetoxyl). Positive FAB-MS *m/z*: 397 $[M + Na]^+$, 375.2029 $[M + H]^+$ (Calcd for $C_{18}H_{31}O_8$; 375.2019), 153 $[M - C_8H_{14}O_7 + H]^+$ (base). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 3. HMBC correlations: H-2ax/C-1, C-6, C-7; H-3ax/C-1, C-2, C-4, C-5, C-8; H-3eq/C-1, C-2, C-4, C-5, C-8; H-5/C-1, C-3, C-4, C-6, C-8; H-6ax/C-1, C-2, C-4, C-5, C-7; H-6eq/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2; H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Glc H-1/C-1; Glc H-6a/O*C*OCH3; Glc H-6b/O*C*OCH_3; OCO*C*H_3/O*C*OCH_3.

3.8. Alkaline hydrolysis of **3**

Glucoside **3** (5 mg) was deacetylated by heating in a water bath with 5% NH_4OH–MeOH for 6 hr. Glucoside **2** (3 mg) was obtained after Sephadex LH-20 (MeOH) column chromatography.

3.9. (1R,2R)-p-Menth-4(5)-ene-1,2-diol 1-O-β-D-(2-O-acetyl) glucopyranoside (**4**)

Amorphous powder, $[\alpha]_D^{22} = -32°$ ($c = 0.4$, MeOH). IRν_{max} cm^{-1}: 3350 (OH), 1720 (acetoxyl). Positive FAB-MS *m/z*: 397 $[M + Na]^+$, 375.2002 $[M + H]^+$ (Calcd for $C_{18}H_{31}O_8$; 375.2019), 153 $[M - C_8H_{14}O_7 + H]^+$ (base). For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 3. HMBC correlations: H-2ax/C-1, C-3, C-4, C-6, C-7; H-3ax/C-2, C-4, C-5, C-8; H-3eq/C-1, C-2, C-4, C-5; H-5/C-1, C-3, C-6, C-8; H-6ax/C-1, C-2, C-4, C-5, C-7; H-6eq/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Glc H-1/C-1; Glc H-2/O*C*OCH_3; OCO*C*H_3/O*C*OCH_3.

3.10. Alkaline hydrolysis of **4**

Glucoside **4** (2 mg) was deacetylated in the same way as described for **3** to afford **2** (1 mg).

3.11. Thymuside A (**5**)

Amorphous powder, $[\alpha]_D^{22} = +2°$ ($c = 0.5$, MeOH). Positive FAB-MS *m/z*: 353.1565 $[M + Na]^+$ (Calcd for $C_{16}H_{26}NaO_7$; 353.1577), 313 $[M - H_2O + H]^+$ (base), 135 $[M - C_6H_{12}O_6 + H]^+$. Negative FABMS *m/z*: 329 $[M - H]^+$ (base), 311 $[M - H_2O - H]^+$, 293 $[M - 2H_2OH]^+$. For ^1H NMR and ^{13}C NMR spectral data, see Tables 1 and 3. HMBC correlations: H-2ax/C-1, C-3, C-6, C-7, Sug C-2; H-3ax/C-1, C-2, C-4, C-5; H-3eq/C-1, C-2, C-4, C-5; H-5/C-1, C-3, C-6, C-8; H-6ax/C-1, C-4, C-5; H-6eq/C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Sug H-1/C-1, Sug C-2, Sug C-5; Sug H-3/Sug C-2, Sug C-4, Sug C-5; Sug H-4/Sug C-3, Sug C-5, Sug C-6; Sug H-5/Sug C-1, Sug C-3, Sug C-4, Sug C-6; Sug H_2-6/Sug C-4, Sug C-5. NOE correlations: H-2ax/H-6ax; H-3ax/H_3-7, H_3-10; H-3eq/H_3-10; H-5/H-8, H_3-9; H-6eq/H_3-7; Sug H-1/Sug H-3, Sug H-5; Sug H-3/Sug H-5.

3.12. (1R,2R)-p-menth-3-ene-1,2-diol 2-O-β-D-glucopyranoside (**6**)

Amorphous powder, $[\alpha]_D^{22} = -23°$ ($c = 1.7$, MeOH). Positive FAB-MS *m/z*: 371.1484 $[M + K]^+$ (Calcd for $C_{16}H_{28}KO_7$; 371.1472), 355.1746 $[M + Na]^+$ (Calcd for $C_{16}H_{28}NaO_7$; 355.1733), 153 $[M - C_6H_{12}O_6 + H]^+$ (base). For ^1H NMR (in pyridine-d_5), see Table 1; (in CD_3OD): 1.02 (6H, *s*, H_3-9 and H_3-10), 1.19 (3H, *s*, H_3-7), 1.73 (1H, *ddd*, $J = 5.0$, 10.5, 13.0 Hz, H-6ax), 1.88 (1H, *ddd*, $J = 3.0$, 5.0, 13.0 Hz, H-6eq), 2.09 (1H, *ddd*, $J = 3.0$, 10.5, 17.0 Hz, H-5ax), 2.11 (1H, *ddd*, $J = 5.0$, 5.0, 17.0 Hz, H-5eq), 2.21 (1H, *septet*. $J = 7.0$, H-8), 4.28 (1H, *br s*, H-2ax), 5.22 (1H, *br s*, H-3), 4.58 (1H, *d*, $J = 7.5$ Hz, Glc H-1). For ^{13}C NMR (in pyridine-d_5), see Table 3; (in CD_3OD): 80.85 (C-1), 74.18 (C-2), 122.56 (C-3), 145.92 (C-4), 25.98 (C-5), 33.73 (C-6), 16.62 (C-7), 35.35 (C-8), 21.70, 21.87 (C-9, C-10), 98.03 (Glc C-1), 75.29 (Glc C-2), 78.15 (Glc C-3), 71.94 (Glc C-4), 77.71 (Glc C-5), 62.85 (Glc C-6). HMBC correlations: H-2ax/ C-1, C-3, C-4, C-7; H-3/ C-1, C-5, C-8; H-5ax/C-1, C-3, C-4, C-6; H-5eq/C-1, C-3, C-4, C-6; H-6ax/C-1, C-2, C-4, C-5, C-7; H-6eq/ C-1, C-2, C-4, C-5, C-7; H_3-7/C-1, C-2, C-6; H-8/C-3, C-4, C-5, C-9, C-10; H_3-9/C-4, C-8, C-10; H_3-10/C-4, C-8, C-9; Glc H-1/C-1. NOE correlations:

H-2ax/H-6ax; H-3/H-8, H_3-10; H-5ax/H_3-7, H_3-9; H-5eq/H_3-9; H-6eq/H_3-7; H_3-7/Glc H-1.

3.13. Enzymatic hydrolysis of **6**

A mixture of **6** (10 mg) and β-glucosidase (3 mg) in water (5 ml) was shaken in a water bath at 37 °C for 7 d. The mixture was applied to a silica gel column [$CHCl_3$–MeOH (9:1 → 1:1)] to afford **6a** (5 mg) and a sugar fraction. From the sugar fraction, D-glucose was detected as described for **2**.

3.14. (1R,2R)-p-menth-3-ene-1,2-diol (**6a**)

Amorphous powder, $[\alpha]_D^{22} = -38°$ ($c = 0.3$, MeOH). For ^{1}H NMR and ^{13}C NMR spectral data, see Tables 1 and 3.

3.15. 2-O-MTPA ester of **6a**

A solution of **6a** (2 mg) in a mixture of $CHCl_3$ and pyridine (each 0.5 ml) was treated with *R*-(−)-MTPA-chloride (5 drops) and the mixture was stirred for 2 h at room temperature. The reaction mixture was treated as described for **1** to give 1-*O*-*S*-(−)-MTPA ester of **6a-S** (**6a**-*S*-(−)-MTPA). In the similar procedure, **6a-R** (**6a**-*R*-(+)-MTPA) was obtained with*S*-(+)-MTPA-chloride.

3.16. (1S,2R,4S)-borneol β-D-glucopyranoside (**10**)

Colorless needles (MeOH), m.p. 126–130 °C, $[\alpha]_D^{24} = -53°$ ($c = 3.2$, MeOH). Positive FAB-MS *m/z*: 339 $[M + Na]^+$, 317.1978 $[M + H]^+$ (Calcd for $C_{16}H_{29}O_6$; 317.1964), 137 $[M - C_6H_{12}O_6 + H]^+$ (base). For ^{1}H NMR and ^{13}C NMR spectral data, see Tables 2 and 3. HMBC correlations: H-2exo/C-1, C-3, C-4, C-6, C-7, C-10; H-3endo/C-1, C-2, C-4, C-5, C-7; H-3exo/C-1, C-2, C-4, C-5, C-7; H-4/C-1, C-2, C-3, C-5, C-6, C-7, C-8, C-9; H-5endo/C-1, C-3, C-4, C-6, C-7; H-5exo/C-1, C-3, C-4, C-6, C-7; H-6endo/C-1, C-2, C-4, C-5, C-7, C-8, C-10; H-6exo/C-1, C-2, C-4, C-5, C-7, C-10; H_3-8/C-1, C-4, C-7, C-9; H_3-9/C-1, C-4, C-7, C-8; H_3-10/C-1, C-2, C-6, C-7; Glc H-1/C-2.NOESY correlations: H-2exo/H_3-9, H_3-10, Glc H-1; H-3endo/H-5endo; H-3exo/H_3-9; H-5exo/H_3-8; H-6endo/H_3-8.

3.17. Enzymatic hydrolysis of **10**

A mixture of **10** (20 mg) and naringinase (10 mg, ICN Biomedicals Inc., Lot 2421c) in water (5 ml) was placed in a glass test tube closed with a glass plug, and shaken in a water bath at 37 °C for 14 d. The precipitated prisms were filtered out and washed with water to give **10a** [(1*S*,2*R*,4*S*)-borneol, colorless prisms (H_2O), m.p. 205–209 °C, $[\alpha]_D^{23} = -33°$ ($c = 0.3$, MeOH); 8 mg]. The filtrate was subjected to silica gel CC [$CHCl_3$–MeOH (6:4)] to give a sugar fraction which was detected as D-glucose as described for **2**.

3.18. (1S,2R,4S,7R)-vicodiol 2-O-β-D-glucopyranoside (**11**)

Amorphous powder, $[\alpha]_D^{23} = -48°$ ($c = 1.7$, MeOH). Positive FAB-MS *m/z*: 371.1485 $[M + K]^+$ (Calcd for $C_{16}H_{28}KO_7$; 371.1472), 355.1724 $[M + Na]^+$ (Calcd for $C_{16}H_{28}NaO_7$; 355.1733), 333 $[M + H]^+$, 315$[M - H_2O + H]^+$, 153 $[M - C_6H_{12}O_6 + H]^+$ (base). For ^{1}H NMR and^{13}C NMR spectral data, see Tables 2 and 3. HMBC correlations: H-2exo/C-4, C-6, C-7, C-10; H-3endo/C-1, C-2, C-4, C-5; H-3exo/C-1, C-2, C-4, C-5, C-7; H-4/C-1, C-2, C-5, C-6, C-7, C-8, C-9; H-5endo/C-1, C-3, C-4, C-6, C-7; H-5exo/C-3, C-4, C-6; H-6endo/C-1, C-2, C-4, C-5, C-7; H-6exo/C-2, C-5, C-7, C-10; H_3-8/C-1, C-4, C-7, C-9; H_2-9/C-1, C-4, C-7, C-8; H_3-10/C-1, C-2, C-6, C-7; Glc H-1/C-2. NOESY correlations: H-2exo/H-9a, H-9b, H_3-10, Glc H-1; H-3endo/H-5endo; H-3exo/H_2-9; H-5exo/H_3-8; H-6endo/H_3-8.

3.19. Enzymatic hydrolysis of **11**

A mixture of **11** (10 mg) and β-glucosidase (5 mg) in water (5 ml) was shaken in a water bath at 37 °C for 30 d. The mixture was applied to a silica gel [$CHCl_3$–MeOH (9:1 → 1:1)] column to afford **11a** [(1*S*,2*R*,4*S*,5*R*)-vicodiol, amorphous powder, $[\alpha]_D^{23} = -16°$ ($c = 0.3$, MeOH), $[\alpha]_D^{23} = -19°$ ($c = 0.3$, $CHCl_3$); 3 mg] and a sugar fraction. From the sugar fraction, D-glucose was detected as described for **2**.

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References

Garg, S.N., Agarwal, S.K., 1988. New monoterpene diols from essential oil of *Ferula jaeschkeana*. Phytochemistry 27, 936–937.

Ishikawa, T., Sega, Y., Kitajima, J., 2001a. Water-soluble constituents of *Glehnia littoralis* fruit. Chem. Pharm. Bull. 49, 583–584.

Ishikawa, T., Sega, Y., Kitajima, J., 2001b. Water-soluble constituents of ajowan. Chem. Pharm. Bull. 49, 840–844.

Kasai, R., Suzuo, M., Asakawa, J., Tanaka, O., 1977. Carbon-13 chemical shifts of isoprenoid-β-D-glucopyranosides and β-D-mannopyranosides. Stereochemical influences of aglycone alcohols. Tetrahedron Lett., 175–178.

Kitajima, J., Okamura, C., Ishikawa, T., Tanaka, Y., 1998. Monoterpenoid glycosides of *Glehnia littoralis* root and rhizoma. Chem. Pharm. Bull. 46, 1595–1598.

Kitajima, J., Suzuki, N., Satoh, M., Watanabe, M., 2002. Sesquiterpenoids of *Torilis japonica* fruit. Phytochemistry 59, 811–815.

Kitajima, J., Ishikawa, T., Satoh, M., 2003. Polar constituents of celery seed. Phytochemistry 64, 1003–1011.

Klyne, W., 1950. The configuration of the anomeric carbon atoms in some cardiac glycoside. Biochem. J. 47, XIi–XIii.

Konishi, T., Tada, A., Shoji, J., Kasai, R., Tanaka, O., 1978. The structures of platycodin A and C, monoacetylated saponins of the roots of *Platycodon grandiflorum* A. DC Chem. Pharm. Bull. 26, 668–670.

Ohtani, I., Kusumi, T., Kashman, Y., Kakisawa, H., 1991. High-Field FT-NMR application of Mosher's method. Absolute configurations of marine terpenoids. J. Am. Chem. Soc. 113, 4092–4096.

Orihara, Y., Furuya, T., 1993. Biotransformation of (−)-borneol by cultured cells of *Eucalyptus perriniana*. Phytochemistry 34, 1045–1048.

Rustaiyan, A., Masoudi, S., Monfared, A., Kamalinejad, M., Lajevardi, T., Sedaghat, S., Yari, M., 2000. Volatile constituents of three *Thymus* species grown wild in Iran. Planta Med. 66, 197–198.

Reddy, M.V.B., Angers, P., Gosselin, A., Arul, J., 1998. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry 47, 1515–1520.

Tori, K., Seo, S., Yoshimura, Y., Atita, H., Tomita, Y., 1977. Glycosylation shifts from aglycone and glucose to glucoside. Tetrahedron Lett., 179–182.

Vasanth, S., Kundu, A.B., Purushothaman, K.K., Patra, A., Pattabhi, V., Connolly, J.D., 1990. Isolation and characterization of vicodiol, a new monoterpenediol from *Vicoa indica*. J. Nat. Prod. 53, 354–358.

Wichtl, M. (Ed.), 1994. Herbal Drugs and Phytopharmaceuticals. CRC Press, Stuttgart, pp. 493–495.

Yamazaki, K., Kasai, R., Masaki, Y., Okihara, M., Tanaka, O., Ohshiro, H., Takagi, S., Yamaki, M., Masuda, K., Nonaka, G., Tsuboi, M., Nishioka, I., 1977. Application of C-13 NMR to the structural elucidation of acylated plant glycosides. Tetrahedron Lett., 1231–1234.