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New Monoterpene Glucoside from the Aerial Parts of Thyme (Thymus vulgaris L.)

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Note

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A new monoterpene glucoside (1) was isolated from a methanol extract of the dried aerial parts of thyme (*Thymus vulgaris* L.), together with known 2- and 5-β-D-glucopyranosylthymoquinols (2 and 3, respectively), and (−)-angelicoidenol-β-D-glucopyranoside (4). The structure of 1 was elucidated to be (*R*)-*p*-cymen-9-yl β-D-glucopyranoside by spectral evidence and enantioselective synthesis from (*R*)- and (*S*)-*p*-cymen-9-ol derived from *p*-cymen-8-ol.

Key words: *Thymus vulgaris* L.; thyme; monoterpene glucoside; (*R*)-*p*-cymen-9-yl β-D-glucopyranoside; enantioselective synthesis

Thyme (*Thymus vulgaris* L.) is a perennial herbaceous plant native to Southern Europe. It is one of the spices widely used as both a food additive and a folk medicine to treat various illnesses in Europe.[1)] Five new biphenyl compounds have previously been isolated from an acetone extract of the leaves of thyme by Nakatani and our colleague.[2,3)] These biphenyls showed more effective deodorizing activity against methyl mercaptan than sodium copper chlorophylline, which is commonly used as an oral deodorizer.[2–4)] The antioxidative activity of flavonoids isolated from the acetone extract of the plant has also been reported by Miura and Nakatani.[5)] In the course of our studies on polar deodorizing compounds and new constituents in the plant, we have previously isolated three known flavonoid glycosides, hesperidin, eriocitrin (= eriodictyol-7-rutinoside) and narirutin, and two phenolic compounds, rosmarinic acid and arbutin, from the *n*-BuOH soluble-fraction of a methanolic extract of the dried aerial parts of thyme.[6,7)] In addition to these five compounds, a new monoterpene glucoside (**1**), together with three known monoterpene glucosides (**2**, **3** and **4**) were obtained in the present study from the same extract. We report here the isolation of these four monoterpene glucosides from the methanol extract of thyme, and the structural elucidation of **1** which was based spectral evidence and enantioselective synthesis.

The dried aerial parts of thyme (5 kg) cultivated in Spain were soaked in MeOH for one week at room temperature. The resulting methanol extract was concentrated, and the residue was sequentially extracted with *n*-hexane, $CHCl_3$, and *n*-BuOH. According to the guidance of coloration on TLC with coloring reagents such as sulfuric acid, thymol-sulfuric acid and ferric chloride, the *n*-BuOH extract (50.8 g) was fractionated by silica gel column chromatography [BW-820H (Fujisilisia Chemical)] eluting with $CHCl_3$ and an increasing ratio of MeOH. The eluates were combined into fifteen fractions on the basis of the TLC pattern. Four known compounds, hesperidin, eriocitrin, narirutin

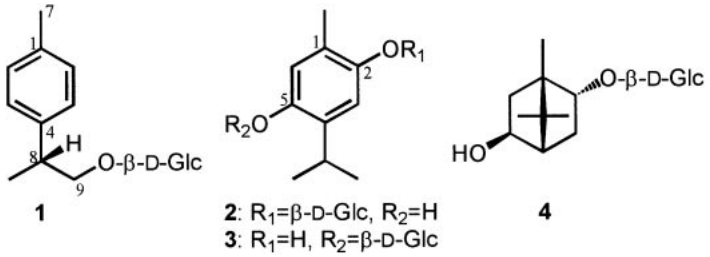

Fig. 1. Structures of the Monoterpene Glucosides from Thyme.

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and arbutin, were isolated from fractions 10–12 as reported previously.[6] After combining fractions 6–9 (10.7 g), rosmarinic acid was removed from the combined fraction by silica gel column chromatography (Kieselgel 60) eluting with EtOAc. The remaining components in the column were then chromatographed by eluting with a mixed solvent of EtOAc–MeOH–H_2O (20:1:0.1, v/v/v). Some of the fractions, including the positive spots by TLC with thymol-sulfuric acid, were subjected to preparative HPLC in a Chemcosorb 5-ODS-H (4.6ϕ × 150 mm) column to afford four monoterpene glucosides, **1** (5.1 mg), **2** [47.5 mg, amorphous powder; ^{1}H-NMR δ_H (CD_3OD, 500 MHz): 1.17, 1.18 (each 3H, d, J = 7.0 Hz), 2.18 (3H, s), 3.20 (1H, m), 3.70 (1H, dd, J = 5.2, 11.9 Hz), 3.87 (1H, dd, J = 1.8, 11.9 Hz), 4.66 (1H, d, J = 7.6 Hz), 6.52 (1H, s), 6.98 (1H, s); ^{13}C-NMR δ_C (CD_3OD): 127.0, 150.3, 116.0, 134.0, 150.4, 117.7, 16.2, 28.0, 23.2, 23.2, 104.3, 75.1, 78.2, 71.5, 78.0, 62.6], **3** [23.1 mg, amorphous powder; ^{1}H-NMR δ_H (CD_3OD, 500 MHz): 1.14, 1.15 (each 3H, d, J = 7.0 Hz), 2.12 (3H, s), 3.47 (1H, m), 3.70 (1H, dd, J = 5.2, 11.9 Hz), 3.88 (1H, dd, J = 2.1, 11.9 Hz), 4.69 (1H, d, J = 7.9 Hz), 6.61 (1H, s), 6.91 (1H, s); ^{13}C-NMR δ_C (CD_3OD): 123.2, 151.8, 113.0, 138.2, 149.2, 120.3, 16.1, 27.0, 23.6, 23.7, 104.4, 75.2, 78.3, 71.6, 78.0, 62.9] and **4** [2.1 mg, amorphous powder; $[\alpha]_D^{20}$ −30° (c 0.02, MeOH); ^{1}H-NMR δ_H (CD_3OD, 500 MHz): 0.85, 0.92, 1.08 (each 3H, s), 1.01 (1H, dd, J = 3.1, 13.4 Hz), 1.31 (1H, br d, J = 13.4 Hz), 1.70 (1H, d, J = 5.2 Hz), 2.18 (1H, ddd, J = 5.2, 9.2, 13.4 Hz), 2.49 (1H, dd, J = 7.9, 13.4 Hz), 3.15–3.33 (4H, m), 3.66 (1H, dd, J = 5.5, 11.6 Hz), 3.85 (1H, dd, J = 2.4, 11.6 Hz), 3.85 (1H, dd, J = 2.7, 7.6 Hz), 4.05 (1H, ddd, J = 1.8, 2.8, 9.2 Hz), 4.22 (1H, d, J = 7.9 Hz); ^{13}C-NMR δ_C (CD_3OD): 51.0, 82.9, 34.3, 53.6, 75.9, 39.7, 48.7, 21.3, 20.4, 13.4, 102.9, 75.1, 78.2, 71.7, 77.9, 62.8]. Their retention times by HPLC were 5.2, 6.8 and 8.4 min for **1**–**3** [eluent: H_2O:MeOH = 70:30 (v/v), flow rate: 1.0 ml/min, UV 280 nm], and 6.8 min for **4** [eluent: H_2O:MeOH = 65:35 (v/v), flow rate: 1.0 ml/min, RI detector], respectively. Compounds **2**, **3** and **4** were respectively identified as 2- and 5-β-D-glucopyranosylthymoquinol, and (−)-angelicoidenol-β-D-glucopyranoside on the basis of a comparison of their spectral data with those shown in the literature.[8,9]

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{20}$ −30° (c 0.05, $CHCl_3$). The molecular formula of **1** was established to be $C_{16}H_{24}O_6$ from HR-CIMS (reaction gas: isobutane); m/z 313 $[M + H]^+$: Calcd. for $C_{16}H_{25}O_6$: 313.1651, Found: 313.1645 (see also the NMR data in Table 1). The UV and IR spectra of **1** revealed the presence of an aromatic ring and hydroxyl groups [UV λ_{max} (MeOH) nm (ε): 258 (270), 264 (350), and 272 (320); IR ν_{max} (KBr) cm^{-1}: 3400, 2920, 1520, 1160, 1080, and 1040]. The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra revealed the presence of one 1,4-disubstituted benzene ring [δ_C 128.2 (d, 2 × C), 129.9 (d, 2 × C), 136.7 (s), and 142.4 (s); δ_H 7.08 (2H, d, J = 8.2 Hz), 7.13 (2H, d, J = 8.2 Hz)], one methine group [δ_C 40.6 (d); δ_H 3.02 (1H, m)], one oxygenated methylene group [δ_C 76.2 (t); δ_H 3.64 (1H, dd, J = 6.0, 9.8 Hz), 3.90 (1H, dd, J = 8.2, 9.8 Hz)] and two methyls [δ_C 21.1 (q), 19.0 (q); δ_H 2.28 (3H, s), 1.27 (3H, d, J = 6.7 Hz)]. The ^{13}C-NMR spectrum also showed the presence of a β-glucopyranosyl moiety (δ_C 104.2, 77.9, 77.8, 74.9, 71.5, and 62.7) by a comparison of the chemical shifts with those of known monoterpene glucosides.[10] The coupling constant (J = 7.9 Hz) between the anomeric proton and H′-2 supported the compound to be a β-glucopyranoside. In the ^{1}H–^{1}H COSY spectrum, correlations among the protons of a methine (δ_H 3.02), oxygenated methylene (δ_H 3.64 and 3.90) and methyl (δ_H 1.27) showed the presence of a –$CH(CH_3)CH_2O$– moiety. The deshielded methyl group (δ_H 2.28) would have been directly attached to the 1,4-di-substituted benzene ring. These spectral data were used to infer that the aglycone moiety of **1** was *p*-cymen-9-ol. The connectivity of these partial structures was confirmed by HMBC data (H-2/C-7, H-3/C-1 and C-8, H-7/C-2, H-9/C-4, C-10, and C-1′, H-1′/C-9). Compound **1** was hydrolyzable by β-glucosidase (emulsin) to give *p*-cymen-9-ol as an aglycone. Consequently, the structure of **1** was elucidated to be *p*-cymen-9-yl β-D-glucopyranoside.

In order to determine the stereochemistry at C-8 of **1**, both (*R*)-*p*-cymen-9-ol [(*R*)-**5**] and (*S*)-*p*-cymen-9-ol [(*S*)-**5**] were synthesized from *p*-cymen-8-ol according to the method of Matsumoto *et al.*[11,12] *p*-Cymen-8-ol was dehydrated with aqueous H_2SO_4 and then hydrated with BH_3–THF and H_2O_2–NaOH to afford racemic **5**. After acetylating racemic **5** with Ac_2O–pyridine, the racemic *p*-cymen-9-yl acetate obtained (5.0 g) was dissolved in methanol (140 ml) and hydrolyzed with lipase (5.0 g, Type-II, Sigma) in 0.1 M phosphate buffer (pH 6.86, 460 ml) at 35 °C for 2.5 h. The reaction mixture was extracted with Et_2O, and the resulting organic layer was evaporated under reduced pressure. The Et_2O extract was subjected to silica gel column chromatography (BW-820H), successively eluting with *n*-hexane-EtOAc (90:10 and then 70:30, v/v). The 30% EtOAc eluate was further purified by silica gel column chromatography (BW-820H), eluting with *n*-hexane–EtOAc (75:25, v/v), to afford (*S*)-**5** (1.80 g, 73% yield), $[\alpha]_D^{20}$ −14.8° (c 9.01, $CHCl_3$), R/S = 5:95. The 10% EtOAc eluate was recrystallized from MeOH to afford (*R*)-*p*-cymen-9-yl acetate (2.14 g, 68% yield), $[\alpha]_D^{20}$ +8.0° (c 10.4, $CHCl_3$). (*R*)-*p*-cymen-9-yl acetate (2.08 g) was hydrolyzed with 5% aqueous sodium hydroxide (12 ml). The reaction mixture was extracted with $CHCl_3$ and then worked up in the usual manner. The $CHCl_3$ extract was applied to silica gel column chromatography (BW-820H), eluting with *n*-hexane–EtOAc (70:30, v/v) to give (*R*)-**5** (1.47 g, 91% yield), $[\alpha]_D^{20}$ +15.7° (c 9.00, $CHCl_3$), R/S = 98:2.

Next, alcohols (*R*)-**5** and (*S*)-**5** were separately glucosidized with Ag_2CO_3 as a catalyst by the modified

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1**, (*S*)-**1**, (*R*)-**5**, (*R*)-**6**, and (*S*)-**6**

Position	**1** [= (*R*)-**1**]*		(*S*)-**1***		(*R*)-**5****	(*R*)-**6****		(*S*)-**6****	
	^{13}C-NMR δ_C	^{1}H-NMR δ_H (integral, mult., *J* Hz)	^{13}C-NMR δ_C	^{1}H-NMR δ_H (integral, mult., *J* Hz)	^{13}C-NMR δ_C	^{13}C-NMR δ_C	^{1}H-NMR δ_H (integral, mult., *J* Hz)	^{13}C-NMR δ_C	^{1}H-NMR δ_H (integral, mult., *J* Hz)
1	136.7	—	136.8	—	136.0	135.7	—	136.0	—
2,6	128.2	7.08 (2H, d, 8.2)	128.3	7.07 (2H, d, 8.2)	127.2	127.1	7.08 (2H, s)	127.2	7.10 (2H, s)
3,5	129.9	7.13 (2H, d, 8.2)	129.9	7.13 (2H, d, 8.2)	129.2	128.9	7.08 (2H, s)	129.1	7.10 (2H, s)
4	142.4	—	142.5	—	140.4	140.7	—	140.3	—
7	21.1	2.28 (3H, s)	21.0	2.28 (3H, s)	21.1	21.0	2.31 (3H, s)	21.0	2.31 (3H, s)
8	40.6	3.02 (1H, m)	41.0	3.02 (1H, m)	42.0	39.3	2.96 (1H, m)	39.1	3.01 (1H, m)
9	76.2	3.64 (1H, dd, 6.0, 9.8)	76.7	3.52 (1H, dd, 8.6, 9.8)	68.7	75.5	3.51 (1H, dd, 7.7, 9.5)	75.7	3.42 (1H, t, 8.9)
		3.90 (1H, dd, 8.2, 9.8)		4.02 (1H, dd, 6.0, 9.8)			3.94 (1H, dd, 6.6, 9.5)		4.02 (1H, dd, 5.2, 9.2)
10	19.0	1.27 (3H, d, 6.7)	19.0	1.28 (3H, d, 7.0)	17.7	18.2	1.22 (3H, d, 7.0)	17.9	1.26 (3H, d, 7.0)
Glc-1	104.2	4.24 (1H, d, 7.9)	104.8	4.27 (1H, d, 7.9)		100.8	4.41 (1H, d, 7.9)	101.0	4.49 (1H, d, 7.9)
2	74.9	3.15 (1H, dd, 7.6, 9.2)	75.1	3.17 (1H, dd, 7.6, 9.2)		71.0	4.95 (1H, dd, 7.9, 9.5)	71.2	5.03 (1H, dd, 7.9, 9.5)
3	77.9	3.3 (1H, m)	78.1	3.3 (1H, m)		72.7	5.14 (1H, t, 9.5)	72.8	5.15 (1H, dd, 9.5, 9.5)
4	71.5	3.3 (1H, m)	71.6	3.3 (1H, m)		68.4	5.06 (1H, t, 9.5)	68.4	5.06 (1H, dd, 9.5, 9.8)
5	77.8	3.26 (1H, m)	77.9	3.25 (1H, m)		71.7	3.64 (1H, ddd, 2.4, 4.6, 9.5)	71.8	3.66 (1H, ddd, 2.4, 4.6, 9.8)
6	62.7	3.66 (1H, dd, 5.2, 11.9)	62.7	3.65 (1H, dd, 5.2, 11.9)		61.9	4.12 (1H, dd, 2.4, 12.3)	61.9	4.11 (1H, dd, 2.4, 12.5)
		3.86 (1H, dd, 1.8, 11.9)		3.85 (1H, dd, 1.5, 11.9)			4.25 (1H, dd, 4.6, 12.3)		4.25 (1H, dd, 4.6, 12.5)
CO						170.5		170.7	
						170.1		170.3	
						169.2		169.4	
						169.0		169.3	
CH_3						20.77	2.08 (3H, s)	20.74	2.08 (3H, s)
						20.63	2.01 (3H, s)	20.61	2.02 (3H, s)
						20.62	1.98 (3H, s)	20.58	2.01 (3H, s)
						20.40	1.81 (3H, s)	20.55	1.98 (3H, s)

The spectra were measured at 270 MHz for ^{1}H and at 67.5 MHz for ^{13}C, with tetramethylsilane used as an internal standard. Coupling constants in Hz are in parentheses.
*Taken in CD_3OD. **Taken in $CDCl_3$.

Königs–Knorr method.[10,13] An Ag_2CO_3/Celite (4.68 g, 43% Ag_2CO_3) catalyst was added to a solution of (*R*)-**5** (1.42 g) and acetobromo-α-D-glucose (4.64 g) in Et_2O (30 ml). The solution was refluxed for 6 h in the dark, before the reaction mixture was cooled to room temperature. After removing the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography (BW-820H) with *n*-hexane–EtOAc (70:30, v/v) and then crystallized from *n*-hexane–EtOAc to give (*R*)-*p*-cymen-9-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (*R*)-**6** (1.54 g, 34% yield), mp 103–104 °C, $[\alpha]_D^{20}$ −21.6° (*c* 5.51, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 2970, 1750, 1520, 1370, 1260 and 1220. ^{1}H- and ^{13}C-NMR data: see Table 1. In the same manner, (*S*)-**5** (1.57 g) was converted to (*S*)-**6** (2.51 g, 50% yield), mp 104–105 °C, $[\alpha]_D^{20}$ −13.3° (*c* 2.12, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 2960, 1740, 1520, 1370, and 1220. The HPLC retention times of (*R*)-**6** and (*S*)-**6** were 10.3 and 11.0 min, respectively [Chemcosorb 5-ODS-H (4.6φ × 150 mm); eluent, H_2O–MeOH (30:70, v/v); flow rate, 1.0 ml/min]. After recrystallization, both (*R*)-**6** and (*S*)-**6** were confirmed to be optically pure by the HPLC analysis. To a solution of (*R*)-**6** (1.24 g) in MeOH (90 ml), 10% KOH (0.3 ml) was added dropwise, and the mixture stirred for 1 h at room temperature. The reaction mixture was concentrated *in vacuo*, and the resulting residue was subjected to silica gel column chromatography (BW-820H) with a mixture of $CHCl_3$–MeOH (70:30, v/v) to give (*R*)-**1** (0.80 g) as colorless needles, mp 85–87 °C, $[\alpha]_D^{20}$ −33.8° (*c* 4.10, $CHCl_3$). The IR, ^{1}H- and ^{13}C-NMR spectral data of (*R*)-**1** were identical with those of **1**. (*S*)-**1** was obtained from (*S*)-**6** in a similar way in a quantitative yield [(*S*)-**1**, mp 119–120 °C, $[\alpha]_D^{20}$ −36.4° (*c* 1.86, $CHCl_3$); ^{1}H- and ^{13}C-NMR data: see Table 1]. The notable difference between (*R*)-**1** and (*S*)-**1** was the chemical shift of the methylene protons at C-9 in their ^{1}H-NMR spectra, although the values for their specific rotation were quite close. The chemical shift of H_2-9 in (*R*)-**1** was δ_H 3.64 and 3.90, while that in (*S*)-**1** was δ_H 3.52 and 4.02. Consequently, the stereochemistry of C-8 in **1** was determined to be (*R*)-configuration.

This study isolated a new compound, (*R*)-*p*-cymen-9-yl β-D-glucopyranoside (**1**), and three known monoterpene glucosides (**2**–**4**) from a methanolic extract of the dried aerial parts of thyme. This is the first report on the isolation of these monoterpene glucosides from thyme. These glucosides seemed to be present as aroma precursors or as protected forms of their aglycones in the plant, although their aglycones have not been detected as volatiles in thyme oil. Eriocitrin and rosmarinic acid have previously shown highly deodorizing activity against methyl mercaptan among the compounds already isolated from the methanol extract,[6,7] although the monoterpene glucosides (**1**–**4**) found here did not show any deodorizing activity.

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