A New Hydroxyjasmonone Glucoside and Its Related Compounds from the Leaf of Thyme

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Received April 5, 2004; accepted June 2, 2004

From the polar portion of the methanol extract of thyme (leaf of *Thymus vulgaris*; Labiatae), which has been used as a stomachic, carminative, a component of prepared cough tea, and as a spice, a new hydroxyjasmonone glucoside, (Z)-5'-hydroxyjasmonone 5'-O- β -D-glucopyranoside was isolated together with five related compounds and four aromatic compounds. The structures of the new compound was clarified by spectral investigation.

Key words thyme; *Thymus vulgaris*; hydroxyjasmonone; 5'-hydroxyjasmonone 5'-O- β -D-glucopyranoside; (1R,2R)-methyl-5'-hydroxyjasmonate

The leaf of thyme [*Thymus vulgaris*; Labiatae] is a most popular herbal medicine and spice used world wide. It is listed in German and British Herbal Pharmacopoeia, and has been used as a stomachic, carminative, diuretic, urinary disinfectant, and vermifuge. Thyme is indigenous to central and southern Europe, and is now widely cultivated as a tea which promotes rest and relaxation.¹⁾ Most of the studies on this leaf were concerned with the essential oil 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 and so on.^{2,3)} However, no report had been published on the polar portion of this leaf. Therefore, we undertook investigation of the polar portion of this leaf, in continuation of our studies on the polar constituents of spices and herbal medicines.⁴⁾ In this paper, we discuss the compounds related to hydroxyjasmonic acid derivative, which are interesting for their metabolic formation and defense responses in higher plants.^{5,6)}

The commercial thyme leaf was extracted with 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, respectively. 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 six compounds related to hydroxyjasmonone (**1**–**6**), three aromatic glucosides, 3-hydroxy-4-methoxyphenethyl 3-O- β -D-glucopyranoside (**7**),⁷⁾ eugenol β -D-glucopyranoside (**8**),⁸⁾ syringin (**9**),⁹⁾ and rosmarinic acid (**10**).^{10,11)} On the other hand, five glucides (glycerol, D-hamamelose, D-fructose, D-glucose and sucrose) were isolated from the water eluate fraction. Among them, **4** is a new compound.

Glucoside **1** (C₁₂H₂₂O₆, an amorphous powder, [α]_D²³ –31°) was identified as (Z)-3-hexenyl 1-O- β -D-glucopyranoside, which was isolated from the leaf of *Pertya glabrescens*.¹²⁾ Recently, aglycone of **1** found to induce the expression of defense genes in uninfected leaves.¹³⁾

Glucosides **2** (C₁₈H₂₈O₉, an amorphous powder, [α]_D²⁴ –72°) and **3** (C₁₉H₃₀O₉, an amorphous powder, [α]_D²¹ –55°) were identified as (1R,2R)-5'-hydroxyjasmonic acid 5'-O- β -D-glucopyranoside, which was isolated from the leaf of *Perilla frutescens*,¹⁴⁾ and (1R,2R)-methyl-5'-hydroxyjasmonate

5'-O- β -D-glucopyranoside, which was isolated as a potato tuber-forming substance from Jerusalem Artichoke,¹⁵⁾ respectively.

Glucoside **4** (C₁₇H₂₆O₇, an amorphous powder, [α]_D²² –24°) showed [M+Na]⁺, [M+H]⁺ and [M+H–C₆H₁₂O₆]⁺ ion peaks at *m/z* 365, 343 and 163 in their positive ion FAB-MS, and its molecular formula was suggested from the accurate mass number of the [M+H]⁺ and [M+Na]⁺ ion peak in the high-resolution positive ion FAB-MS. Acid hydrolysis of **4** gave glucose, and its ¹H-, ¹³C-NMR and ¹H–¹³C COSY spectral data (see Experimental) showed the presence of one *tert*-methyl, four methylenes, one hydroxylated methylene, one disubstituted double bond, one tetrasubstituted double bond, and one carbonyl group, in addition to the β -glucopyranosyl moiety.^{16,17)} From comparison of the NMR spectral data with those of **1** to **3**, and analysis of its HMBC spectral data (see Experimental), the aglycone of **4** was found to be 5'-hydroxyjasmonone and the location of the glucosyl group was C-5'. The glucose was suggested to be D-form by comparison of its [M]_D value (–82°) with those of **1** (–81°) and methyl β -D-glucopyranoside (–62°).¹⁸⁾ The pentenyl double bond was determined to be *Z* by its coupling constant between H-2' and H-3' (*J*=10.5 Hz). So, **4** was characterized as (Z)-5'-hydroxyjasmonone 5'-O- β -D-glucopyranoside.

Compound **5** (C₁₂H₁₈O₄, an amorphous powder, [α]_D²⁴ –67°) was identified as (1R,2R)-5'-hydroxyjasmonic acid first isolated from the culture filtrate of the tropical fungus *Botryodiplodia theobromae*,¹⁹⁾ and **6** (C₁₃H₂₀O₄, an amor-

Fig. 1. Structures of **1**–**6**

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phous powder, $[\alpha]_D^{21} -49^\circ$) was identified as (1*R*,2*R*)-methyl-5'-hydroxyjasmonate, which was derived by Kramell *et al.*²⁰⁾

The aglycone of **4** and compound **6** were reported for the first time from the plant.

Experimental

Melting points were determined on a Yanagimoto micromelting 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 ¹³C-NMR spectra were taken on a JEOL A-500 spectrometer with tetramethylsilane as an internal standard, and chemical shifts were recorded in δ value. ¹H-¹³C COSY, HMBC spectra were obtained with standard JEOL software. Column chromatography (C.C.) was carried out under TLC monitoring by using Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 μ m, 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-H₂SO₄ reagent. HPLC separation was carried out with Symmetryprep C₁₈ 7 μ m [Waters; column size, 7.8 $\times$ 300 mm; ODS] and carbohydrate analysis [Waters; column size, 3.9 $\times$ 300 mm; CHA] columns.

Extraction and Separation Commercial thyme leaves (the leaf of *Thymus vulgaris* L.; purchased from Asaoka Spices Ltd., Lot. No. 00011801; 2.0 kg) were extracted with methanol (5 $\times$ 2) for 2 weeks, and the extract (419.1 g) was partitioned between ether/water. An aqueous phase was extracted with ethyl acetate. Removal of the solvents from each phase gave ether (140.3 g), ethyl acetate (20.9 g) and aqueous (257.9 g) fractions. The aqueous fraction was chromatographed over Amberlite XAD-II (H₂O $\rightarrow$ MeOH) to give water eluate (106.6 g) and methanol eluate (143.4 g) fractions. The methanol eluate fraction was subjected to Sephadex LH-20 (MeOH) to give five fractions (frs. A–E). Fraction B (124.3 g) was chromatographed over silica gel [CHCl₃–MeOH–H₂O (17:3:0.2 $\rightarrow$ 4:1:0.1 $\rightarrow$ 7:3:0.5) $\rightarrow$ MeOH] to give 13 fractions (frs. B₁–B₁₃). Fraction B₃ (6.7 g) was passed through a Lobar RP-8 column [MeCN–H₂O (3:17 $\rightarrow$ 3:7)] to give 25 fractions (frs. B_{3,1}–B_{3,25}), and fr. B_{3,8} was subjected to HPLC [CHA, MeOH–H₂O (19:1)] to give **1** (9 mg) and **4** (23 mg). Fraction B_{3,19} was subjected to HPLC [ODS, MeCN–H₂O (1:1)] to give **6** (2 mg) and **8** (51 mg). Fraction B₄ (5.5 g) was passed through a Lobar RP-8 column [MeCN–H₂O (3:17 $\rightarrow$ 3:7)] to give 21 fractions (frs. B_{4,1}–B_{4,21}), and fr. B_{4,2} was subjected to silica gel column chromatography [CHCl₃–MeOH–H₂O (9:1:0.1)] to give **5** (34 mg). Fraction B_{4,9} was subjected to HPLC [CHA, MeCN–H₂O (49:1)] to give **3** (23 mg). Fraction B₅ (4.3 g) was passed through a Lobar RP-8 column [MeCN–H₂O (3:17 $\rightarrow$ 3:7)] to give 20 fractions (frs. B_{5,1}–B_{5,20}), and fr. B_{5,4} was subjected to HPLC [ODS, MeCN–H₂O (1:3)] to give **9** (10 mg). Fraction B₆ (5.8 g) was passed through a Lobar RP-8 column [MeCN–H₂O (3:17)] to give 15 fractions (frs. B_{6,1}–B_{6,15}), and fr. B_{6,5} was subjected to HPLC [CHA, MeCN–H₂O (19:1)] to give **7** (46 mg). Fraction B₇ (1.2 g) was passed through a Lobar RP-8 column [MeCN–H₂O (3:17 $\rightarrow$ 3:7)] to give 10 fractions (frs. B_{7,1}–B_{7,10}), and fr. B_{7,5} was subjected to silica gel column chromatography [CHCl₃–MeOH–H₂O (7:3:0.5)] to give **2** (11 mg). Fraction D (7.6 g) was chromatographed over silica gel [CHCl₃–MeOH (3:2) $\rightarrow$ MeOH] to give **10** (1.5 g). A part of the water eluate fraction (50.8 g) was subjected to Sephadex LH-20 (MeOH) to give eight fractions (frs. F–M). Fraction H (35.7 g) was chromatographed over silica gel [CHCl₃–MeOH–H₂O (4:1:0.1 $\rightarrow$ 15:5:0.4 $\rightarrow$ 7:3:0.5 $\rightarrow$ 6:4:0.5 $\rightarrow$ 1:1:0.1) $\rightarrow$ MeOH] to give ten fractions (frs. H₁–H₁₀). Fraction H₂ (7.8 g) was subjected to a Lobar RP-8 column (H₂O) to give glycerol (70 mg). Fraction H₇ (7.8 g) was subjected to a Lobar RP-8 column (H₂O) and HPLC [CHA, MeCN–H₂O (14:1)] to give D-hamamelose (97 mg), D-fructose (1.4 g) and D-glucose (1.1 g). Fraction H₈ (7.3 g) was subjected to a Lobar RP-8 to give sucrose (182 mg).

The following compounds were identified by comparison of physical and NMR data with those reported: (Z)-3-hexenyl 1-*O*- β -D-glucopyranoside (**1**), (1*R*,2*R*)-5'-hydroxyjasmonic acid 5'-*O*- β -D-glucopyranoside (**2**), (1*R*,2*R*)-methyl-5'-hydroxyjasmonate 5'-*O*- β -D-glucopyranoside (**3**), (1*R*,2*R*)-5'-hydroxyjasmonic acid (**5**), (1*R*,2*R*)-methyl-5'-hydroxyjasmonate (**6**), 3-hydroxy-4-methoxyphenethyl 3-*O*- β -D-glucopyranoside (**7**), eugenol β -D-glucopyranoside (**8**), syringin (**9**), and rosmarinic acid (**10**).

(Z)-5'-Hydroxyjasmonate 5'-*O*- β -D-Glucopyranoside (4**)** An amor-

phous powder, $[\alpha]_D^{22} -24^\circ$ ($c=0.9$, MeOH). Positive ion FAB-MS m/z : 365.1562 [M+Na]⁺ (Calcd for C₁₇H₂₆NaO₇; 365.1576), 365.1562 [M+Na]⁺ (Calcd for C₁₇H₂₆NaO₇; 365.1576), 343.1754 [M+H]⁺ (Calcd for C₁₇H₂₆O₇; 343.1757), 163 [M–C₆H₁₂O₆+H]⁺ (base). ¹H-NMR (in pyridine-*d*₅): 2.22 and 2.22 (each 2H, ddd, $J=6.0, 9.5, 19.0$ Hz, H₂-4 and H₂-5), 3.00 (2H, br d, $J=7.0$ Hz, H₂-1'), 5.48 (1H, dt, $J=10.5, 7.0$ Hz, H-2'), 5.56 (1H, dt, $J=10.5, 7.0$ Hz, H-3'), 2.66 (2H, dt, $J=7.0, 7.0$ Hz, H₂-4'), 3.78 and 4.17 (each 1H, dt, $J=9.5, 7.0$ Hz, H-5'a and H-5'b), 1.84 (3H, s, H₃-1'), 4.90 (1H, d, $J=8.0$ Hz, Glc H-1), 4.07 (1H, brt, $J=8.0$ Hz, Glc H-2), 4.29 (1H, brt, $J=8.0$ Hz, Glc H-3), 4.26 (1H, brt, $J=8.0$ Hz, Glc H-4), 3.98 (1H, m, Glc H-5), 4.40 (1H, dd, $J=12.0, 5.0$ Hz, Glc H-6a), 4.58 (1H, dd, $J=12.0, 2.0$ Hz, Glc H-6b). ¹³C-NMR (in pyridine-*d*₅): 170.33 (C-1), 138.88 (C-2), 208.15 (C-3), 34.41 (C-4), 31.57 (C-5), 21.79 (C-1'), 128.19 (C-2'), 126.81 (C-3'), 28.57 (C-4'), 69.28 (C-5'), 16.99 (C-1''), 104.71 (Glc C-1), 75.22 (Glc C-2), 78.60 (Glc C-3), 71.73 (Glc C-4), 78.54 (Glc C-5), 62.85 (Glc C-6). HMBC Correlations: H₂-4'/C-1, C-2, C-3, C-5; H₂-5'/C-1, C-2, C-3, C-4; H₂-1'/C-1, C-2, C-3, C-2', C-3'; H-2'/C-2, C-1', C-4'; H-3'/C-1', C-4', C-5'; H-5'a/C-3', C-4', Glc C-1; H-5'b/C-3', C-4', Glc C-1; H₃-1''/C-1, C-2, C-5; Glc H-1/C-5'.

Acid Hydrolysis of **4** Glucoside **4** (3 mg) was heated at 95 °C with 5% H₂SO₄ (2 ml) for 1 h. After neutralization with Amberlite IRA-400 (OH[–] form), the reaction mixture was concentrated to give 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, solv.; MeCN–H₂O (17:3), 2 ml/min; t_R 4.50 min (same location as that of authentic sample of D-glucose)] show the presence of glucose.

Acknowledgment The authors thank Mr. Takase Y., Dr. Suzuki H. and Miss Kiyotani T. of the Analytical Center of this University for NMR and MS measurements.

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