

Precursor of α -Methylene- γ -butyrolactone Involved in the Insecticidal Activity of Thunberg Spiraea, *Spiraea thunbergii*

Chul-Sa KIM,[†] Probal Kanti DATTA, Tetsuro HARA, Eiji ITOH,
and Michio HORIIKE

Department of Bioresources Science, Faculty of Agriculture, Kochi University,
B200 Monobe, Nankoku 783-8502, Japan

Received August 17, 1998; Accepted September 22, 1998

6-Tuliposide A {6-*O*-(4-hydroxy- α -methylenebutyl)-*D*-glucopyranose} was isolated from thunberg spiraea (*Spiraea thunbergii*) leaves. Acid-hydrolysis of this compound generated tulipalin A (α -methylene- γ -butyrolactone). This compound is thus considered as a precursor of insecticidal tulipalin A.

Key words: insecticidal activity; α -methylene- γ -butyrolactone (tulipalin A); thunberg spiraea (*Spiraea thunbergii*); 6-tuliposide A {6-*O*-(4-hydroxy- α -methylenebutyl)-*D*-glucopyranose}

During our studies on the host selection of *Thrips palmi* (Thysanoptera),^{1–6)} we found that thunberg spiraea (*Spiraea thunbergii*; Rosaceae) contained a potent insecticidal component against this insect, which was isolated and identified as α -methylene- γ -butyrolactone (tulipalin A) in our previous paper.⁷⁾ However, tulipalin A could not be found in the intact leaf, and was released only when the leaf was subjected to injury by solvents, crumpling, etc.⁷⁾ We report here the identification of a precursor of tulipalin A in thunberg spiraea.

A methanol extract of thunberg spiraea was dissolved in water and defatted with ethyl acetate in order to remove α -methylene- γ -butyrolactone generated during the extraction procedure. An aliquot of the obtained water-soluble fraction hydrolyzed by hydrochloric acid generated tulipalin A. This result obviously indicates that a precursor of tulipalin A existed in the water-soluble fraction. Next, the water-soluble fraction was chromatographed on ODS resin eluted with water (100 ml \times 10) to give 10 aqueous fractions. Of these 10 fractions, the hydrolysates of fractions 2–8 contained tulipalin A, with that of fraction 4 being richest in tulipalin A. The HPLC profile of this fraction 4 is shown in Fig. 1. Of the hydrolysates of these components in the profile, only those of compounds **1** and **2** contained tulipalin A. These two components were, therefore, respectively isolated from the water-soluble fraction of thunberg spiraea by reverse-phase HPLC.

Leaving an aqueous solution of isolated compound **1** at room temperature readily generated compound **2** in the aqueous solution. Similarly, the aqueous solution of only compound **2** gave a mixture of compounds **1** and **2** after standing. The ratio of compounds **1** and **2** was

approximately 3:2. Hydrolysis of compound **1** by hydrochloric acid generated equimolar amounts of α -methylene- γ -butyrolactone and *D*-glucose ($[\alpha]_D^{25} + 50^\circ$, *c* 0.1 H₂O). In addition to these products, two types of anomeric protons were observed at 4.51 ppm (β form: $J=7.8$ Hz) and 5.07 ppm (α form: $J=3.6$ Hz) in the ratio 3:2 in the ¹H-NMR spectrum of compound **1**. The molecular weights of compounds **1** and **2** were respectively determined to be 278 (4-hydroxy- α -methylenebutyric acid + glucose – H₂O = 278) from LCMS data. There was no difference between the MS data for **1** and **2**. Judging from these data, it is considered that compounds **1** and **2** were anomers to each other and both precursors of tulipalin A.

In the ¹³C-NMR spectrum of compounds **1** and **2**, the C-6 carbons of the glucose moiety were shifted downfield by 2.8 ppm (β form) and by 3.0 ppm (α form), respectively, compared with those of glucose. On the other hand, the C-5 carbons were shifted upfield by 2.5 ppm (β form) and by 1.7 ppm (α form), respectively, compared with those of glucose (shown in the Table). In the ¹H-NMR spectrum of compounds **1** and **2**, all protons (both of α and β forms) of C-6 of the glucose moiety were also observed at 4.20–4.36 ppm {(4.20 ppm, β -H6a), (4.24, α -H6a), (4.32, β -H6b), (4.36, α -H6b)}.

Table ¹³C-NMR Assignment of the Mixture of Compounds **1** and **2** and of *D*-Glucose in D₂O

Carbon No.	Compounds 1 and 2 * δ_c (multiplicity)	<i>D</i> -Glucose * δ_c (multiplicity)
1	169.3 (s)	—
2	137.0 (s)	—
3	34.9 (t)	—
4	60.8 (t)	—
5	129.8 (t)	—
α 1	92.9 (d)	92.9 (d)
α 2	72.2 (d)	72.3 (d)
α 3	73.4 (d)	73.5 (d)
α 4	70.0 (d)	70.4 (d)
α 5	70.5 (d)	72.2 (d)
α 6	64.4 (t)	61.4 (t)
β 1	96.8 (d)	96.7 (d)
β 2	74.8 (d)	74.9 (d)
β 3	76.3 (d)	76.5 (d)
β 4	70.4 (d)	70.4 (d)
β 5	74.2 (d)	76.7 (d)
β 6	64.3 (t)	61.5 (t)

* Chemical shifts are given in ppm.

[†] To whom correspondence should be addressed. Fax: +81-888-64-5219; E-mail: cs-kim@cc.kochi-u.ac.jp

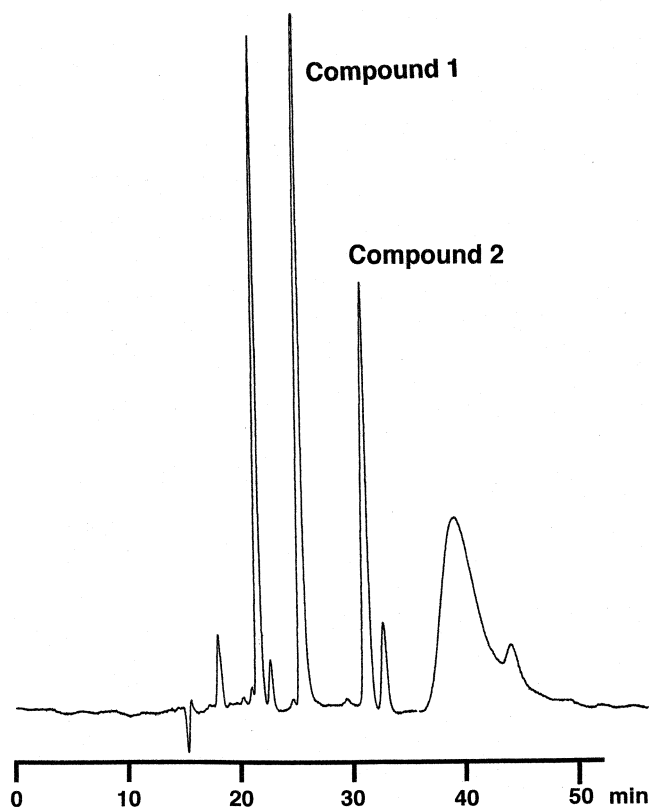


Fig. 1. HPLC Profile of Fraction 4 Separated by an ODS Open Column.

Conditions: column, Shiseido Capcellpak ODS SG 120 (250 mm \times 10 mm i.d.); solvent, 10% methanol in water; flow rate, 0.9 ml/min; detection, UV 208 nm.

These results indicate typical acylation^{8,9} at the C-6 position of the glucose moiety. The above-mentioned results as well as diagnostic ^1H - and ^{13}C -NMR and mass spectra enabled compounds 1 and 2 to be identified as 6-*O*-(4-hydroxy- α -methylenebutyryl)- β -D-glucopyranose and 6-*O*-(4-hydroxy- α -methylenebutyryl)- α -D-glucopyranose, respectively, that is, 6-tuliposide A (Fig. 2). The specific rotation of a mixture of 1 and 2 after equilibration ($[\alpha]_D^{25} + 36.8^\circ$, *c* 1.55 methanol) approximately matches that in the literature ($[\alpha]_D^{25} + 45.5^\circ$, *c* 1.0 methanol).¹⁰

The content of 6-tuliposide A in fresh leaf of thunberg spiraea was estimated to be 3.75 mg (M.W. $278 \times 1.35 \times 10^{-5}$ mole) in 1 g of fresh leaves, since 1.32 mg (1.35×10^{-5} mole) of tulipalin A (α -methylene- γ -butyrolactone) was detected by GC from 1 g of fresh leaves equivalent of the methanol extract by per-hydrolysis.

This is the first time that 6-tuliposide A has been found in a plant family and genus other than *Alstroemeria*, *Erythronium*, and *Tulipa* of Liliaceae.¹¹⁻¹³ 6-Tuliposide A and its hydrolysate (tulipalin A) seem to be inherent protective compounds against fungal invasion in the Liliaceae family. Our studies also suggest that this protective mechanism would operate in many plants besides the liliaceous family and be effective against not only fungal invasion but also animal attack, including in-

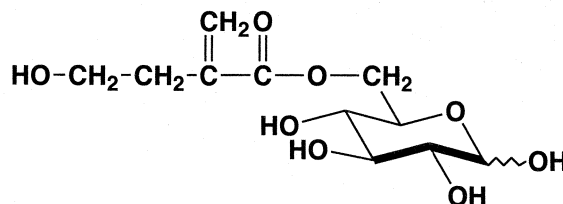


Fig. 2. Structure of 6-Tuliposide A.

sects. To confirm these proposals, studies on the compound's activity against other species of insects and its distribution in the plant kingdom must be carried out. We also point out the possibility that thunberg spiraea leaves cause dermatitis as the leaves of *Tulipa* and *Alstroemeria* do,¹⁴ because 6-tuliposide A gave positive reactions at 0.01% in the patch-test.¹⁵

Experimental

Instruments. LCMS data were measured with a JEOL MS600 mass spectrometer by the flow injection method. GCMS data for the ether-soluble hydrolysate were recorded with a Shimadzu QP-2000 instrument and measured at 20 eV. GC analyses were done with a Shimadzu GC-14A chromatograph fitted with a fused silica column (HR1701, 0.25 μm thickness, 25 m \times 0.2 mm i.d.) and programmed from 45°C (1-min hold) to 200°C at a rate of 5°C/min. The water-soluble hydrolysate was analyzed with an amino column (Shiseido, Capcellpak NH₂ SG 80A, 250 mm \times 4.6 mm i.d.) eluted with 75% acetonitrile in water at a flow rate of 0.5 ml/min. ^1H - and ^{13}C -NMR data for the mixture of compounds 1 and 2 were measured with a JEOL Lambda 400 spectrometer (400 MHz). 3-(Trimethylsilyl)propionic-2, 2, 3, 3-*d*₄ acid sodium salt was used as the internal standard. Letters (br.) s, (d) d, t, q, and m represent (broad) singlet, (double) doublet, triplet, quartet, and multiplet, respectively, and coupling constants are given in Hz. Specific rotation values were recorded with a Horiba High Sensitive Polarimeter SEPA-200.

Isolation of compounds 1 and 2. Fresh thunberg spiraea leaves (250 g) were extracted immediately after plucking with 80% methanol in water (2 l) for 3 days in darkness at room temperature. After evaporating the solvent under reduced pressure at 40°C, a thunberg spiraea methanol extract was obtained. Aliquots of this methanol extract (188.0 g of fresh leaf weight equivalent) of thunberg spiraea leaves was dissolved in 250 ml of water, and the solution was then partitioned between ethyl acetate (250 ml \times 2) and water. The water-soluble fraction was then chromatographed on ODS resin (260 mm \times 30 mm i.d., Chromatorex DM1020T, Fuji Silysia Chemical) eluted with water (100 ml \times 10) to give 10 aqueous fractions. An aliquot of each of these 10 fractions was then hydrolyzed to check for generated tulipalin A by GC. Of these 10 fractions, the hydrolysates of fractions 2-8 contained tulipalin A, that of fraction 4 being richest in tulipalin A. Fraction 4 was then separated into peaks by HPLC (Shiseido, Capcellpak ODS SG

120, 250 mm \times 10 mm i.d.), eluting with 10% methanol in water at a flow rate of 0.9 ml/min and detecting at UV 208 nm. Compounds **1** and **2** were isolated at t_R =25.5 min and 31.3 min, respectively. The combined yield of both compounds was 31.0 mg.

Hydrolysis of compounds 1 and 2. A mixture of compounds **1** and **2** (7.4 mg) was dissolved in 2 ml of 1 *N* HCl and then heated at 80°C for 5 h. The ether-soluble fraction obtained by extraction with ether (5 ml \times 3) was passed through a Sep-pak silica cartridge (Waters), eluting with 10 ml of ether. After the eluate had been concentrated until the total volume of the solution was 10 ml, GC and GCMS analyses were carried out in order to detect tulipalin A (t_R =9.91 min) and to determine its content in the plant. The aqueous layer was dried and then dissolved in a small amount of water. The solution was passed through a Sep-pak C₁₈ cartridge (Waters), eluting with 10 ml of water. The water-soluble hydrolysate was isolated with an amino column (NH₂) at t_R =12.5 min.

Compounds 1 and 2 (6-Tuliposide A). [α]_D²⁵ + 36.8° (c 1.55, methanol), t_R =25.5 and 31.3 min (by HPLC). LCMS (FRIT-FAB⁺) m/z (%): 279(M+H⁺, 78), 273(13), 261(41), 243(18), 189(13), 155(13), 145(18), 141(14), 119(12), 117(17), 105(17), 99(100), 85(20), 82(12), 81(17), 71(14). ¹H-NMR δ_H (D₂O): 2.42 (2H, t, J =6.2, H-4), 3.00–4.00 (6H, m, H-4, 2', 3', 4', and 5'), 4.20 (1H, dd, J =12.2 and 5.0, β -H6a), 4.24 (1H, dd, J =12.2 and 4.6, α -H6a), 4.32 (1H, dd, J =12.2 and 2.2, β -H6b), 4.36 (1H, dd, J =12.2 and 2.2, α -H6b), 4.51 (1H, d, J =7.8, β -H1), 5.07 (1H, d, J =3.6, α -H1), 5.65 (s, H5a), 6.16 (d, J =3.4, H5b). ¹³C-NMR data are shown in the Table.

α -Methylene- γ -butyrolactone (Tulipalin A). t_R =9.91 min (by GC). GCMS (EI⁺) m/z (%): 98(M⁺, 29), 68(86), 54(12), 43(29), 42(100), 41(75).

D-Glucose. [α]_D²⁵ + 50° (c 0.1, H₂O), t_R =12.5 min (in HPLC). ¹H-NMR δ_H (D₂O): 3.04–3.74 (m, β -2–6 and α -2–6), 4.48 (d, J =8.0, β -1), 5.07 (d, J =3.9, α -1). ¹³C-NMR data are shown in the Table.

Acknowledgment

We are grateful to Center for Joint Research and De-

velopment at Kochi University for measuring the NMR spectral data.

References

- 1) Hirano, C., Jinbo, Y., Yasumi, K., and Horiike, M., *Thrips palmi*: Indiscriminative invasion into green-houses with host and non-host plants. *Proc. Assoc. Pl. Protec. Shikoku* (in Japanese), **25**, 53–55 (1990).
- 2) Hirano, C., Jinbo, Y., Itoh, E., Yasumi, K., and Horiike, M., Oviposition of *Thrips palmi* KARNY (Thysanoptera: Thripidae) onto tomato leaves. *Proc. Assoc. Pl. Protec. Shikoku* (in Japanese), **26**, 81–83 (1991).
- 3) Hirano, C., Itoh, E., Yasumi, K., and Horiike, M., Short-distance walking responses of *Thrips palmi* KARNY (Thysanoptera: Thripidae). *Appl. Ent. Zool.*, **28**, 233–234 (1993).
- 4) Yasumi, K., Syoumae, Y., Horiike, M., and Hirano, C., Survival of *Thrips palmi* KARNY (Thysanoptera: Thripidae) on leaves of egg-plant, cucumber, and tomato. *Proc. Assoc. Pl. Protec. Shikoku* (in Japanese), **26**, 77–79 (1991).
- 5) Yasumi, K., Shinohara, H., Horiike, M., and Hirano, C., Effect of tomato leaf constituents on survival of *Thrips palmi* KARNY (Thysanoptera: Thripidae). *Jpn. J. Appl. Ent. Zool.* (in Japanese), **35**, 311–316 (1991).
- 6) Hirano, C., Yasumi, K., Itoh, E., Kim, C.-S., and Horiike, M., A feeding deterrent for *Thrips palmi* KARNY (Thysanoptera: Thripidae) found in tomato leaves: Isolation and identification. *Jpn. J. Appl. Ent. Zool.* (in Japanese), **38**, 109–120 (1994).
- 7) Kim, C.-S., Hara, T., Datta, P. K., Itoh, E., and Horiike, M., Insecticidal component in thunberg spiraea, *Spiraea thunbergii*, against *Thrips palmi*. *Biosci. Biotechnol. Biochem.*, **62**, 1546–1549 (1998).
- 8) Couperus, P. A., Clague, A. D. H., and van Dongen, J. P. C. M., Carbon-13 chemical shifts of some model carboxylic acids and esters. *Org. Magn. Reson.*, **11**, 590–597 (1978).
- 9) Wenkert, E., Gasic, M. J., Hagaman, E. W., and Kwarl, L. D., Long range substituent effects reflected in the ¹³C-NMR spectra of allyl alcohols and their derivatives. *Org. Magn. Reson.*, **7**, 51–53 (1975).
- 10) Tschesche, R., Kämmerer, F.-J., and Wulff, G., Über die antibiotisch wirksamen substanzen der tulpe (*Tulipa gesneriana*). *Tetrahedron Letters*, **6**, 701–706 (1968).
- 11) Slob, A., Tulip allergens in *Alstroemeria* and some other Liliiflorae. *Phytochemistry*, **12**, 811–815 (1973).
- 12) Slob, A., Jekel, B., Jong, B. de, and Schlattmann, E., On the occurrence of tuliposides in the Liliiflorae. *Phytochemistry*, **14**, 1997–2005 (1975).
- 13) Christensen, L. P., and Kristiansen, K., A simple HPLC method for the isolation and quantification of the allergens tuliposide A and tulipalin A in *Alstroemeria*. *Contact Dermatitis*, **32**, 199–203 (1995).
- 14) Cronin, E., Sensitivity to tulip and Alstroemeria. *Contact Dermatitis Newsletter*, **11**, 286 (1972).
- 15) Santucci, B., Picardo, M., Iavarone, C., and Trogolo, C., Contact dermatitis to Alstroemeria. *Contact Dermatitis*, **12**, 215–219 (1985).