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Ovipositional Deterrent in the Sweet Pepper, *Capsicum annuum*, at the Mature Stage against *Liriomyza trifolii* (Burgess)

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***Liriomyza trifolii* (Burgess), the American serpentine leafminer fly, is well known as a serious pest throughout the world. This insect attack over 21 different plant families including solanaceae plants. The mature sweet pepper, *Capsicum annuum* (Solanaceae), however, shows resistance to this leafminer fly. This resistance is based on the ovipositional deterrent in the sweet pepper leaf against the fly species. Based on bioassay-guided fractionation, luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside was isolated and identified as the ovipositional deterrent against this insect species. This compound completely deterred *L. trifolii* females from laying their eggs on a host plant leaf treated at 4.90 μg/cm^2.**

Key words: *Liriomyza trifolii*; ovipositional deterrent; *Capsicum annuum*; luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside

Liriomyza trifolii (Burgess), the American serpentine leafminer fly, is well known as a serious pest to many vegetables and crops throughout the world. The fly has a broad host range and can attack over 120 plant species in more than 21 families.[1] Females of *L. trifolii* puncture the leaf surface with their ovipositor for feeding and oviposition. The larvae, after hatching from the eggs, feed on the mesophyll tissue in the leaf and form a serpentine mine which can significantly reduce the photosynthetic capacity of the plant.[2]

The fly, which originated in North America, has quickly spread its distribution area throughout the world and was first found in Japan in 1990 in Shizuoka prefecture.[3,4] In addition to the broad host range, the leafminer shows resistance to most insecticides and can develop all year round in a greenhouse,[3,5–7] making control of the leafminer pest more difficult. The development of an integrated pest control method against this species is therefore necessary.

Sweet pepper, *Capsicum annuum* (Solanaceae) has been reported, along with many other solanaceae plants, as one of the hosts.[8] In fact, this insect species fiercely attacks young *C. annuum*. However, we have observed that *L. trifolii* seldom attacked and laid their eggs on mature sweet pepper in our greenhouse.

We report in this paper the factors that contributed to the resistance of mature *C. annuum* against *L. trifolii*.

A fresh kidney bean leaf was soaked in a methanol solution of *C. annuum* at the mature stage (1 g of fresh leaf equivalent/ml) and then submitted to a bioassay. Only a few ovipositional marks (1.85 marks/cm^2 ± 0.72; mean ± S. E.) were observed on the treated leaf. On the other hand, a significant number of marks (64.83 marks/cm^2 ± 2.85) were observed on the control leaf. There were no larvae hatching from the treated leaves after keeping them for a few days in the incubator. These results are similar to our previous observations in the greenhouse and clearly show that the leaf of *C. annuum* at the mature stage exerted an ovipositional deterrent against this insect species. During the bioassay, the female flies landed briefly on the kidney bean leaf that had been treated with the methanol extract. Upon landing, the females walked over the leaf and drummed the surface of the leaf, before attempting to insert their ovipositors into the leaf.

The active methanol extract was separated into the hexane, diethyl ether, ethyl acetate, water-saturated butanol and water layers by liquid-liquid partition. Of these, the hexane (9.03 marks/cm^2 ± 1.88) and the water layers (7.31 marks/cm^2 ± 0.13) showed intense activity. The other fractions (diethyl ether: 49.35 marks/cm^2 ± 4.27, ethyl acetate: 45.71 marks/cm^2 ± 4.04, and water-saturated butanol: 37.53 marks/cm^2 ± 6.38) did not show any activity against this insect species (Fig. 1). The active compound in the hexane layer has recently been determined to be the terpene alcohol, (−)-phytol. This compound showed ovipositional deterrent activity against the insect species at 35.2 μg/cm^2 (5.91 marks/cm^2 ± 2.04). Since (−)-phytol is a common compound in various plants and sweet pepper contained as much of the phytol as that in kidney

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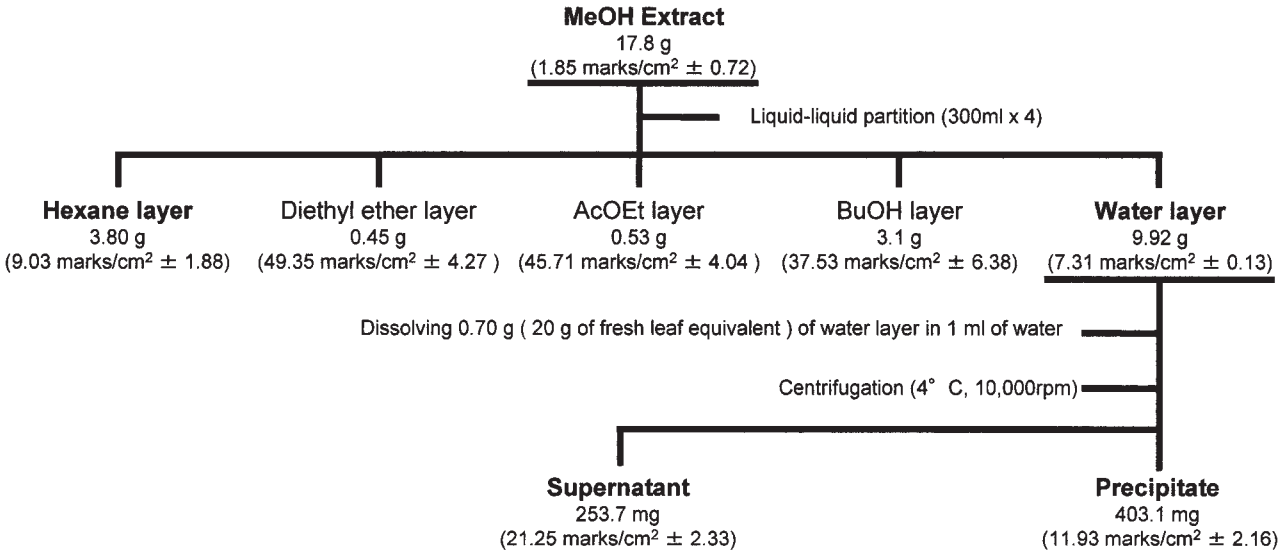

Fig. 1. Scheme for Fractionation of the Methanol Extract of *C. annuum*.

bean, this compound may have been a by-product generated during the extraction procedure with methanol.[9] When the water layer (0.70 g: 20 g of fresh leaf equivalent) was dissolved in 1 ml of water, as educt was generated. After separating this educt by centrifugation, the precipitate and supernatant were submitted to a bioassay. The precipitate (11.93 marks/cm^2 ± 2.16) and supernatant (21.25 marks/cm^2 ± 2.33) both showed strong activity against this insect species. The precipitate was separated into three fractions (A, B and C) by reverse-phase HPLC. Of these three fractions, only B showed high activity (A, 52.93 marks/cm^2 ± 5.80; B, 3.87 marks/cm^2 ± 0.95; C, 41.69 marks/cm^2 ± 5.74). Fr. B was a yellow powder and gave a single peak (compound **1**) in the HPLC analysis. However, this compound was hardly detectable in the supernatant, indicating that several active compounds, which deterred this insect from laying its eggs on the leaf, were present in the leaf of mature *C. annuum*.

The LC–MS data (positive *m/z* 581 $[M+H]^+$; negative *m/z* 579 $[M-H]^-$), indicated the molecular weight of compound **1** to be 580. Since a set of ABX systems at δ 6.96 (d, $J = 8.8$, H-5′), δ 7.48 (d, $J = 1.2$, H-2′) and δ 7.50 (dd, $J = 8.8$, and 1.2, H-6′), two aromatic proton signals (δ 6.48, $J = 2.0$, H-6; and 6.81, $J = 2.0$, H-8) and one aromatic proton signal (δ 6.80, s, H-3) were observed in the ^{1}H-NMR spectrum of compound **1**, this compound was considered to be a luteolin derivative. As shown in Table 1, 11 carbon signals, including two anomeric carbons (98.1 ppm and 108.8 ppm), were observed, in addition to those signals due to the luteolin moiety, in the ^{13}C-NMR spectrum. This compound seemed to be a luteolin diglycoside. A comparison of the ^{13}C-NMR spectrum of **1** with that of luteolin[10] showed significant downfield shifts at C-6 (+0.2 ppm), C-8 (+0.5 ppm) and C-10 (+1.2 ppm), and upfield shifts at C-5 (−0.9 ppm), C-7 (−0.2 ppm) and C-9 (−0.9 ppm). Such shifts are typical of *O*-glycosylation at the C-7 position. It is therefore reasonable that this compound had a luteolin aglycon with a di-*O*-glycoside at position 7. Comparing the ^{13}C-NMR spectrum with

Table 1. Chemical Shifts Values for Compound **1** and Analogous Flavonoids in Their ^{13}C-NMR Spectra

C-Position	Compound **1**	Luteolin[10]	luteolin 7-*O*-glucoside[11]	Apiin[12]
Aglycon	Luteolin	Luteolin	Luteolin	Apigenin
C-2	164.5	164.5	164.9	164.3
C-3	103.2	103.3	103.6	103.1
C-4	181.9	182.2	182.4	181.9
C-5	161.2	162.1	161.6	161.3
C-6	99.4	99.2	100.0	99.4
C-7	162.7	164.7	163.4	162.7
C-8	94.7	94.2	95.2	94.8
C-9	157.0	157.9	157.4	156.9
C-10	105.4	104.2	105.8	105.4
C-1′	121.4	119.3	121.8	121.0
C-2′	113.5	113.8	114.0	128.5
C-3′	145.8	146.2	146.3	116.0
C-4′	150.0	150.1	150.4	161.1
C-5′	116.0	116.4	116.5	116.0
C-6′	119.2	122.1	119.7	128.5
Glucose				
C-1″	98.1		100.3	98.2
C-2″	75.9		73.8	75.9
C-3″	76.8		76.8	76.1
C-4″	69.8		70.0	69.8
C-5″	77.0		77.6	77.0
C-6″	60.6		61.1	60.6
Apiose				
C-1‴	108.8			108.7
C-2‴	76.0			76.7
C-3‴	79.3			79.1
C-4‴	74.0			73.9
C-5‴	64.2			64.2

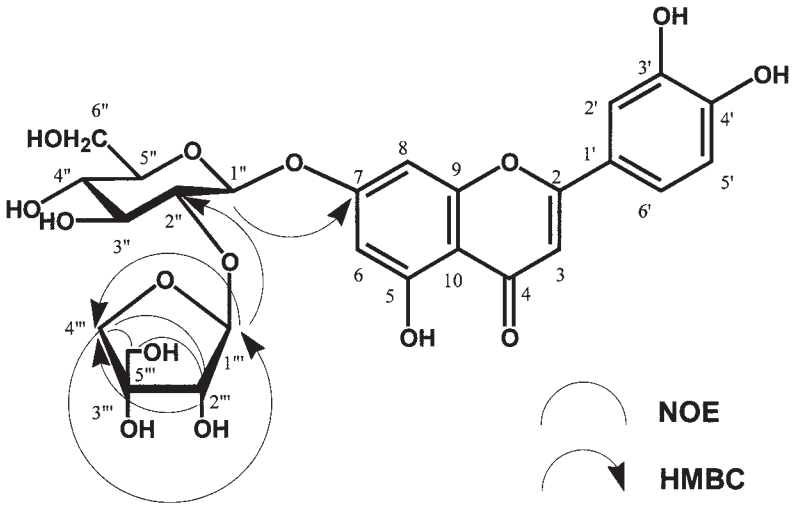

Fig. 2. Structure of Compound **1**.
NOEs and HMBC relationships observed are shown by solid lines and arrows, respectively.

that of apiin (apigenin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside),[12] the chemical shift values of the sugar moieties were very similar. Hence, this compound was determined to be luteolin 7-*O*-*β*-apiofuranosyl-(1→2)-*β*-glucopyranoside. As shown in Fig. 2, the H–H and C–H COSY, HMQC and HMBC data indicated the same structure. Methanolysis of compound **1** was conducted to confirm the absolute configuration of both sugars. The obtained mixture of 1-*O*-methyl-*α*- and *β*-glucopyranose and 1-*O*-methyl-*β*-apiofuranose showed specific rotation values of +86.0° and −88.8°, respectively. These values are similar to those of an authentic sample ($[\alpha]_D$ +87.1°) and literature data (−95°).[13] Each absolute configuration was determined as being D-form from these results. This compound was therefore determined to be luteolin 7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside, which completely deterred the fly from laying its eggs on a leaf treated at 4.90 μg/cm^2 (Fig. 3). On the other hand, luteolin 7-*O*-*β*-D-glucopyranoside isolated from the same plant leaves did not show any activity against this fly insect species at all. These results indicate that the apiose moiety was very important to the activity.

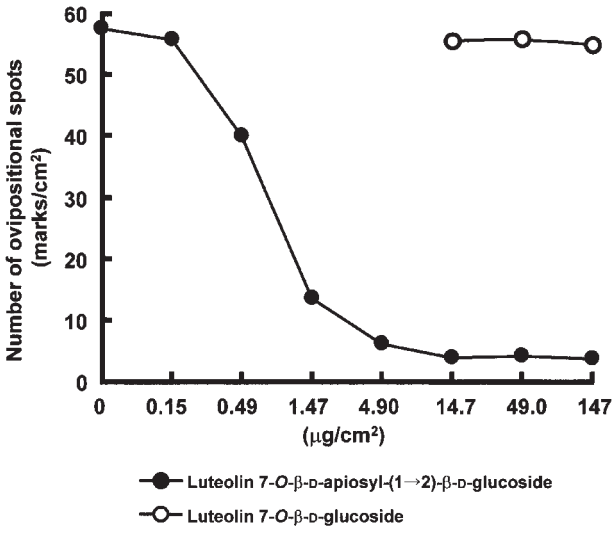

Fig. 3. Ovipositional Deterrent Activities of Luteolin.
7-*O*-*β*-D-Apiosyl-(1→2)-*β*-D-glucoside and Luteolin 7-*O*-*β*-D-glucoside against *L. trifolii*.

As shown in Table 2, the concentration and calculated distribution of compound **1** in the leaves of *C. annuum* increased with plant growth. The calculated distribution of compound **1** in the cotyledon and leaves at the four-leaf stage was 0.1 and 0.7 μg/cm^2, respectively. These levels were not strong enough to have any ovipositional deterrent effect towards the adult females of *L. trifolii*. In contrast, the distribution of compound **1** in the leaves at the mature stages (twenty-leaf stage and fruiting stage) was 130–3000 times as much as that at the young stages. This dose range was enough to exert an oviposi-

Table 2. Concentration and Calculated Distribution of Compound **1** in the Leaves at Various Stages and in the Fruit of the Sweet Pepper

	Cotyledons at four-leaf stage	Four-leaf stage	Six-leaf stage	Ten-leaf stage	Twenty-leaf stage	Fruiting stage	Fruit
Concentration of compound **1** (μg/g)	4.1	61.2	2116.3	4147.7	8250.3	12,135.6	417.9
Calculated distribution of compound **1** (μg/cm^2)	0.1	0.7	26.3	123.4	222.3	329.5	—

tional deterrent effect against this insect spices.

Compound **1** was initially isolated and identified from parsley (*Petroselinum crispum*) as graveobioside A by Nordström.[14] The biological activity and spectroscopic data for this compound are reported here for the first time. This flavonoid glycoside is present in a large amount (more than 12,000 ppm) in mature *C. annuum* leaves. The fruit of sweet pepper also contains 417.9 μg of compound **1** per 1 g of fresh weight. This compound can be very easily isolated and is consumed in daily food. These results might contribute to the development of an integrated pest control method against this insect that is safe and of suitable concentration for the natural environment. Active compounds in the supernatant from the water-soluble fraction now need to be investigated, and the mechanism for changing the resistance of *C. annuum* with growth stage also needs to be elucidated.

Experimental

Instruments. LC–MS data were recorded with a Shimadzu LCMS-2010 mass spectrometer when using a reversed-phase column (SHISEIDO CAPCELLPAK C_{18} UG120 Å, 250 mm × 4.6 mm i.d.) eluted with 10–90% acetonitrile in water containing 1% acetic acid in the APCI-positive and APCI-negative modes. Optical rotation was measured with a HORIBA SEPA-200 spectropolarimeter. ^{1}H-NMR and ^{13}C-NMR spectra, including two-dimensional correlation spectra, were measured with a JEOL JNM-AL 400 (400 MHz) instrument. TMS was used as an internal standard. Letters (br.)s, d, t, q, and m represent (broad)singlet, doublet, triplet, quartet, and multiplet, respectively, and coupling constants are given in Hz.

Insect and plant. Stock colonies of *L. trifolii* were collected from Kochi Pref. Agricultural Center and successively reared on 10- to 14-d-old seedlings of the kidney bean, *Phaseolus vulgaris* L. (Daikintoki) at 27 ± 2 °C and a relative humidity of 60–70% with 16:8(L:D) illumination. After emerging, 1-d-old females were used for the bioassay.

Forty seeds of *C. annuum* var. *angulosum* (Sakigake 2-go) were planted in a plastic tray (20 cm × 27 cm in length, 1 cm in depth) containing nursery soil in a plant-growth incubator at 27 ± 2 °C. After four weeks, the seedlings were transplanted into individual pots (6.6 cm in height, 5.0 cm in i.d.) containing nursery soil and grown in a greenhouse without any application of insecticides. Plants at the twenty-leaf stage (approximately 17 weeks old) were used for the extract. Ten- to 14-d-old seedlings of *P. vulgaris* were used for the bioassay.

Preparation of the plant extract. Fresh sweet pepper leaves (420 g) were twice extracted with 80% methanol in water (1 liter) for 3 d in darkness. After concentrating under vacuum, the residue (33.9 g) was dissolved at a concentration of 1 g of fresh leaf equivalent/ml in methanol.

Bioassay of the plant extract. A fresh kidney bean leaf was dipped into the test solution (1 g of fresh leaf equivalent/ml) for 30 s. After removing the solvent, the leaf was put onto a moistened filter paper at the bottom of a Petri dish (10 mm in high, 90 mm in i.d) to maintain the humidity. Five adult female flies at least 24 h old were put into a screw vial (28 mmϕ) and it was placed on treated or control leaves so that the mouth of the vial touched the leaf and allowed to oviposit for 24 h at 27 °C under 16:8(L:D) illumination. The number of ovipositional marks was counted on each leaf under a microscope. Each test was replicated five times. The control leaf was treated in the same manner with only a methanol solution.

*Isolation of compound **1**.* The methanol extract (17.8 g, 320 g of fresh leaf equivalent) was dissolved in water (430 ml), and then successively extracted with hexane (300 ml × 4, 3.80 g), diethyl ether (300 ml × 4, 0.45 g), ethyl acetate (300 ml × 4, 0.53 g) and water-saturated butanol (300 ml × 4, 3.1 g). The water-soluble fraction (0.70 g: 20 g of fresh leaf equivalent) was dissolved in 1 ml of water and then divided into the supernatant and precipitate by centrifuging (10,000 rpm at 4 °C for 10 min). The precipitate was separated into three fractions, A (Rt = 0–11.1 min), B (Rt = 11.1–12.8 min) and C (Rt = 12.8–15.0 min), by reversed-phase HPLC (SHISEIDO CAPCELLPAK C_{18} UG120 Å column, 250 mm × 10 mm i.d.), eluting with 20% acetonitrile in water at a flow rate of 2 ml/min and detecting at UV 254 nm. Compound 1 was isolated at Rt = 11.8 min from fraction B. The leaf of sweet pepper at the twenty-leaf stage contained compound 1 at 8,250 μg/g of fresh leaf equivalent.

*Methanolysis of compound **1**.* Compound **1** (120 mg) was mixed with 1 ml of 5% hydrochloric acid in methanol, and heated at 80 °C for 1 h. After vacuum concentration, the reaction mixture was dissolved in 10 ml of water and extracted three times with 10 ml of ethyl acetate. The water layer diluted with water (20 ml) was passed through a Sep-pak C_{18} cartridge (Waters) and successively eluted with 10 ml of water and 10 ml of 40% aqueous methanol. The 1-*O*-methylsugars were recovered from the water eluate, and luteolin (45.6 mg) was obtained from the 40% aqueous methanol eluate and the ethyl acetate layer. 1-*O*-Methylapiofranose (2.7 mg) and 1-*O*-methylglucopyranose (16.8 mg) were isolated by HPLC (Nakarai tesque waters Cosmosil 5NH2-MS column; 10 mmϕ × 250 mm, 70% acetonitrile in water at 3 ml/min; refractive index detector).

α- and β-D-1-*O*-Methylglucopyranose were also prepared from an authentic D-glucose sample by the same procedure.

Compound ***1*** *(luteolin 7-O-β-D-apiofranosyl-(1→2)-β-D-glucopyranoside).* $[\alpha]_D^{22}$ −82.0° (*c* 1.0, DMSO). LC–MS (APCI-positive) *m/z* (%): 581 (87.5, $M + H^+$), 449 (8.6, $M + H^+$-apiose), 319 (17.3), 287 (74.1, luteolin + H^+), 157 (100.0). LC–MS (APCI-negative) *m/z* (%): 579 (72.2, $M^- - H$), 447 (9.6, $M^- - H$-apiose), 285 (100.0, luteolin$^-$ − H). ^{1}H-NMR (DMSO-d_6) δ: 7.50 (1H, dd, *J* = 8.8, and 1.2, H-6′), 7.48 (1H, d, *J* = 1.2, H-2′), 6.96 (1H, d, *J* = 8.4, H-5′), 6.81 (1H, d, *J* = 2.0, H-8), 6.80 (1H, s, H-3), 6.48 (1H, d, *J* = 2.0, H-6), 5.41 (1H, s, H-1‴), 5.23 (1H, d, *J* = 6.8, H-1″), 3.97 (1H, d, *J* = 9.2, H-4‴-a), 3.79 (1H, s, H-2‴), 3.77 (1H, d, *J* = 9.6, H-6″-a), 3.72 (1H, d, *J* = 9.2, H-4‴-b), 3.58 (1H, t, *J* = 9.2, H-3″), 3.56 (1H, dd, *J* = 9.6 and 9.2, H-6″-b), 3.55 (1H, t, *J* = 9.2, H-5″), 3.52 (1H, dd, *J* = 9.0 and 6.8, H-2″), 3.34 (2H, s, H-5‴), 3.25 (1H, t, *J* = 9.2, H-4″). ^{13}C-NMR (DMSO-d_6): see Table 1.

Luteolin from compound ***1***. ^{1}H-NMR (DMSO-d_6) δ: 7.50 (1H, dd, *J* = 8.8, and 1.2, H-6′), 7.48 (1H, d, *J* = 1.2, H-2′), 6.96 (1H, d, *J* = 8.4, H-5′), 6.81 (1H, d, *J* = 2.0, H-8), 6.80 (1H, s, H-3), 6.48 (1H, d, *J* = 2.0, H-6). ^{13}C-NMR (DMSO-d_6) δ: 181.9 (s, C-4), 164.5 (s, C-2), 162.7 (s, C-7), 161.2 (s, C-5), 157.0 (s, C-9), 150.0 (s, C-4′), 145.8 (s, C-3′), 121.4 (s, C-1′), 119.2 (d, C-6′), 116.0 (d, C-5′), 113.5 (d, C-2′), 105.4 (s, C-10), 103.2 (d, C-3), 99.4 (d, C-6), 94.7 (d, C-8).

α- and β-D-1-O-Methylglucopyranose from compound ***1***. *Rt* = 7.42 min, $[\alpha]_D^{22}$ +86.0° (*c* 1.0, H_2O). ^{13}C-NMR (D_2O) δ: 104.3 (d, α-1), 100.3 (d, β-1), 77.0 (d, α-3), 76.8 (d, α-5), 74.2 (d, β-3), 74.2 (d, α-2), 72.7 (d, β-5), 72.3 (d, β-2), 70.7 (d, α-4), 70.6 (d, β-4), 61.8 (t, α-6), 61.6 (t, β-6), 58.3 (q, α-*O*-Me), 56.1 (q, β-*O*-Me).

α- and β-D-1-O-Methylglucopyranose from an authentic sample. Rt = 7.42 min, $[\alpha]_D^{22}$ +87.1° (*c* 1.0, H_2O). ^{13}C-NMR (D_2O) δ: 104.3 (d, α-1), 100.3 (d, β-1), 77.0 (d, α-3), 76.9 (d, α-5), 74.2 (d, β-3), 74.2 (d, α-2), 72.7 (d, β-5), 72.3 (d, β-2), 70.8 (d, α-4), 70.7 (d, β-4), 61.9 (t, α-6), 61.7 (t, β-6), 58.4 (q, α-*O*-Me), 56.2 (q, β-*O*-Me).

β-D-1-O-Apiofuranose. Rt = 6.16 min, $[\alpha]_D^{22}$ −88.8° (*c* 0.18, H_2O). ^{13}C-NMR (D_2O) δ: 109.8 (d, A-1), 79.8 (s, A-3), 77.0 (d, A-2), 74.0 (t, A-4), 63.9 (t, A-5), 56.6 (q, -*O*-Me).

Acknowledgments

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References

1) Minkenberg, O. P. J. M., and van Lenteren, J. C., The leafminers *Liriomyza trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. *Agric. Univ., Wageningen Papers*, **86**, 1–50 (1986).
2) Chandler, L. D., and Thomas, C. E., Seasonal population trends and foliar damage of agromyzid leafminers on cantaloup in Lower Rio Grande Valley. *Texas J. Ga. Entomol. Soc.*, **18**, 112–120 (1983).
3) Saito, T., Outbreak of *Liriomyza trifolii* Burgess in Japan and its control. *Plant Protection* (in Japanese with English abstract), **47**, 123–124 (1993).
4) Sasakawa, M., Notes on Japanese Agromyzidae (Diptera), 1. *Jpn. J. Ent.*, **61**, 149–155 (1993).
5) Saito, T., Outbreak of *Liriomyza trifolii* Burgess in Japan and its control. *Plant Protection* (in Japanese), **46**, 103–106 (1992).
6) Isawa, A., Saito, T., and Ikeda, F., Effect of host plant and temperature on reprodution of American serpentine leafminer, *Liriomyza trifolii* (Burgess). *Jpn. J. Appl. Entomol. Zool.* (in Japanese with English abstract), **43**, 41–48 (1999).
7) Saito, T., Oishi, T., Isawa, A., and Ikeda, F., Effect of temperature, photoperiod, and host plant and on development and oviposition of *Liriomyza trifolii* (Burgess). *Jpn. J. Appl. Entomol. Zool.* (in Japanese with English abstract), **39**, 127–134 (1995).
8) Chandler, L. D., Flight activity of *Liriomyza trifolii* (Diptera: Agromyzidae) in relationship to placement of yellow traps in Bell pepper. *J. Eco. Entomol.*, **78**, 825–828 (1985).
9) Kashiwagi, T., Mikagi, E., Daniel, B. M., Aman, D. B., Tebayashi, S., and Kim, C.-S., Ovipositional deterrent in the sweet pepper on mature stage, *Capsicum annuum*, against *Liriomyza trifolii* (Burgess). *Z. Naturf.*, **60C**, in press.
10) Agrawal, P. K. A., "Carbon-13 NMR of Flavonoids", Elsevier Science, Oxford, p. 134 (1989).
11) Nissler, L., Gebhardt, R., and Berger, S., Flavonoid binding to a multi-drug-resistance transporter protein: an STD-NMR study. *Anal. Bioanal. Chem.*, **379**, 1045–1049 (2004).
12) Yoshikawa, M., Uemura, T., Shimoda, H., Kishi, A., Kawahara, Y., and Matsuda, H., Medicinal, foodstuffs. XVIII. Phytoestrogens from the aerial part of *Petroselinum crispum* mILL. (PARSLEY) and structures of 60-acetylapiin and a new monoterpene glycoside, petroside. *Chem. Pharm. Bull.*, **48**, 1039–1044 (2000).
13) Kitagawa, I., Hori, K., Sakagami, M., Hashiuchi, F., Yoshikawa, M., and Ren, J., Saponin and sapogenol. XLIX. On the constituents of the roots of *Glycyrrhiza inflate* BATALIN from Xinjiang, China. Characterization of two sweet oleanane-tp triterpene oligoglycosides, apoglycyrrhizin and araboglycyrrhizin. *Chem. Pharm. Bull.*, **41**, 1350–1357 (1993).
14) Nordström, C. G., and Swain, T., The flavonoid glycosides of *Dahlia variabiles*. Part I, General introduction. Cyanidin, apigenin, and luteolin glycosides from the variety "dandy". *J. Chem. Soc.*, 2764–2773 (1953).