A New Oviposition Deterrent to the Leafminer, *Liriomyza trifolii*: Cucurbitane Glucoside from *Momordica charantia*

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A new cucurbitane glucoside, 23-*O*-*β*-D-glucopyranosyl-7-hydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al, named momordicine V, has been isolated from *Momordica charantia* leaves, along with the previously reported compounds, momordicines I, II, IV and 3-*O*-malonylmomordicine I. The structure of the new compound was established on the basis of spectral analysis, as well as by its conversion to momordicine II by alkaline catalyzed hydrolysis. Momordicine V deterred significantly the oviposition by *L. trifolii* on host plant leaves treated at 26.16 μg/cm^2 leaf surface.

Key words: Liriomyza trifolii, Oviposition Deterrent, Momordicine V

Introduction

Bitter gourd, *Momordica charantia* L., belongs to the Cucurbitaceae family and is widely cultivated in tropical Africa and Asia as a vegetable crop (Yasui *et al*., 1998). The fruit of *M. charantia* has been used as a bitter stomachic in southern Japan, and as an anthelmintic and a laxative for children in India (Kirtikar and Basu, 1918). An alcoholic extract of *M. charantia* fruits has also been reported in India as a remedy for diabetes (Okabe *et al*., 1980). It is also well known that the leaves of *M. charantia* are rarely attacked by polyphagous insects including the leafminer fly, *Liriomyza trifolii* (Burgess), Diptera Agromyzidae (Mekuria *et al*., 2005, 2006), which is a well known serious pest of a wide variety of vegetables and ornamentals throughout the world and attacks tomato, cucumber, lettuce, melons (Dogimont *et al*., 1999), celery, chrysanthemums, and so on (Parrella and Robb, 1982).

Cucurbitane and its glucosides, that are momordicines I–IV (Mekuria *et al*., 2005, 2006; Yasui *et al*., 1998) and 3-*O*-malonylmomordicine I (Mekuria *et al*., 2005), have been isolated and identified from the leaves of *M. charantia* as defense substances against polyphagous insects.

As part of our continuous work on bioassay-guided isolation and characterization of oviposition deterrents from plants, here we report the isolation and structural elucidation of a new oviposition deterrent cucurbitane glucoside, 23-*O*-*β*-D-glucopyranosyl-7-hydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al, named momordicine V, from the polar fraction of *M. charantia* leaves.

Materials and Methods

Instruments

^{1}H and ^{13}C NMR data were recorded by a JEOL JNM-L400 spectrometer in pyridine-d_5 with TMS 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. LC-MS data were measured by a Shimadzu LC-MS 2010 instrument in the electrospray ionization (ESI) mode. HPLC was carried out with a Hitachi L-6200 intelligent pump equipped with a Hitachi L-4000 UV detector and a Hitachi D-2500 chromato-integrator. Wakogel C-300 (Wako Chemical Ltd., Japan) was used for column chromatography.

Plant material

Seeds of *M. charantia* (var. Satsuma futonaga reishi) were initially sown in 8-cm-diameter pots (2 seeds per pot) with holes in the bottom and vermiculite soil. The plants were grown in a glasshouse for seven weeks until four to six true leaves had fully expanded. The *M. charantia* seedlings were then transplanted and grown in an open field at the Faculty of Agriculture of Kochi University, Japan. All plants were grown in the glasshouse or field without any application of insecticides. Fresh leaves were collected for extraction from matured *M. charantia* (the fruiting stage had already passed) fourteen weeks after transplanting to the open field.

Insect and plant

Stoke colonies of *L. trifolii* were obtained from Kochi Prefecture Agricultural Center and reared successively by feeding 10- to 14-day-old seedlings of kidney bean, *Phaseolus vulgaris*, at (27 ± 2) °C, a relative humidity of 60–70% with a 16 h : 8 h (L : D) illumination. One-day-old females were collected randomly and used for the bioassay.

Extraction and isolation

Fresh leaves (1.2 kg) of *M. charantia* were cut into pieces and extracted with MeOH (8 l × 3) at room temperature for 3 d under darkness. The extracts were combined, filtered and then the solvent was removed under reduced pressure (40.5 g; 3.33% after extraction). The residue was re-dissolved in water (1 l) and successively partitioned with hexane (1 l × 2; 1.61 g), diethyl ether (1 l × 2; 2.78 g), water-saturated butanol (1 l × 2; 19.72 g) and an aqueous layer (16.42 g). Each fraction was dried under reduced pressure. A portion of the butanol fraction (10 g) was adsorbed on silica gel (5 g) and evaporated to dryness *in vacuo*. Three fractions were collected when the adsorbed butanol fraction had been chromatographed in a silica gel column, eluting in sequence with an increasing content of MeOH in ethyl acetate to obtain the ethyl acetate fraction (1 g), 50% MeOH in ethyl acetate fraction (4 g), and MeOH fraction (4.7 g). Further separation of the 50% MeOH fraction (2 g) by HPLC led to the isolation of compounds 1 (340 mg) and 2 (25 mg). HPLC was performed in a column of Cosmosil-5C_{18}-AR-II (Nacalai tesque, Inc.; 250 mm × 10 mm i.d.) at 1.5 ml/min flow rate, using an isocratic solvent system of 80% MeOH in water for 30 min. Compounds **1** and **2** were detected at 210 nm, and, respectively, eluted at Rt = 15.44 and 22.03 min.

Bioassay

A kidney bean leaf was dipped in the test solution prepared at a concentration of 1 g of leaf equivalent (gle)/ml for 30 s. A filter paper was placed in a Petri dish (10 mm high, 90 mm i.d.) and moistened with distilled water to maintain humidity. After removing the solvent by air-drying, the treated kidney bean leaf was then put on the moistened filter paper at the bottom of the Petri dish to maintain humidity exposing its upper surface. In a 50 ml screw vial (28 mm i.d.) five adult female flies were introduced and placed on treated and control leaves by turning the screw vials upside down and allowed to oviposit for 24 h at 27 °C under 16 h : 8 h (L : D) illumination. The number of leaf punctures made by the flies was counted after 24 h. Each test was replicated four times.

Momordicine V (23-*O*-β-D-glucopyranosyl-7-hydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al, **1**): Amorphous solid; $[\alpha]_D^{22}$ + 100.0° (c = 0.2, MeOH). – Positive-ion ESI-MS: m/z = 743 $[M+Na]^+$, 759 $[M+K]^+$. – Negative-ion ESI-MS: m/z = 719 $[M-H]^-$. – ^{1}H NMR (pyridine-d_5): δ = 0.80, 0.87, 1.12, 1.43 (each 3H, s), 1.18 (3H, d, J = 6.4 Hz, H_3-21), 1.66, 1.74 (each 3H, s, H_3-26 and H_3-27), 1.19, 1.55, 1.56, 1.59, 1.91, 1.93, 1.97, 2.72 (each 1H), 1.34, 1.57, 1.92 (each 2H), 2.06 (1H, m, H-20), 2.33 (1H, s, H-8), 2.67 (1H, m, H-10), 3.65 (2H, s, malonyl-CH_2), 3.89 (1H, brs, Glc-5), 4.01 (1H, brt, Glc-2), 4.22 (1H, d, Glc-3), 4.22 (1H, d, Glc-4), 4.34 (1H, d, J = 4.6 Hz, H-7), 4.34 (1H, dd, J = 9.1 and 5.2 Hz, Glc-6_a), 4.46 (1H, dd, J = 9.1 and 1.9 Hz, Glc-6_b), 4.94 (1H, d, J = 7.8 Hz, H-23), 4.94 (1H, d, J = 7.9 Hz, Glc-1), 5.06 (1H, brs, H-3), 5.59 (1H, d, J = 7.8 Hz, H-24), 6.19 (1H, d, J = 4.5 Hz, H-6), 10.59 (1H, s, H-19). – ^{13}C NMR (pyridine-d_5): see Table I.

*Alkaline hydrolysis of compound **1***

A solution of **1** (11 mg) in 5% aqueous KOH (3 ml) was stirred for 4 h at room temperature. After removing the solvent, the reaction mixture was neutralized with 1% HCl and extracted with ethyl acetate to give a product (5 mg) which has ^{1}H and ^{13}C NMR spectra and specific rotation identical with those of compound **2**.

Momordicine II (**2**): $[\alpha]_D^{20}$ + 46.2° (*c* = 0.3, MeOH). – Positive-ion ESI-MS: *m/z* = 657 $[M+Na]^+$, 673 $[M+K]^+$. – Negative-ion ESI-MS: *m/z* = 633 $[M-H]^-$. – ^{1}H NMR (pyridine-d_5): δ = 0.86, 0.88, 1.16, 1.47 (each 3H, s), 1.19 (3H, d, *J* = 6.6 Hz, H_3-21), 1.69, 1.75 (each 3H, s, H_3-26 and H_3-27), 1.18, 1.53, 1.57, 1.58, 1.93, 1.94, 1.96, 2.71 (each 1H), 1.35, 1.55, 1.93 (each 2H), 2.06 (1H, m, H-20), 2.36 (1H, s, H-8), 2.71 (1H, m, H-10), 3.80 (1H, brs, H-3), 3.88 (1H, brs, Glc-5), 4.01 (1H, brt, Glc-2), 4.21 (1H, d, Glc-3), 4.22 (1H, d, Glc-4), 4.33 (1H, d, *J* = 4.6 Hz, H-7), 4.34 (1H, dd, *J* = 9.1 and 5.2 Hz, Glc-6_a), 4.46 (1H, dd, *J* = 9.1 and 1.9 Hz, Glc-6_b), 4.94 (1H, d, *J* = 7.8 Hz, H-23), 4.94 (1H, d, *J* = 7.8 Hz, Glc-1), 5.61 (1H, d, *J* = 7.8 Hz, H-24), 6.27 (1H, d, *J* = 4.4 Hz, H-6), 10.73 (1H, s, H-19). – ^{13}C NMR (pyridine-d_5): see Table I.

Results and Discussion

Oviposition effect of L. trifolii on kidney bean leaves treated with momordicine V

In the previous studies we have reported that adult female flies of *L. trifolii* were deterred from oviposition on *M. charantia* leaves (Mekuria *et al*., 2005, 2006). The kidney bean leaves treated with an MeOH extract of *M. charantia* leaves significantly decreased the number of leaf punctures [(2.57 ± 0.27) marks/cm^2; mean ± S. E.] compared to the control (Mekuria *et al*., 2005). The crude MeOH extract was then partitioned between hexane, diethyl ether, butanol and water fractions. The butanol fraction reduced the number of oviposition marks [(2.88 ± 0.51) marks/cm^2] (Mekuria *et al*., 2005) while other fractions failed to deter the oviposition by this species. The butanol extract was subjected to CC over silica gel eluting with MeOH/EtOAc mixtures yielding three fractions (EtOAc, 50% MeOH/EtOAc and MeOH). The 50% MeOH/EtOAc fraction declined the number of oviposition marks [(2.91 ± 0.49) marks/cm^2] (Mekuria *et al*., 2005). The other two fractions did not show any activity. Further isolation of the 50% MeOH/EtOAc fraction by HPLC yielded compound **1** along with other previously reported oviposition deterrent compounds. A few ovipositional marks [(4.15 ± 0.56) marks/cm^2] were observed on the kidney bean leaves treated with compound **1** at 1 gle/ml MeOH. On the other hand, a significant number of marks [(59.20 ± 1.97) marks/cm^2] were observed on the control leaf (Tukey-Kramer multiple range test). This result clearly indicated that *L. trifolii* was deterred from oviposition on kidney bean leaves treated with compound 1.1.13 mg (26.16 μg/cm^2) of compound **1** was contained in the 1 g fresh leaves of *M. charantia*.

Structural determination

Compound **1** was assigned the molecular formula $C_{39}H_{60}O_{12}$ determined on the basis of its positive ESI mass data (*m/z* 743 $[M+Na]^+$ and 759 $[M+K]^+$) and its ^{1}H and ^{13}C NMR spectra. Compound **1** gave a positive reaction in the Liebermann-Burchard test, and the UV spectrum showed an absorption maximum at 210 nm. These along with the NMR spectra suggested that compound **1** is a triterpenoid. The ^{1}H and ^{13}C NMR spectroscopic data indicated that compound **1** possesses the same moiety as compound **2**, which was previously reported as momordicine II from *M. charantia* leaves (Mekuria *et al*., 2006), but differes in the presence of additional signals due to a methylene signal (δ_C 42.7 and δ_H 3.6) and two carbonyl carbon atoms (δ_C 167.2 and 169.4). The pseudomolecular ions of **1** in the positive ESI-mass spectrum appeared 86 mass units higher than those observed in **2**, indicating the presence of a malonyl moiety as a part of the molecule. The ^{13}C NMR (Table I) and DEPT spectra showed signals for 39 different carbon atoms corresponding to seven methyl groups, nine methylene groups, fourteen methane groups including two olefinic carbon atoms, six quaternary carbon atoms, two ester carbonyl groups and an aldehyde carbonyl carbon atom. Alkaline hydrolysis of compound **1** yielded a product, which had an optical rotation value of $[\alpha]_D^{20}$ + 46.2° (*c* = 0.3, MeOH) and NMR data coinciding with those of compound **2**. Thus, compound **1** had an identical configuration at C-3 as compound **2**. The linkage position of the malonyl moiety was established by the ^{1}H and ^{13}C NMR spectra of compound **1**. The downfield shift of a signal corresponding to hydroxylmethine H-3 in **1** was observed (from δ_H 3.82 in **2** to 5.06 in **1**). Similarly, the downfield shift of the signal in **1** corresponding to the C-3 was observed (from δ_C 75.6 in **2** to 79.4 in **1**). Thus these data indicated that esterification had occurred at C-3. The position of the malonyl moiety was verified by the HMBC spectrum of **1**, which showed a ^3J correlation between H-3 (δ_H 5.06) and the carbonyl carbon atom (malonyl-1, δ_C 167.2). Other important correlations were obtained from the HMBC spectrum,

Table I. ^{13}C NMR spectral data of compounds **1** and **2** in C_5D_5N.

C	**1**	**2**
1	21.8 (CH_2)	21.7 (CH_2)
2	25.8 (CH_2)	29.9 (CH_2)
3	79.4 (CH)	75.6 (CH)
4	40.0 (C)	41.8 (C)
5	143.5 (C)	145.7 (C)
6	124.6 (CH)	124.3 (CH)
7	65.3 (CH)	65.7 (CH)
8	50.4 (CH)	50.6 (CH)
9	51.2 (C)	50.6 (C)
10	36.2 (CH)	36.8 (CH)
11	22.5 (CH_2)	22.7 (CH_2)
12	29.4 (CH_2)	29.6 (CH_2)
13	45.7 (C)	45.9 (C)
14	48.1 (C)	48.3 (C)
15	34.7 (CH_2)	34.9 (CH_2)
16	27.5 (CH_2)	27.8 (CH_2)
17	50.6 (CH)	51.2 (CH)
18	14.9 (CH_3)	14.9 (CH_3)
19	207.3 (CH)	207.5 (CH)
20	32.5 (CH)	32.7 (CH)
21	19.3 (CH_3)	19.4 (CH_3)
22	43.4 (CH_2)	43.8 (CH_2)
23	75.1 (CH)	75.3 (CH)
24	128.8 (CH)	129.1 (CH)
25	132.2 (C)	132.2 (C)
26	18.3 (CH_3)	18.3 (CH_3)
27	26.2 (CH_3)	26.2 (CH_3)
28	25.2 (CH_3)	25.8 (CH_3)
29	26.8 (CH_3)	26.3 (CH_3)
30	18.0 (CH_3)	18.2 (CH_3)
23-Glc-1	104.4 (CH_2)	104.2 (CH_2)
23-Glc-2	75.5 (CH)	75.7 (CH)
23-Glc-3	78.9 (CH)	78.9 (CH)
23-Glc-4	71.6 (CH)	71.8 (CH)
23-Glc-5	78.6 (CH)	78.3 (CH)
23-Glc-6	62.7 (CH)	63.0 (CH_2)
Malonyl-1	167.2 (C)	–
Malonyl-2	42.7 (CH_2)	–
Malonyl-3	169.4 (C)	–

Assignments are in ppm.

which showed cross peaks between the sp^2 quaternary carbon atom (C-5, δ_C 143.5) and two protons (H-3, δ_H 5.06; H-7, δ_H 4.34). Therefore, the structure of the new compound **1** was elucidated as 23-*O*-β-D-glucopyranosyl-7-hydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al, named momordicine V (Fig. 1).

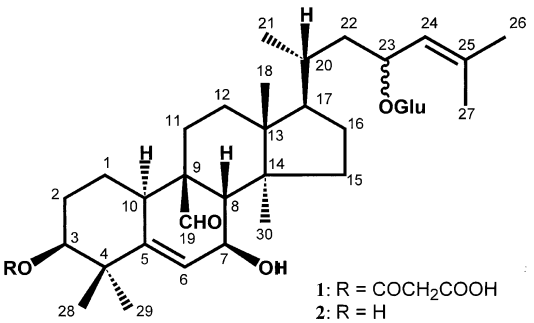

Fig. 1. Structures of compounds **1** and **2**.

The finding of the present investigation revealed that the leaf extract of *M. charantia* possesses oviposition deterrent activity against *L. trifolii*, which is a notorious pest of flower and vegetable crops in greenhouses and open fields. The biological activity of the plant extract is due to the presence of compound **1** along with other cucurbitane triterpenoids previously reported (Mekuria *et al*., 2005, 2006) in the plant. These compounds may jointly or independently contribute to oviposition deterrent activity against *L. trifolii*. There are only a few reports (Kashiwagi *et al*., 2005a, b), dealing with the chemical aspects of plant defense based on ovipositional deterrence. It will be, therefore, more important to study the host selection based on ovipositional deterrence. These compounds may be used as an alternative against insecticides and as a guide for the design of safe and environmental control methods for this species.

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