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Antifeedants of Finger Millet, *Eleusine coracana* GAERTN, Against Brown Planthopper, *Nilaparvata lugens* (STÅL)

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Nine compounds isolated from finger millet as antifeedants for brown planthopper have been identified as known compounds, L-malic acid, isocitric acid, 4-hydroxybenzoic acid, vanillic acid, 4-hydroxybenzaldehyde, and vitexin, and new constituents, 2-*O*-[4-hydroxy-(*Z* and *E*)-cinnamoyl]glyceric acid and 8-*C*-*β*-D-[6″-*O*-(3-hydroxy-3-methyl)glutaroyl]glucopyranosylapigenin.

During a study of the feeding behavior of three species of planthopper, the brown planthopper, *Nilaparvata lugens* (STÅL), the white-back planthopper, *Sogatella furcifera* (HORVÁTH), and the smaller brown planthopper, *Laodelphax striatellus* (FALLÉN), finger millet (*Eleusine coracana* GAERTN) was found to be resistant to *N. lugens* and *S. furcifera*. Through biological and chemical studies, we have come to the conclusion that resistance of this millet to each planthopper species is due mainly to the presence of multiple antifeedants. This paper deals with isolation and identification of antifeedants of *N. lugens* in finger millet and presents feeding responses to the isolated compounds.

When a methanol extract of finger millet was dissolved in water and washed with *n*-hexane, the antifeedants were found in the aqueous phase, which was separated by column chromatography on cation and anion exchange resins. Only the acidic fraction had activity for feeding deterrent to *N. lugens*. The acidic fraction was then chromatographed on a reverse phase open column (ODS, 100–200 mesh, Fuji Davision Chemical Ltd.) eluted with water, 20% and 40% aqueous methanol, and methanol, successively. Bioassay showed that the combined fraction of water, 20% and 40% aqueous methanol eluents had the same level of activity as the original acidic fraction, while each fraction alone was much less active, as reported in our previous paper.[1] This indicates that the inhibitory activity for feeding is not attributable to a single component but to several components combined.

The water fraction, mainly composed of organic acids, was analyzed with a capillary GLC after methylation using methanolic hydrogen chloride. The 20% and 40% aqueous methanol fractions were analyzed with HPLC (column: Cosmosil 5Ph, 250 mm × 10 mm i.d., flow rate: 2 ml/min, detected at 254 nm) eluted with a mixture of water, methanol, acetonitrile, and acetic acid (70 : 20 : 10 : 1). Rice plants were also treated through the same procedure and the corresponding water, 20% and 40% aqueous methanol fractions of the acidic part were obtained. The results of GLC and HPLC analyses of each fraction are shown in Figs. 1 and 2. All analyses were done with a concentration equivalent to a 10% solution of the original extract, but a two-fold concentration was used in the GLC analysis of the water fraction of the rice plant extract. Nine compounds (**1**–**9**) were predominant in the finger millet extract, most of which were not detected or were at low levels in the rice plant extract.

As described above, the antifeedants of finger millet against *N. lugens* were composed of multiple compounds. We therefore tried to isolate compounds which were predominant in the active fractions of the finger millet extract, and tested the feeding inhibitory activity of the isolated compounds to *N. lugens*.

Compound **2** was esterified with methanolic hydrogen chloride and isolated as a single compound by HPLC (column: YMC pack, Silicagel, 250 mm × 8 mm i.d., flow rate: 2 ml/min, detected by refractive index) eluted with a mixture of 40% ethyl acetate in *n*-hexane. Other compounds were isolated as a single compound through chromatography on ODS open column and repeated preparative HPLC eluted with 1% acetic acid in 0–40% aqueous methanol.

Compound **1** ($[\alpha]_D^{24}$ −6°, *c* 10, MeOH), **3**, **4**, and **5** were identified as L-malic acid, 4-hydroxybenzoic acid, vanillic

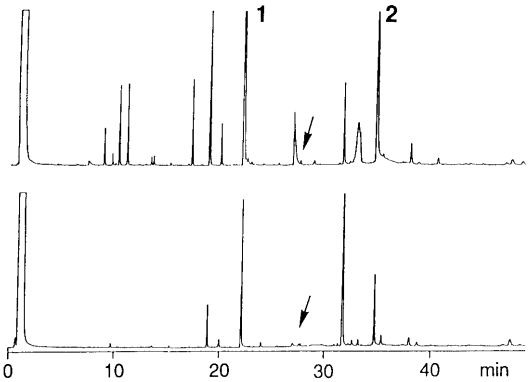

Fig. 1. GLC Chromatograms of the Methyl Esters of the Water Eluted Fraction of Finger Millet Extract (Above) and Rice Plant Extract (Below).
The arrow designate the position of trimethyl ester of *trans*-aconitic acid.

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acid, and 4-hydroxybenzaldehyde, respectively, by comparing ^{1}H-NMR and ^{13}C-NMR, MS, and optical rotation with those of authentic ones.

The methyl ester of compound **2** ($[\alpha]_D^{24}$ +12.5°, *c* 1, MeOH) was identified as trimethylisocitrate, by comparing ^{1}H-NMR, ^{13}C-NMR, and GC-MS with those of the authentic compound. Compound **2** was therefore identified as (+)-isocitric acid.

The molecular weights of compound **6** ($[\alpha]_D^{24}$ +70°, *c* 1.5, MeOH) and **7** ($[\alpha]_D^{24}$ −55°, *c* 1.5, MeOH) were measured from secondary ion mass spectra (SIMS) to be the same (M+H$^+$, 253). The ^{1}H-NMR spectrum of **7** had characteristic A_2B_2 type doublet signals at δ 6.81 and δ 7.48 (each 2H, J=8.4 Hz), *trans*-olefinic signals at δ 6.41 and δ 7.74 (each 1H, J=15.8 Hz), and a methylene doublet at δ 3.98 coupled with a methine triplet at δ 5.18 (J=4.4 Hz).

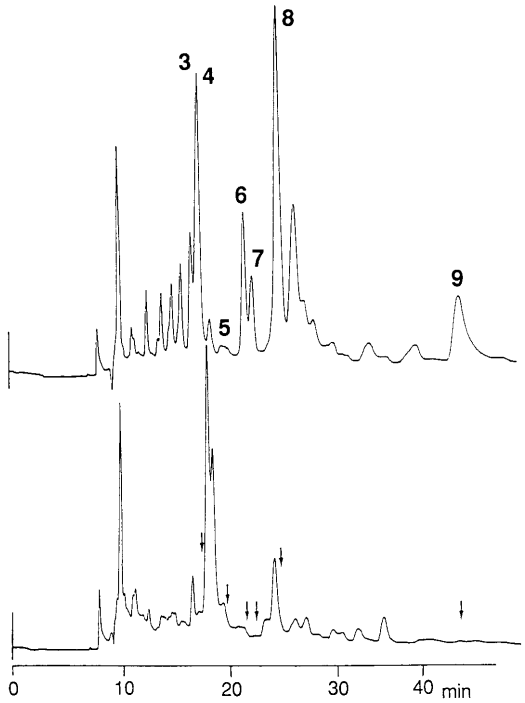

Fig. 2. HPLC Chromatograms of the ODS Eluent of 20% and 40% Aqueous Methanol Fractions of Finger Millet Extract (Above) and Rice Plant Extract (Below).

When ^{1}H-NMR and ^{13}C-NMR spectral data (see Table I) of **7** were compared with those of glyceric acid, significant differences occured in the C-2 and C-3 proton signals of **7**. That is to say, C-2 and C-3 protons had shifted by 1.22 ppm and 0.43 ppm, respectively. In addition, analysis of the carbon signals showed that the C-2 carbon of glyceric acid moiety had shifted downfield by 2.2 ppm and the C-3 carbon had shifted upfield by 2.8 ppm. Such shifts typically indicate acylation[2,3] at the C-2 position of glyceric acid. Accordingly, compound **7** is assigned the structure (−)-2-[4-hydroxy-(*E*)-cinnamoyl]glyceric acid.

The ^{1}H-NMR, ^{13}C-NMR, and EIMS spectra of compound **6** gave very similar patterns to those of compound **7** (Table I). Analysis of ^{1}H-NMR of compound **6** also found typical acylation[2,3] at the C-2 position of glyceric acid, and with a smaller coupling constant (J= 12.9 Hz) at the olefinic signals than that observed for compound **7**. These data indicated that compounds **6** and **7** differed only in the geometric environment at the olefinic position. When the methanol solution of this compound **6** was exposed to ultraviolet light (254 nm), the presence of compound **7** was detected afterwards by HPLC analysis. The newly generated compound **7** was isolated through chromatography on HPLC and its structure identified by ^{1}H-NMR, ^{13}C-NMR, and SIMS. Compound **6** was also generated from compound **7** through the same treatment at almost equimolar quantity. Though both compounds have a reverse optical rotation value, these compounds are at equilibrium under ultraviolet light exposure. Accordingly, compound **6** is assigned the structure (+)-2-[4-hydroxy-(*Z*)-cinnamoyl]glyceric acid. Though the absolute configurations of these compounds are unknown, they are new natural compounds (Fig. 3).

From SIMS, the molecular weights of compounds **8** and **9** were found to be 432 and 576, respectively. Compound **8** was identified by ^{1}H-NMR and ^{13}C-NMR spectra with

Compound **7**

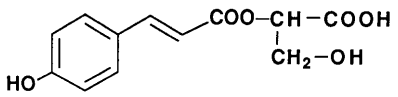

Fig. 3. Structure of Compound **7**.

Table I. ^{13}C- and ^{1}H-NMR Assignments of Comound **6**, **7**, and Glyceric Acid

C[a]/H[b]	C-Position No.	Compound **6**		Compound **7**		Glyceric acid	
		C	H	C	H	C	H
Glyceric acid	1	169.6	—	169.6	—	174.2	—
	2	74.0	5.11, (t, 4.4)	73.9	5.18, (t, 4.4)	71.7	3.96, (t, 4.5)
	3	60.7	3.94, (d, 4.4)	60.8	3.98, (d, 4.4)	63.6	3.55, (d, 4.5)
4-Hydroxy-cinnamoyl	1	165.4	—	166.0	—		
	2	114.6	5.89, (d, 12.9)	113.7	6.41, (d, 15.8)		
	3	144.6	6.78, (d, 12.9)	145.4	7.74, (d, 15.8)		
	1′	125.3	—	130.2	—		
	2′,6′	132.7	7.49, (d, 8.5)	130.2	7.48, (d, 8.4)		
	3′,5′	114.8	6.74, (d, 8.5)	115.8	6.81, (d, 8.4)		
	4′	158.9	—	159.9	—		

[a] Chemical shifts in δ (in DMSO-d_6).
[b] Chemical shifts in δ (in CD_3OD), multiplicities and J-values in Hz.

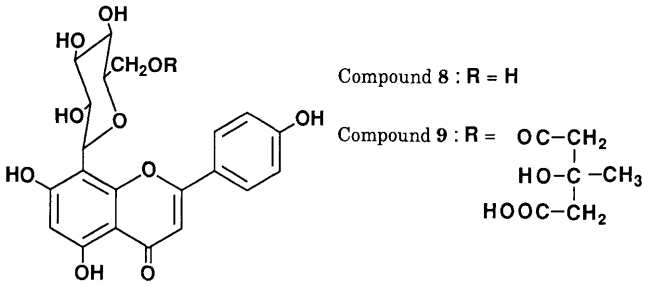

Fig. 4. Structures of Compound **8** (Vitexin) and **9**.

Table II. ^{13}C-NMR (in DMSO-d_6) Assignments of Compound **9** and Vitexin (**8**)

	C-Position	Compound **9** δ	Vitexin (**8**) δ
Apigenin	4	182.0 (s)	182.0 (s)
	2	163.7 (s)	163.9 (s)
	7	162.6 (s)	162.5 (s)
	4′	161.2 (s)	161.1 (s)
	5	160.5 (s)	160.4 (s)
	9	155.9 (s)	155.9 (s)
	2′,6′	128.5 (d)	128.8 (d)
	1′	121.4 (s)	121.5 (s)
	3′,5′	115.9 (d)	115.9 (d)
	8	104.0 (s)	104.6 (s)
	10	104.0 (s)	104.0 (s)
	3	102.4 (d)	102.4 (d)
	6	98.1 (d)	98.1 (d)
Glucosyl	1	73.5 (d)	73.4 (d)
	2	70.6 (d)	70.8 (d)
	3	78.3 (d)	78.6 (d)
	4	70.5 (d)	70.8 (d)
	5	78.3 (d)	81.7 (d)
	6	64.1 (t)	61.3 (t)
3-Hydroxy-3-methyl-glutaroyl	1	170.6 (s)	—
	2	44.9* (t)	—
	3	68.9 (s)	—
	4	45.3* (t)	—
	5	172.6 (s)	—
	3-Me	27.2 (q)	—

* Interchangeable.

the aid of an authentic sample, *i.e.*, vitexin (8-*C*-β-D-glucopyranosylapigenin,[4,5] as shown in Fig. 4).

Compounds **9** and **8** resembled each other in their ^{1}H-NMR and ^{13}C-NMR spectra in the field of flavone and sugar regions. Acid hydrolysis of compound **9** led to 3-hydroxy-3-methylglutaric acid, identified with an authentic specimen after methylation with methanolic hydrogen chloride (t_R=9.58 min), and likewise vitexin by direct comparison with its corresponding authentic specimen. The ^{13}C-NMR and two-dimensional NMR (H–H COSY and C–H COSY) spectral analyses of the sugar carbon signals with reference to those of vitexin (Table II) showed that the only significant differences occurred in the C-5 and C-6 carbon signals of the glucose (G-5 and G-6, respectively). Comparing ^{13}C-NMR spectra of **8** and **9**, the G-5 carbon had shifted upfield by 3.4 ppm and the G-6 carbon had shifted downfield by 2.8 ppm in that of **9**. Such shifts typically indicate acylation[2,3] at the G-6 position. Since

Table III. The Amounts (mg%) of Antifeedants in 10% Solutions of Each Plant Extract

Compound No.	Compound	Finger millet	Rice plant
1	L-Malic acid	1075	23.3
2	Isocitric acid	616.7	10.0
3	4-Hydroxybenzoic acid	10.4	—
4	Vanilic acid	4.2	—
5	4-Hydroxybenzaldehyde	2.6	—
6	2-*O*-[4-Hydroxy-(*Z*)-cinnamoyl]glyceric acid	10.3	—
7	2-*O*-[4-Hydroxy-(*E*)-cinnamoyl]glyceric acid	8.4	—
8	Vitexin	41.7	—
9	6″-*O*-(3-Hydroxy-3-methyl-glutaroyl)vitexin	14.6	—

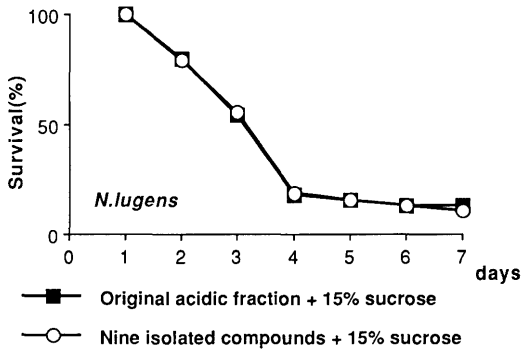

Fig. 5. Survival of the 3rd Instar Nymphs of *N. lugens* on the Original Acidic Fraction and the Nine Isolated Compounds.

there is no indication of acylation at any other sites in compound **9**, it is concluded that this compound is mono-acylated and thus defined as 8-*C*-β-D-[6″-*O*-(3-hydroxy-3-methyl)glutaroyl]glucopyranosylapigenin, a new natural compound (Fig. 4).

Contents of these compounds contained in the 10% solution of each original plant extract with estimated by HPLC using an authentic specimen or an isolated compound as an internal standard, as shown in Table III. As far as L-malic acid and isocitric acid are concerned, these compounds were also contained in the rice plant extract, but the amounts were far less than in the finger millet extract as shown in Table III.

The combined solution of the identified compounds as listed in Table III, and the original acidic fraction of the finger millet extract were neutralized with KOH and subjected to bioassay as described in our previous paper.[1]

As shown in Fig. 5, the nine isolated compounds showed the same level of activity on feeding of *N. lugens* as the original acidic fraction of the finger millet extract only when all of them were combined together. Although the activity of each compound alone was not assayed, it can be assumed that individually, they are much less active, as described above.

As shown in Fig. 1, it is characteristic to note that *trans*-aconitic acid, the antifeedant of barnyard grass against *N. lugens*,[6,7] was not detectable in both the finger millet extract and the rice plant extract, while this acid has been shown to be common in many species of plants.[8,9,10]

Through this study, it may be mentioned here that the antifeedant of barnyard grass against *N. lugens* must be reinvestigated and the participation of other components other than *trans*-aconitic acid must be evaluated.

It is concluded that the nine compounds listed in Table III were isolated from the finger millet as antifeedants of *N. lugens*, and that they showed the same level of activity as the finger millet extract only when they were combined together.

Experimental

Instruments. SIMS, EIMS, and GC-MS were recorded with a Hitachi M-80 mass spectrometer. SIMS were measured at 8 kV with Xe as the primary beam gas, and the samples were put on a silver sample stage mixed with glycerol. EIMS and GC-MS were measured at 70 eV (in beam). GLC analyses were done with a Hewlett Packard 5790A with fused silica column (25 m × 0.2 mm i.d.) coated with OV-101 0.25 μm thickness, programmed from 70°C (2 min holding) to 300°C at a rate of 4°C/min. Optical rotation was measured with a JASCO ORD Model J-5 spectropolarimeter. ^{1}H-NMR and ^{13}C-NMR of compound **9** including the two-dimensional correlation spectra were measured with a Brucker AC-250 (250 MHz) instrument, and those of other compounds were measured with a JEOL JNMFX90Q spectrometer (90 MHz). TMS was used as the 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.

Hydrolysis of compound **9**. Compound **9** (500 μg) was mixed with 1 ml of 5% hydrochloric acid in water, and was heated at 80°C for 1 h. The solution was passed through a Sep-pak C_{18} cartridge (Waters) eluted with 10 ml of water, and then with 10 ml of 40% aqueous methanol. 3-Hydroxy-3-methylglutaric acid was recovered from the water eluent and vitexin was obtained from the 40% aqueous methanol eluent.

Compound **6** [(+)-2-[4-hydroxy-(*Z*)-cinnamoyl]glyceric acid]. $[\alpha]_D^{24}$ +70° (*c* 1.5, MeOH). EIMS *m/z* (%): 207 (0.3), 164 (27), 148 (11), 147 (100), 119 (23), 91 (19), 65 (11). SIMS *m/z* (%): 253 ($M+H^+$, 48), 207 (22), 147 (100). ^{1}H-NMR and ^{13}C-NMR: listed in Table I.

Compound **7** [(−)-2-[4-hydroxy-(*E*)-cinnamoyl]glyceric acid]. $[\alpha]_D^{24}$ −55° (*c* 1.5, MeOH). EIMS *m/z* (%): 164 (31), 148 (10), 147 (100), 119 (22), 91 (14.). SIMS *m/z* (%): 253 ($M+H^+$, 48), 207 (22), 147 (100). ^{1}H-NMR and ^{13}C-NMR: listed in Table I.

Compound **9** [8-*C*-β-D-[6″-*O*-(3-hydroxy-3-methyl)glutaroyl]glucopyranosylapigenin]. SIMS *m/z* (%): 577 ($M+H^+$, 5), 313 (14), 369 (matrix peaks, 4 × glycerol + H^+, 100). ^{1}H-NMR (250 MHz, DMSO-d_6 + D_2O) δ: 7.96 (2H, d, *J*=8.6, H-2′, 6′), 6.95 (2H, d, *J*=8.6, H-3′, 5′), 6.78 (1H, s, H-3), 6.28 (1H, s, H-6), 4.75 (1H, d, *J*=9.9, H-1″), 4.39 (1H, d, *J*=11.4, H-6″b), 4.05 (1H, dd, *J*=5.7 and 11.4, H-6″a), 3.91 (1H, t, *J*=9.0, H-4″), 3.48 (1H, dd, *J*=9.0 and 5.7, H-5″), 3.41 (1H, dd, *J*=9.9 and 8.5, H-2″), 3.27 (1H, dd, *J*=8.5 and 9.0, H-3″), 2.49 (2H, br s, H-2‴), 2.40 (2H, br s, H-4‴), 1.16 (3H, s, H-6‴). ^{13}C-NMR: lited in Table II.

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