Antifeedants of Indian Barnyard Millet, *Echinochloa frumentacea* Link, against Brown Planthopper, *Nilaparvata lugens* (Stål)

Chul-Sa Kim*, Kabir Md Alamgir, Sachi Matsumoto, Shin-ichi Tebayashi, and Hen-Sik Koh

Life Chemistry, Faculty of Agriculture, Kochi University, B200 Monobe, Nankoku, 783–8502, Japan. Fax: +81-888-64-5186. E-mail: cs-kim@kochi-u.ac.jp

* Author for correspondence and reprint requests

Z. Naturforsch. **63 c**, 755–760 (2008); received February 7/March 10, 2008

Eight compounds isolated from Indian barnyard millet have been identified as L-malic acid, *trans*-aconitic acid, (+)-isocitric acid, 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, isocarlinoside, 2″-*O*-rhamnosylisoorientin, and 7-*O*-(2″-*O*-glucuronosyl)glucuronosyltricin, respectively. These compounds showed high antifeeding activity against brown planthopper only when they were combined.

Key words: Nilaparvata lugens, Antifeedant, *Echinochloa frumentacea*

Introduction

Three species of planthopper, brown planthopper [*Nilaparvata lugens* (Stål)], white-black planthopper [*Sogatella furcifera* (Horváth)], and smaller brown planthopper [*Laodelphax striatellus* (Fallén)], are well known as notorious rice pests in many Asian countries including Korea, China, and Japan. Continuous study of the feeding behaviour of these planthoppers led to find out that Indian barnyard millet, *Echinochloa frumentacea* Link, is resistant to *N. lugens* and *S. furcifera,* but susceptible to *L. striatellus* (Kim *et al*., 1994a). 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 antifeedants (Kim *et al*., 1994a). This paper deals with isolation and identification of antifeedants of *N. lugens* in Indian barnyard millet and presents feeding responses to the isolated compounds.

Materials and Methods

Insect

The brown planthopper has been reared successively on rice seedlings at 25 °C, under 14 h illumination.

Plant

Indian barnyard millet was grown in a greenhouse with no pesticides.

Separation of the antifeedants of N. lugens

Fresh stems and leaves of Indian barnyard millet (4.8 kg) were cut in rather large pieces (about 10 cm long), immersed in methanol (7 l) for 4 d, and decanted. This procedure was repeated three times. The combined methanol extract was evaporated under reduced pressure. The residue was dissolved in water (2.5 l) and washed three times with hexane (1.5 l × 3). The aqueous phase was evaporated to dryness, leaving a brownish oil (108.6 g). This oil (51.6 g) was separated into neutral (16.35 g), basic (4.82 g), and acidic (10.09 g) fractions by column chromatography on cation exchange resin (Dowex 50W X 8, H^+ form, 200–400 mesh) eluted with 2 N NH_4OH and subsequently on anion exchange resin (Dowex 1 X 8, formate form, 200–4000 mesh) eluted with 20 N formic acid. The acidic fraction was then chromatographed on a reverse phase open column (ODS, 100–200 mesh, Fuji Silysia Chemical Ltd.) eluted with water, 20% aqueous methanol, 40% aqueous methanol, and methanol to obtain ODS H_2O (2.84 g), ODS 20% MeOH (0.58 g), ODS 40% MeOH (0.33 g), and ODS MeOH (0.08 g) fractions.

Instruments

SI-, EI- and GC-mass spectra were recorded with a Hitachi M-80 mass spectrometer. SIMS data 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. GC-MS was

measured at 70 eV. GLC analyses were done with a Hewlett Packard 5790A instrument with a fused silica column (25 m × 0.2 mm i. d.) coated with OV-101 (0.25 mm thickness), programmed from 70 °C (2 min holding) to 300 °C at a rate of 4 °C/min. The 20% aqueous methanol fraction was analyzed by HPLC (column, Cosmosil 5Ph, 250 mm × 10 mm i. d.; flow rate, 2 ml/min; detection at 254 nm) eluted with a mixture of water, methanol, acetonitrile, and acetic acid (85:10:5:1 v/v/v). The 40% aqueous methanol fraction was analyzed by HPLC (column, Cosmosil 5Ph, 250 mm × 10 mm i. d.; flow rate, 2 ml/min; detection at 254 nm) eluted with a mixture of water, methanol, acetonitrile, and acetic acid (70:20:10:1 v/v/v). Optical rotation was measured with a JASCO ORD Model J-5 spectropolarimeter. ^{1}H NMR and ^{13}C NMR spectra including two-dimensional correlation spectra were measured with a JNM AL400 (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.

L-Malic acid (**1**): t_R = 21.6 min. – $[\alpha]_D^{24}$ −6.0° (*c* 10, MeOH). – ^{1}H NMR (D_2O): δ = 4.66 (1H, t, *J* = 5.8), 2.95 (2H, d, *J* = 5.8). – GC-MS (as methyl ester): *m*/*z* (%) = 130(4.0), 103(100), 71(13), 59(25), 43(56).

trans-Aconitic acid (**2**): t_R = 26.7 min. – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 2.49 (2H, s), 6.70 (1H, s). – ^{13}C NMR (DMSO-d_6 + D_2O): δ = 32.8 (t), 128.9 (d), 140.3 (s), 166.6 (s), 167.4 (s), 171.1 (s). – GC-MS (as methyl ester): *m*/*z* (%) = 184(98), 157(45), 156(94), 153(100), 125(53), 113(29), 59(80).

(+)-Isocitric acid (**3**): t_R = 34.2 min. – $[\alpha]_D^{24}$ +12.5° (*c* 1.0, MeOH). – ^{13}C NMR ($CDCl_3$ as methyl ester): δ = 32.2 (t), 45.0 (d), 52.0 (q), 52.3 (q), 52.9 (q), 70.7 (d), 171.3 (s), 172.1 (s), 173.4 (s). – GC-MS (as methyl ester): *m*/*z* (%) = 175(20), 146(16), 143(66), 115(100), 83(17), 59(21).

5-O-Caffeoylquinic acid (**4**): SIMS: *m*/*z* (%) = 355 ($M+H^+$, 3.8), 185 (matrix peaks, 2× glycerol+H^+, 100), 163(20). – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 7.47 (Ca-3, 1H, d, *J* = 16.0), 7.03 (Ca-2′, 1H, d, *J* = 1.6), 6.98 (Ca-6′, 1H, dd, *J* = 1.6, 8.1), 6.77 (Ca-5′, 1H, d, *J* = 8.1), 6.21 (Ca-2, d, *J* = 16.0), 5.19 (Q-5, 1H, m), 3.86 (Q-3, 1H, m), 3.55 (Q-4, 1H, m), 2.2 ~ 1.7 (Q-2, 6, 4H, m). – ^{13}C NMR (DMSO-d_6 + D_2O): δ = 175.2 (Q-COOH, s), 169.1 (Ca-COO-, s), 149.4 (Ca-4′, s), 146.9 (Ca-3, d), 146.8 (Ca-3′, s), 128.1 (Ca-1′, s), 122.9 (Ca-6′, d), 116.5 (Ca-5′, d), 115.9 (Ca-2′, d), 115.3 (Ca-2, d), 75.5 (Q-1, s), 74.9 (Q-4, d), 73.0 (Q-5, d), 68.4 (Q-3, d), 41.5 (Q-2, t), 36.8 (Q-6, t) (Ca, caffeic acid; Q, quinic acid).

4-O-Caffeoylquinic acid (**5**): SIMS: *m*/*z* (%) = 355 ($M+H^+$, 8.7), 185 (matrix peaks, 2× glycerol+H^+, 41), 163(100). – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 7.51 (Ca-3, 1H, d, *J* = 16.0), 7.05 (Ca-2′, 1H, d, *J* = 1.2), 7.01 (Ca-6′, 1H, dd, *J* = 1.2, 8.1), 6.78 (Ca-5′, 1H, d, *J* = 8.1), 6.29 (Ca-2, d, *J* = 16.0), 4.71 (Q-4, 1H, dd, *J* = 3.0, 7.8), 4.11 (Q-3, 1H, m), 2.2 ~ 1.7 (Q-2, 6, 4H, m). – ^{13}C NMR (DMSO-d_6 + D_2O): δ = 175.2 (Q−COOH, s), 169.1 (Ca−COO−, s), 149.4 (Ca-4′, s), 146.9 (Ca-3, d), 146.8 (Ca-3′, s), 128.1 (Ca-1′, s), 122.9 (Ca-6′, d), 116.5 (Ca-5′, d), 115.9 (Ca-2′, d), 115.3 (Ca-2, d), 76.4 (Q-1, s), 79.3 (Q-4, d), 69.7 (Q-5, d), 65.1 (Q-3, d), 42.7 (Q-2, t), 38.5 (Q-6, t) (Ca, caffeic acid; Q, quinic acid).

Isocarlinoside (6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylluteolin) (**6**): SIMS: *m*/*z* (%) = 581 ($M+H^+$, 1.0), 505 (1.0), 185 (matrix peaks, 2× glycerol+H^+, 100), 131(5.0). – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 7.41 (Lu-2′, 6′, 2H, m), 6.94 (Lu-5′, 1H, d, *J* = 8.4), 6.61 (Lu-3, 1H, s), 5.0 ~ 3.2 (G-1 ~ 6 and A-1~5, 11H, m). – ^{13}C NMR: δ = 182.0 (Lu-4, s), 164.1 (Lu-2, s), 164.7 (Lu-7, s), 158.0 (Lu-5, s), 154.9 (Lu-9, s), 149.6 (Lu-4′, s), 145.7 (Lu-3′, s), 121.8 (Lu-6′, d), 119.3 (Lu-1′, s), 115.5 (Lu-5′, d), 114.0 (Lu-2′, d), 103.6 (Lu-10, s), 102.5 (Lu-3, d), 108.0 (Lu-6, s), 104.9 (Lu-8, s), 73.2 (G-1, d), 70.7 (G-2, d), 78.9 (G-3, d), 70.1 (G-4, d), 82.0 (G-5, d), 61.5 (G-6, t), 73.8 (A-1, d), 68.4 (A-2, d), 74.1 (A-3, d), 69.5 (A-4, d), 70.8 (A-5, t) (Lu, luteolin; G, glucose; A, arabinose).

2″-O-Rhamnosylisoorientin (**7**): SIMS: *m*/z (%) = 595 ($M+H^+$, 14), 185 (matrix peaks, 2× glycerol+H^+, 100), 131 (1.0). – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 7.41 (Lu-2′, 6′, 2H, m), 6.96 (Lu-5′, 1H, d, *J* = 8.8), 6.63 (Lu-3, 1H, s), 6.56 (Lu-8, 1H, s), 5.07 (R-1, 1H, br.s), 4.75 (G-1. 1H, d, *J* = 9.8), 4.25 (G-2, 1H, m), 3.2 ~ 3.7 (G-3,4,5,6, 5H, m), 2.43 (R-5, 1H, dq, *J* = 9.4, 6.0), 0.62 (R-6, 3H, d, *J* = 9.4). – ^{13}C NMR: δ = 181.9 (Lu-4, s),

163.4 (Lu-2, s), 162.7 (Lu-7, s), 161.4 (Lu-5, s), 156.6 (Lu-9, s), 149.4 (Lu-4′, s), 145.5 (Lu-3′, s), 121.7 (Lu-1′, s), 119.1 (Lu-6′, d), 116.2 (Lu-5′, d), 113.3 (Lu-2′, d), 108.6 (Lu-6, s), 103.7 (Lu-10, s), 103.0 (Lu-3, d), 93.9 (Lu-8, d), 80.9 (G-5, d), 79.5 (G-3, d), 75.7 (G-2, d), 71.6 (G-1, d), 70.5 (G-4, d), 61.3 (G-6, t), 100.6 (R-1, d), 71.7 (R-4, d), 70.5 (R-2, d), 70.4 (R-3, d), 68.2 (R-5, d), 17.4 (R-6, q) (Lu, luteolin; G, glucose; R, rhamnose).

7-O-(2″-O-Glucuronosyl)glucuronosyltricin (**8**): SIMS: *m*/z (%) = 683 ($M+H^+$, 14), 329(100), 229(45), 185 (matrix peaks, 2× glycerol+H^+, 100). – ^{1}H NMR (DMSO-d_6 + D_2O): δ = 7.26 (T-2′, 6′, 2H, s), 6.82 (T-6, 8, 2H, m), 6.38 (T-3, 1H, s), 3.93 (OMe-3′, 5′, 6H, s), 6.38 (Gl-1, 1H, d, J = 6.7), 4.03 (Gl-5. 1H, d, J = 9.5), 3.50 (Gl-2′, 3′, 2H, m), 3.42 (Gl-4, 1H, t, J = 9.5), 4.54 (Gl-1′, 1H, d, J = 7.8), 3.65 (Gl-5′, d, J = 9.3), 3.2 ~ 3.3 (Gl-3′,4′, 2H, m), 3.00 (Gl-2′, 1H, dq, J = 7.8, 8.3). – ^{13}C NMR: δ = 182.0 (T-4, s), 164.1 (T-2, s), 162.1 (T-7, s), 160.8 (T-5, s), 156.8 (T-9, s), 148.0 (T-3′, 5′, s), 139.6 (T-4′, s), 120.3 (T-1′, s), 105.4 (T-10, s), 104.3 (T-2′, 6′, d), 103.8 (T-3, d), 99.0 (T-6, d), 95.2 (T-8, d), 56.2 (T-3′, 5′-OMe, q), 170.0 (Gl-6, s), 97.8 (Gl-1, d), 82.3 (Gl-2, d), 74.6 (Gl-3, d), 74.6 (Gl-5, d), 70.5 (Gl-4, d), 170.0 (Gl-6′, s), 104.2 (Gl-1′, d), 75.5 (Gl-5′, d), 75.2 (Gl-3′, d), 73.8 (Gl-2′, d), 71.4 (Gl-4′, d) (T, tricin; Gl, glucuronic acid).

Hydrolysis of compound ***8***

Compound **8** (500 mg) 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 C18 cartridge (Waters) eluted with 10 ml of water, and then with 10 ml of 40% aqueous methanol. Glucuronic acid was recovered from the water eluent and tricin was obtained from the 40% aqueous methanol eluent.

Results and Discussion

The aqueous phase showed high antifeeding activity against *N. lugens* after the methanol extract of Indian barnyard millet stems and leaves was defatted with hexane. This phase was separated into acidic, neutral, and basic fractions by column chromatography on cation and anion exchange resins. Of these fractions, only the acidic fraction had activity as feeding deterrent to *N. lugens* (Fig. 1A). The acidic fraction was then chromatographed on a reverse phase open column (ODS, 100–200 mesh) eluted with water, 20% and 40% aqueous methanol, and methanol, successively. The 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. This indicates that the inhibitory activity for feeding is not attributable to a single component but to several components combined (Fig. 1A).

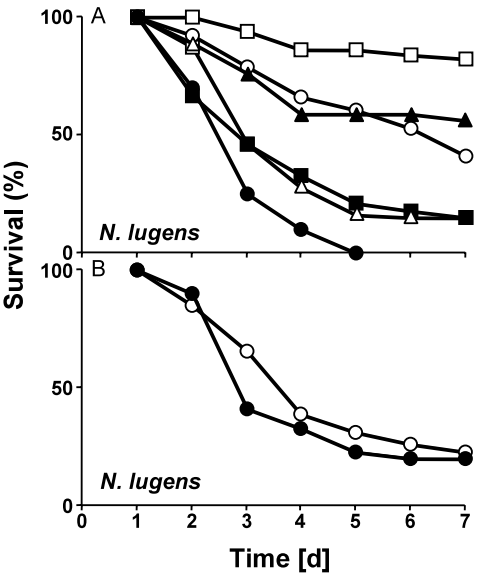

Fig. 1. Survival of the 3rd instar nymphs of *N. lugens* on the several fractions. (A) (●) Original aqueous phase; (■) acidic fr.; (○) ODS H_2O fr.; (▲) ODS 20% + 40% MeOH; (□) ODS MeOH fr.; (△) ODS H_2O + ODS 20% + 40% MeOH. (B) (●) Acidic fr.; (○) isolated compounds **1**–**8**.

The water fraction, mainly composed of organic acids, was analyzed by capillary GLC after methylation using methanolic hydrogen chloride. The 20% and 40% aqueous methanol fractions were analyzed by HPLC (column, Cosmosil 5Ph, 250 mm × 10 mm i. d.; flow rate, 2 ml/min; detection at 254 nm) eluted with a mixture of water, methanol, acetonitrile, and acetic acid. Rice plant was also treated through the same procedure, and the corresponding water, 20% and 40% aqueous methanol fractions of the acidic part were obtained. 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. Repeated bioassays guided to isolate compounds **1**–**8** (Fig. 2) as antifeedants against *N.*

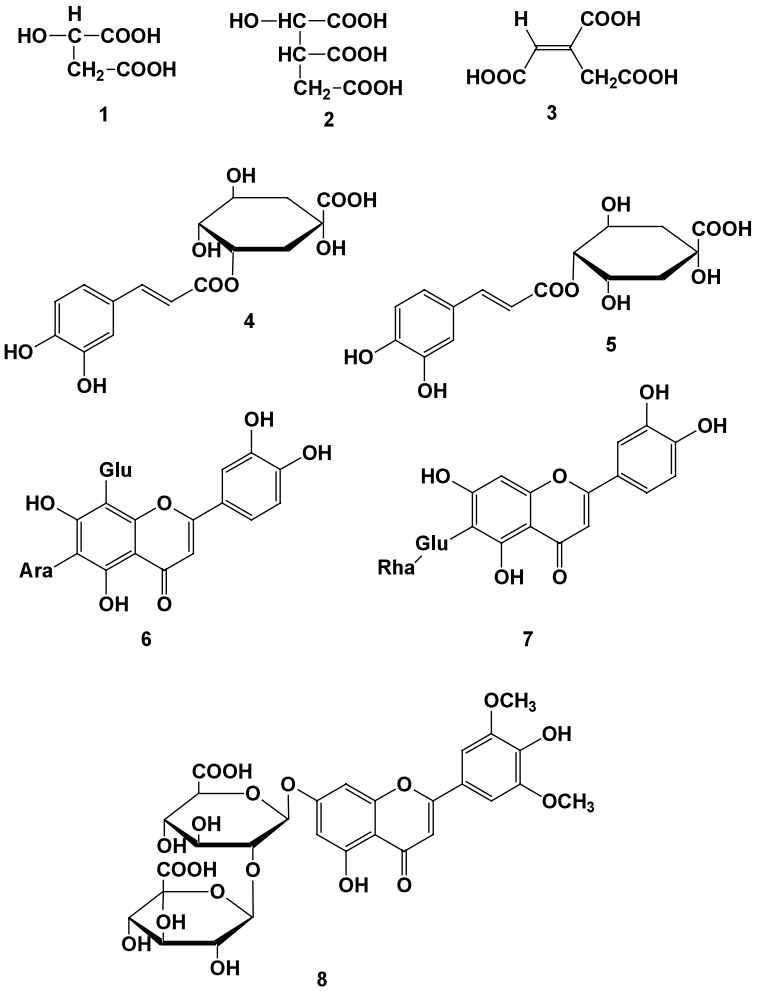

Fig. 2. Chemical structures of the isolated compounds.

lugens from the Indian barnyard millet extract, most of which were not detected or were at low levels in the rice plant extract.

Compounds **1**, **2**, and **3** in the water eluate were identified as L-malic acid, *trans*-aconitic acid, and (+)-isocitric acid, respectively, by comparing ^{1}H and ^{13}C NMR and mass spectra, and optical rotation values with those of authentic samples after methylation by using 5% hydrogen chloride in methanol or being intact. L-Malic acid and (+)-isocitric acid were reported as a part of the antifeedants against *N. lugens* from finger millet, *Eleusine coracana* Gaertn. (Kim *et al.*, 1994b). *trans*-Aconitic acid was also reported as an antifeedant against *N. lugens* from barnyard grass, *Echinochloa oryzicola* Vasing (Kim *et al.*, 1975, 1976).

The ^{1}H and ^{13}C NMR, and secondary ion mass spectra (SIMS) of compounds **4** and **5** gave very similar patterns, and the molecular weights of compounds **4** and **5** were estimated from the SIMS spectra to be the same ($M+H^{+}$, 355). The ^{1}H NMR spectra of **4** and **5** had characteristic 1,3,4-substituted benzene ring signals, *trans*-olefinic signals with the coupling constant J = 16.0 Hz, and quinic acid moieties at δ 3.86 ~ δ 5.19 (compound **4**) and δ 3.86 ~ δ 4.71 (compound **5**), respectively. The methine proton of position 5 in the quinic acid moiety of compound **4** and that of position 4 of compound **5** were shifted downfield in the ^{1}H NMR spectra. These downfield shifts clearly indicate the typical acylation effect on these positions, respectively (Couperus *et al.*, 1978; Wenkert *et al.*,

1978). Compounds **4** and **5** were, therefore, identified as 5-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid by comparing the spectral data of compounds **4** and **5** with those of authentic chlorogenic acid (3-*O*-caffeoylquinic acid). Chlorogenic acid is well known as one of the widely distributed common phenylpropanoid derivatives in many plants, but could not be detected in either rice plant or Indian barnyard millet.

Compounds **6**, **7**, and **8** were expected from their ^{1}H NMR and ^{13}C NMR spectra in the fields of aromatic and oxygen-attached regions as flavonoid glycosides. The molecular weights of compounds **6**, **7**, and **8** were guessed from the SIMS spectra to be 580, 594, and 682, respectively. Compounds **6** and **7** were identified as isocarlinoside and 2″-*O*-rhamnosylisoorientin by comparing the spectral data of compounds **6** (Wenkert *et al.*, 1978) and **7** (Agrawal *et al.*, 1989). The two-dimensional NMR (H-H COSY, C-H COSY, and HMQC) data of these compounds also suggested these identifications.

Compound **8** has a tricin moiety as an aglycon as judged from the ^{1}H and ^{13}C NMR spectra in the aromatic field and is an *O*-glycoside because three protons at positions 3, 6, and 8 in the aglycon were observed. Two mole of the glucuronic acid were obtained after acid hydrolysis. The carbon atom at position 2 in one of the glucuronic acid moieties was shifted downfield in the ^{13}C NMR spectrum, and correlations were observed between the anomeric proton and the methine carbon atom at position 2 and between the other anomeric proton and the carbon atom at position 7 in the aglycon by two-dimensional NMR spectroscopy, respectively. Compound **8** was, therefore, identified as 7-*O*-(2″-*O*-glucuronosyl)glucuronosyltricin. This compound is a very rare flavonoid glycoside, which has been only found in alfalfa, *Medicago sativa* L. (Stochmal *et al.*, 2001).

Contents of these isolated compounds contained in the 10% solution of each original plant extract were estimated by HPLC, using an authentic specimen or an isolated compound as an internal standard, as shown in Table I. As far as L-malic acid (**1**) and (+)-isocitric acid (**3**) are concerned, these compounds were also contained in the rice plant extract, but the amounts were far less than those in the Indian barnyard millet extract as shown in Table I.

The combined solution of the identified compounds, as listed in Table I, and the original acidic fraction of Indian barnyard millet were neutralized with KOH and subjected to a bioassay as described in our previous paper (Kim *et al.*, 1994a).

Table I. Amounts (μg) of antifeedants in 1 ml of 10% solutions of each plant extract.

Compound	Indian barnyard millet	Rice plant
L-Malic acid (**1**)	2950	1380
trans-Aconitic acid (**2**)	6800	ND
(+)-Isocitric acid (**3**)	3420	570
5-*O*-Caffeoylquinic acid (**4**)	270	ND
4-*O*-Caffeoylquinic acid (**5**)	220	ND
Isocarlinoside (**6**)	50	ND
2″-*O*-Rhamnosylisoorientin (**7**)	70	ND
7-*O*-(2″-*O*-Glucuronosyl)-glucuronosyltricin (**8**)	130	ND

ND, not detectable.

As shown in Fig. 1B, the eight isolated compounds showed the same level of activity on feeding of *N. lugens* as the original acidic fraction of the Indian barnyard millet extract only when all of them were combined.

It is characteristic to note that *trans*-aconitic acid (**2**), the antifeedant of barnyard grass against *N. lugens* (Kim *et al.*, 1975, 1976), was also detectable in the Indian barnyard millet extract but not in the rice plant extract. The amount of **2** in Indian barnyard millet seems to be enough to show antifeeding activity against *N. lugens* because it is almost the same amount as that in barnyard grass (Kim *et al.*, 1975, 1976). Though the water eluate of Indian barnyard millet contains enough amount of *trans*-aconitic acid, the fraction did not show strong activity alone. This result clearly indicates that the antifeedant of barnyard grass against *N. lugens* must be reinvestigated and that the participation of components other than *trans*-aconitic acid must be evaluated, though this acid has been shown to be common in many species of plants including gramineous plants (Burau, 1969; Clark, 1969; Stout *et al.*, 1967) and may be still one of the key active compounds involved in the resistance of gramineous plants against *N. lugens.*

In our previous report (Kim *et al.*, 1994b), we isolated and identified nine compounds as antifeedants against *N. lugens* from finger millet, *i.e.*, L-malic acid and (+)-isocitric acid were isolated from the water eluate of finger millet extract, five derivatives of phenylpropanoid and benzoic acid from the 20% aqueous methanol eluate, and two

flavonoid glycosides from the 40% aqueous methanol eluate. These compounds show high antifeeding activity against *N. lugens* only when they are combined, but each of these compounds does not show strong activity like those of Indian barnyard millet. Antifeedants against *N. lugens* isolated from finger millet and those from Indian barnyard millet are quite different except for L-malic acid and (+)-isocitric acid. Both results, however, may indicate that the antifeeding activity against this insect species expresses only when enough amounts of organic acid and appropriate phenylpropanoid derivatives and flavonoid glycosides exist and all of them are combined.

In near future, we will try to elucidate the resistance of Japanese barnyard millet, *Echinochloa utilis* Ohwi et Yabuno, which is resistant to *N. lugens*, but susceptible to *S. furucifera*, and the resistances of Indian barnyard millet and finger millet against *S. furucifera*, respectively.

Acknowledgements

We wish to thank Prof. H. Ohigashi, Kyoto University, Japan, for ORD spectra.

Agrawal P. K., Thakur R. S., and Bansal M. C. (1989), Flavonoids. Carbon-13 NMR of Flavonoids. Elsevier Science Publishers, Amsterdam, pp. 564.

Burau R. G. (1969), Polarographic estimation of aconitates in plant materials. J. Agric. Food Chem. **17**, 1322–1334.

Clark R. B. (1969), Organic acids from leaves of several crop plants by gas chromatography. Crop. Sci. **9**, 341–343.

Couperus P. A., Clague A. D. H., and van Dongon J. P. C. M. (1978), Carbon-13 chemical shifts of some model carboxylic acids and esters. Org. Magn. Reson. **11**, 590–597.

Kim M., Koh H., Ichikawa T., Fukami H., and Ishi S. (1975), Antifeedant of barnyard grass against the brown planthopper, *Nilaparvata lugens* (Stål). Appl. Entomol. Zool. **10**, 116–122.

Kim M., Koh H., Obata T., Fukami H., and Ishi S. (1976), Isolation and identification of *trans*-aconitic acid as the antifeedant in barnyard grass against the brown planthopper, *Nilaparvata lugens* (Stål). Appl. Entomol. Zool. **11**, 53–57.

Kim C.-S., Koh H., and Fukami H. (1994a), Antifeedants of rice planthoppers in some millets. Appl. Entomol. Zool. **29**, 71–79.

Kim C.-S., Koh H., Fukami H., and Irie R. (1994b), Antifeedants of finger millet, *Eleusine coracana* Gaertn., against brown planthopper, *Nilaparvata lugens* (Stål). Biosci. Biotechnol. Biochem. **58**, 380–383.

Stochmal A., Ana M., Macias F. A., and Olezex W. (2001), Alfalfa (*Medicago sativa* L.) flavonoids. 2. Tricin and chrysoeriol glycosides from aerial parts. J. Agric. Food Chem. **49**, 5310–5314.

Stout P. R., Brounell J., and Burau R. G. (1967), Occurrences of *trans*-aconitate in substituent effects reflected in the ^{13}C NMR spectra of allyl alcohols and their derivatives. Org. Magn. Reson. **7**, 51–53.

Wenkert E., Gasic M. J., Hagaman E. W., and Kwarl L. D. (1978), Long range substituent effects reflected in the ^{13}C NMR spectra of allyl alcohols and their derivatives. Org. Magn. Reson. **7**, 51–53.