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Isolation and Identification of *trans*-Aconitic Acid as the Antifeedant in Barnyard Grass Against the Brown Planthopper, *Nilaparvata lugens* (STÅL) (Homoptera : Delphacidae)

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The antifeedant of barnyard grass (var. *oryzicola*) against *N. lugens* was isolated and characterized as *trans*-aconitic acid. This acid was detected in barnyard grass but not in rice plant. *Trans*-aconitic acid, neutralized to pH 7 with calcium hydroxide, showed potent inhibitory activity for feeding of *N. lugens* at concentrations of 0.25% and 0.5% while the *cis*-isomer showed much less inhibitory activity.

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (STÅL), one of the serious pests of rice crops in Asian countries, often occurs in large numbers during outbreaks and causes considerable damage (referred to as "hopperburn") to rice plants during the ripening season. In a previous paper (KIM, et al., 1975) it was reported that barnyard grass, *Echinochloa crus-galli* (LINN.) Beauv. var *oryzicola* (Vasing.) Ohwi, did not suffer damage from *N. lugens* in a paddy field where serious "hopperburn" was observed on rice plant; this difference speculated to be due to the presence of some antifeedant(s) against the planthopper in barnyard grass. We now report here the isolation and characterization of *trans*-aconitic acid as a potent antifeedant against *N. lugens* in barnyard grass.

MATERIAL AND METHODS

Insects and plants. The materials used here were the same as those described in the previous paper (KIM, et al., 1975).

Bioassay. Five 3rd-instar nymphs of *N. lugens* were introduced into a feeding apparatus which consisted of two glass tubes and allowed the planthopper nymphs to suck test solution through a thin polyethylene film (stretched Parafilm "M", American Can Company) as described previously (KIM, et al., 1975). The test solution, composed of 15% sucrose containing the test material at a definite concentration, was renewed every other day. The inhibitory activity of the test material was estimated by comparing nymph survival rates during a 7-day feeding test at 25°C and 14-hr illumination per day.

Gas liquid chromatography. Gas chromatogram of trimethylsilyl derivatives of organic acids from each plant extract was obtained with Yanaco G–80, glass column

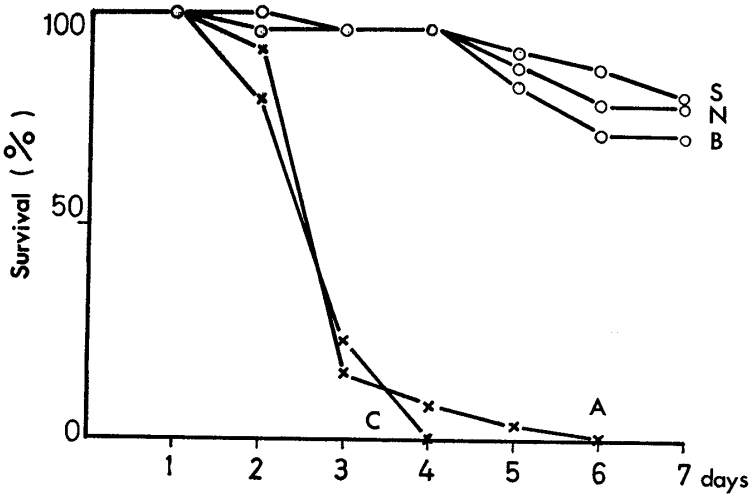

Fig. 1. Survival of the 3rd instar nymphs of *N. lugens* on each fraction of the active oil. S, 15% sucrose; N, 15% sucrose plus the neutral fraction; B, 15% sucrose plus the basic fraction; A, 15% sucrose plus the acidic fraction adjusted to pH 7 with calcium hydroxide; C, 15% sucrose plus 5% the active original oil.

(ϕ 3 mm × 75 cm) packed with 1% OV–1 on Chromosorb W, column temperature 160°C, injection temperature 250°C, carrier gas flow rate 25 ml helium/min, flame ionization detector. The following physicochemical data were obtained: melting point (uncorrected) Yanaco MP–S3 microscopic hot plate; UV, IR, PMR, and mass spectra; Shimadzu UV–200, Shimadzu IR–27G, Hitachi R–22 (operating at 90 MHz), and Hitachi RMS–4, respectively.

RESULTS AND DISCUSSION

Experimental results of rearing *N. lugens* on rice plant or barnyard grass (KIM et al., 1975) showed that the planthopper can survive only on the former but not on barnyard grass. Further study on extracts of these plants clearly indicated the presence of potent antifeedant(s) against *N. lugens* in barnyard grass.

To isolate the antifeedant(s), barnyard grass (4 kg, fresh weight) was dipped in methanol and kept under room temperature for about a year until use. Methanol was evaporated and the residue was dissolved in water and washed successively with *n*-hexane, chloroform, and ethyl acetate to separate inactive substances. The aqueous solution was evaporated to yield an active brown oil (106 g). An aliquot (1 g) of this oil was separated into neutral (261 mg), acidic (204 mg), and basic (128 mg) fractions, by successive elution through a column of cation exchange resin (Dowex 50W × 8, H^+-form, 200–400 mesh) and an anion exchange resin (Dowex 1 × 8, formate form, 200–400 mesh), with 2*N*-NH_4OH and 24*N*-formic acid as the respective eluting systems. Of these fractions, only the acidic one showed feeding inhibition activity at a concentration equivalent to a 5% solution of the original oil even after neutralization with calcium hydroxide (Fig. 1). Preliminary chromatographic analysis of the acidic fraction on a silica gel plate (Kieselgel GF_{254} Nach Stahl, Merck, developed with *n*-butanol/acetic acid/water, 4 : 1 : 1 by volume) showed an intense spot at Rf=0.83

(detected by ultraviolet light or by bromocresol green reagent); this was not detected on the plate of the corresponding fraction obtained from rice plant according to the same procedure. This spot was scraped off from the plate, extracted with methanol and subjected to bioassay, the results of which indicated potent inhibitory activity for feeding of *N. lugens*. The total acidic fraction (204 mg) was then chromatographed on a column of silicic acid (5 g Mallinckrodt, 100 mesh, eluted with ethyl acetate) and the active fraction was collected by monitoring on tlc and evaporated to give a crystalline mass (104 mg), which was recrystallized from benzene–ether, mp. 145°C.

The isolated active compound showed a UV-absorption band at 207 nm (in MeOH solution) and the following four signals in the PMR spectrum (in d_5-pyridine); 3H singlet at δ 3.67 (–CO–O*CH*$_3$), 2H singlet at δ 4.53 (=C–*CH*$_2$–CO–), 1H singlet at δ 7.38 (=*CH*–COOR), and a broad 2H signal at δ 10.38 (2 COO*H*). These spectral data suggested mono-methyl ester of aconitic acid. Unequivocal evidence for its structure was provided by exhaustive esterification with methanolic hydrogen chloride forming the tri-methyl ester, which showed a molecular ion peak at m/e 216 ($C_9H_{12}O_6$) in the mass spectrum and five distinct singlet signals in the PMR spectrum (in $CDCl_3$); three 3H singlets at δ 3.60, 3.69, 3.73 (each-COO*CH*$_3$), a 2H singlet at δ 3.87 (=C–*CH*$_2$–COOCH$_3$), and a 1H singlet at δ 6.89 (=*CH*–COOCH$_3$), and was identical with the authentic tri-methyl ester of *trans*-aconitic acid in all respects in terms of IR-, PMR-, and mass spectral data. From these experimental results the isolated active compound was concluded to be a mono-methyl ester of aconitic acid, but its geometric isomerism remains undecided since *cis*-aconitic acid and its derivatives can easily be converted into *trans*-isomers under the acidic condition of esterification (McKeown, et al., 1965).

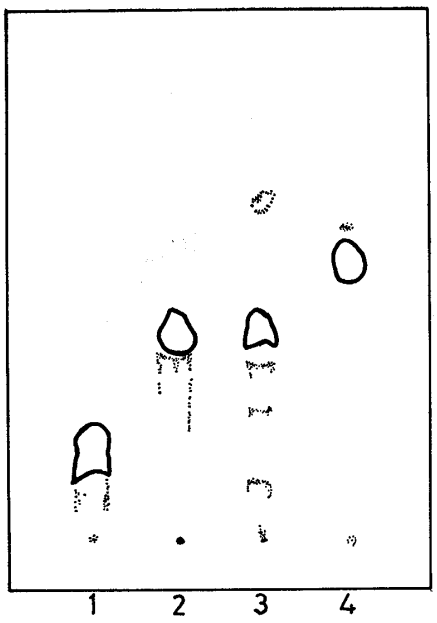

Fig. 2. Thin-layer chromatogram of authentic acids and barnyard grass extracts with benzene/methanol/acetic acid solvent (45 : 16 : 4 by volume). 1, *cis*-aconitic acid; 2, *trans*-aconitic acid; 3, the water extract of barnyard grass; 4, the methanol extract of barnyard grass.

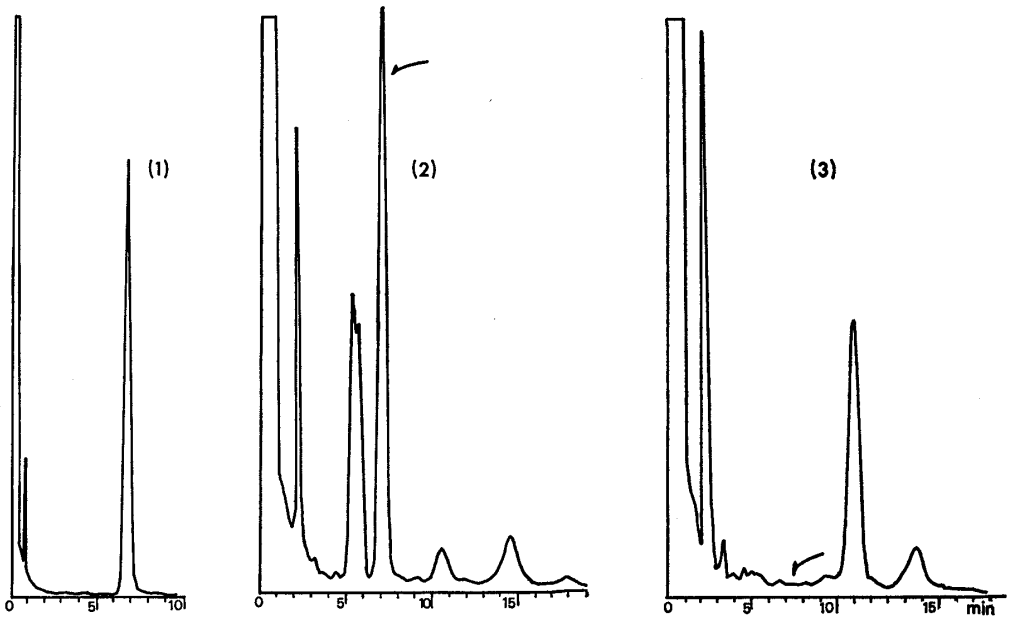

Fig. 3. Gas chromatogram of trimethylsilyl derivatives of *trans*-aconitic acid and organic acids from each plant extract. 1, *trans*-aconitic acid; 2, the acidic fraction of barnyard grass extract; 3, the acidic fraction of rice plant extract.

Although a mono-methyl ester of aconitic acid was isolated as the antifeedant against *N. lugens* from the methanol extract of barnyard grass as mentioned above, further investigation revealed the fact that the antifeeding activity in the plant was due to *trans*-aconitic acid itself, rather than a mono-methyl ester of aconitic acid which might be produced as an artifact during extraction of the plant with methanol, and was isolated since it unexpectedly showed inhibitory activity for feeding of *N. lugens*. This fact was confirmed by the following experimental results. Barnyard grass (20 g in fresh weight) was well ground and extracted with water (250 ml) at 70°C for 4 hr. The dried extract was suspended in water (80 ml) and centrifuged at 10,000×g for 10 min. The supernatant was passed through each ion exchange resin column described above to obtain the active acidic fraction. Rice plant was also extracted and separated by the same procedure to obtain the corresponding acidic fraction. As shown in Fig. 2 the silica gel tlc analysis of the acidic fraction obtained from the water extract of barnyard grass did not indicate a spot corresponding to a mono-methyl ester of aconitic acid but did demonstrate the presence of *trans*-aconitic acid in the fraction. This finding was also confirmed more clearly by glc analysis of the fraction of each plant after their trimethylsilylation using N, O-bis (trimethylsilyl) acetamide. As shown in Fig. 3, the results of glc analysis indicated that *trans*-aconitic acid was detected in barnyard grass but not in rice plant. Bioassay using the geometric isomers of aconitic acid was conducted at concentrations of 0.5% and 0.25% after neutralization to pH 7 with calcium hydroxide. Results are given in Fig. 4 which reveal that *trans*-aconitic acid showed inhibitory activity for feeding of *N. lugens* while the *cis*-isomer showed much less activity.

From the above-mentioned experimental results it is concluded that the resistance of barnyard grass to feeding of *N. lugens* is attributed to the presence of *trans*-aconitic

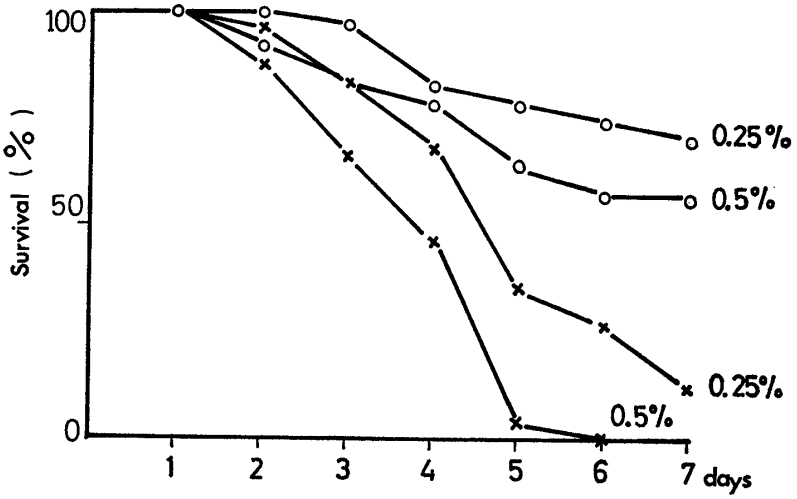

Fig. 4. Survival of the 3rd instar nymphs of *N. lugens* on a 15% sucrose solution containing the geometric isomer of aconitic acid adjusted to pH 7 with calcium hydroxide. ○—○, *cis*-aconitic acid; ×—×, *trans*-aconitic acid.

acid in the grass as a potent antifeedant to the planthopper. It is also interesting in regard to host selection of *N. lugens* that *trans*-aconitic acid has been known to be common in many kind of plants (STOUT, et al., 1967, BURAU., 1969, CLARK., 1969) but not detectable in rice plant. Although many kind of antifeedants to chewing insects have been isolated from plants, no information has been available on sucking insects, especially planthopper and leafhopper, until the present study.

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