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Constituents of Planthopper Attractant in Rice Plant[1]

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The chemical factor in rice plant attracting *Nilaparvata lugens* was extractable with ether and proved to be composed of multiple neutral compounds. The gas chromatogram of the active fraction indicated the presence of more than 27 compounds which were separated by chromatographic technique and identified as shown in Table 1. The planthoppers were well attracted to the mixture of these 27 compounds (14 esters, 7 carbonyl compounds, 5 alcohols, and 1 isocyanurate) and their activity was located in the carbonyl compounds plus isocyanurate, but the attractancy of these compounds was lower than that of the original active fraction. The gas chromatogram of the mixture was superimposable on that of the active fraction except for some minor differences as shown in Fig. 4, suggesting that minor components may play an important role for attractancy of rice plant against *N. lugens*.

INTRODUCTION

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), are the most destructive pests of rice plant in Asian countries. Many records of outbreaks of *N. lugens* and *S. furcifera* are found in Japan and other countries in temperate Asia. It is also well known that *N. lugens* and *S. furcifera* migrate from the southeastern area of Asia to Japan and Korea every year.

In the course of studies on the chemical basis of host selection of these planthoppers, we isolated the antifeedant of barnyard grass against *N. lugens* and identified it as *trans*-aconitic acid (KIM et al., 1975, 1976). In these studies, we have been interested in the orientation behavior of planthoppers to the rice plant. Although various factors may be concerned in their orientation to the host plant, volatile chemicals in the plant must play some important role in the host selection of many phytophagous insects. In the previous paper (OBATA et al., 1981), it was reported that the airborne attractant for three species of planthopper is contained in rice plant and the active principle for attractancy is a neutral substance(s) of the ether extract. It was also proposed that receptors for the attractant are located in the planthopper antennae, based on the fact

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that antennectomized planthoppers showed no response to the plant.

The present paper deals with the isolation of constituents of planthopper attractant in the rice plant, and also presents the response of *N. lugens* to those isolated constituents.

MATERIALS AND METHODS

Bioassay. The attractancy of the tested extract or compound(s) against the planthopper was estimated according to the assay procedure using a two-way choice apparatus described in the previous paper (OBATA et al., 1981). One–three day old macropterous females and males of *N. lugens* were used for bioassay. Fifteen sheets of filter paper (Toyo filter paper 5A, diameter 5.5 cm) were dipped in an ether solution of the sample equivalent to the amount contained in 10 g (fresh weight) of rice plant and placed in the sample side after drying at room temperature. Filter paper similarly treated without sample was placed on the blank side. Fifty insects were placed in the central part of the assay apparatus, and kept for 3hr in the dark with air flow (5–6 *l*/min) at 25°C, 60% relative humidity. Insects migrating to each side of the apparatus were counted. The test was replicated four times. The activity of the test sample was evaluated by the percentage given by $100A/(A+B)$, A being the number migrating to the sample side and B to the blank side. Since activity between 57 and 58% was estimated to be significant at the 5% level as calculated by the χ^2 test, attractancy was tentatively classified as follows: $\pm$ (from 50% to 57%), + (from 58% to 65%), ++ (from 66% to 70%), and +++ (over 70%).

Analytical apparatus. Gas chromatographic (GC) analyses were made on a Yanaco G80 gas chromatograph equipped with a flame ionization detector and a glass column (4 mm × 70 cm) packed with 5% PEG 20M. Column temperature was programmed from 80°C to 200°C at a rate of 4°C/min. Preparative gas chromatography was performed with the same instrument. Gas chromatographic identification of each compound was carried out using a Yanaco G880 with glass capillary columns (0.25 mm × 20 m) coated with PEG 20M and DEGS, respectively. The instrument was operated isothermally at an appropriate temperature between 70°C and 190°C.

Combined gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a Hitachi Model RMU-4 mass spectrometer interfaced with a Hitachi K-53 gas chromatograph using a glass column (4 mm × 70 cm) packed with 5% PEG 20M. A temperature program of 4°C/min beginning at 80°C was used. For all gas chromatographic analyses, helium was used as a carrier gas.

Infrared (IR) spectra were measured on a Shimazu IR-400 spectrometer. Proton magnetic resonance (PMR) spectra were obtained on a Hitachi R-22 instrument in $CDCl_3$ solution with tetramethylsilane as an internal standard.

Authentic samples. Triallyl isocyanurate was synthesized by the method already reported (TANIMOTO et al., 1966). 6, 10, 14-Trimethyl-pentadecan-2-one was prepared by ozonolysis of vitamin K_1. 2-Pentadecanone and 2-heptadecanone were easily synthesized by the reaction of the corresponding *n*-alkylbromide with ethyl acetoacetate, and subsequent hydrolysis and decarboxylation, respectively. Other compounds were obtained from commercial sources.

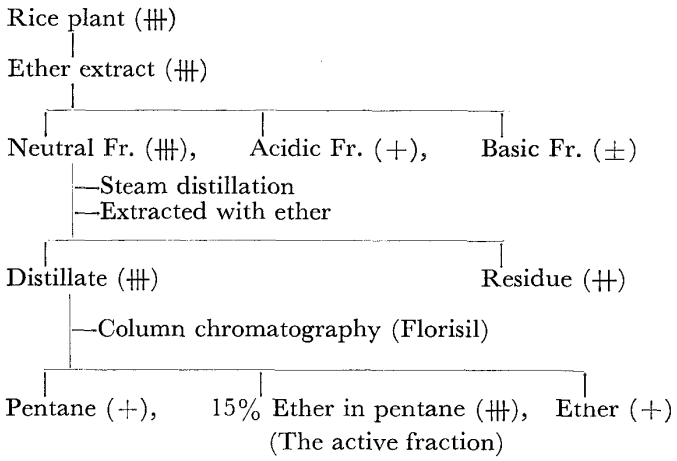

Fig. 1. Fractionation of planthopper attractant in rice plant. Activity of each fraction is shown in parentheses.

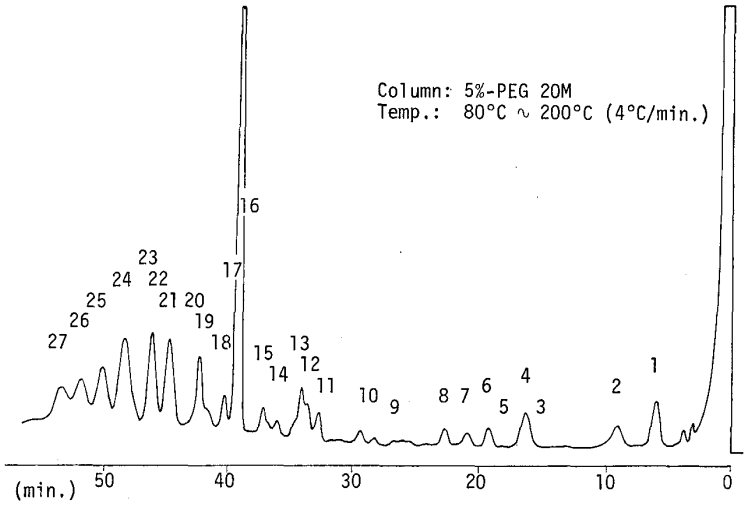

Fig. 2. Analytical gas chromatogram of the active fraction of rice plant.

RESULTS AND DISCUSSION

Purification of planthopper attractant in rice plant

As reported previously (OBATA et al., 1981), planthopper attractant in rice plant was effectively extracted with ether and its activity was found only in the neutral fraction of the ether extract. This result was obtained not only with rice seedlings but also with 70-day old rice plant so that, in this experiment, the attractant was fractionated from the ether extract of 70-day old rice plant as shown in Fig. 1. Rice plant (*cv.* Nihonbare) was collected in September in the paddy field where no pesticide had been applied. Ten kg of the plant was cut in chips of about 10 cm length and dipped in ether (150 *l*) for 5 days at 5°C. The ether layer was extracted with 1% HCl and 5% NaOH to remove inactive basic and acidic fractions. The neutral fraction was subjected to steam distillation and the distillate was extracted with ether. The distillate

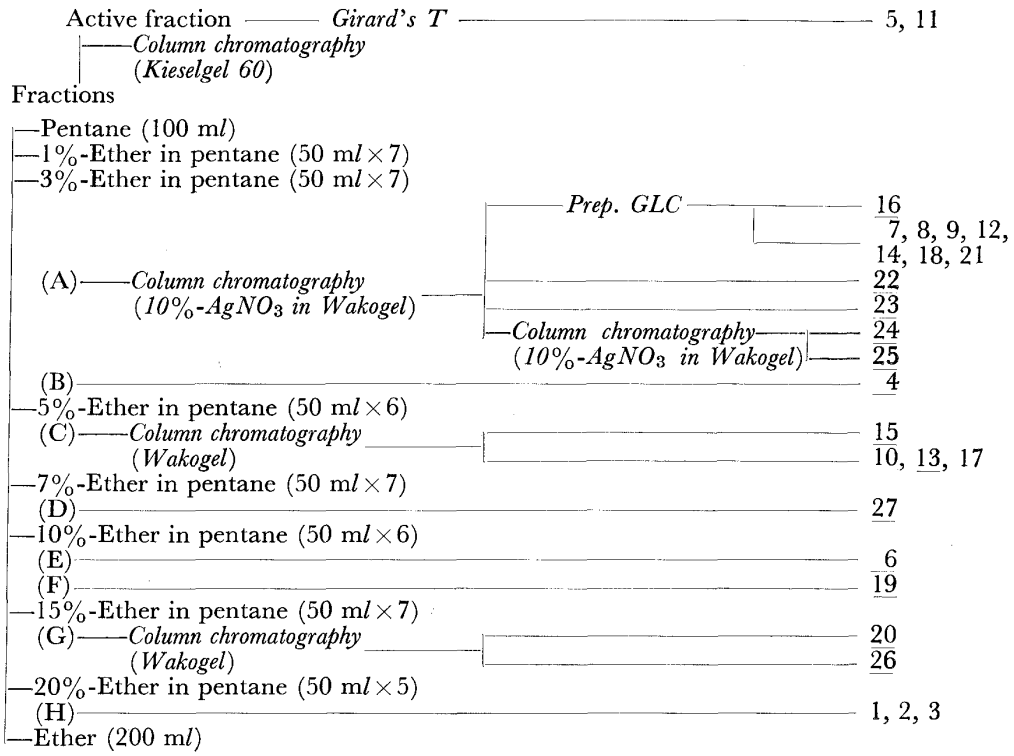

Fig. 3. Isolation of constituents of the active fraction. Numbers show the serial number on the chromatogram of Fig. 2 and underlined numbers represent the compounds isolated as a single component.

was as potently active as fresh rice plant on the bioassay, while the residue also exhibited some activity.

The distillate (1.18 g) was chromatographed on Florisil (100 g) eluted with pentane (500 ml), 15% ether in pentane (1,000 ml), and ether (500 ml), successively. The eluate of 15% ether in pentane showed high activity and was tentatively called the active fraction.

A portion of the active fraction was separated into three fractions by column chromatography on silicic acid (Wakogel C-200) with 1% ether in pentane, 5% ether in pentane, and ether. Bioassay results of these fractions showed that the activity was found in all of these fractions, suggesting that attractancy of rice plant against *N. lugens* is not evoked by a single component but is caused by multiple components. This suggestion was consistently supported by assay results of three fractions (*Rf* 0–0.4, *Rf* 0.4–0.6, and *Rf* 0.6–1.0) separated from the active fraction by preparative thin layer chromatography on silica gel GF_{254} (Merck, Type 60) with benzene.

A gas chromatogram of the active fraction on PEG 20M is shown in Fig. 2 where more than thirty peaks are found; the major peaks have been given the serial number 1–27. The whole eluate was collected in a glass tube by the preparative GC under the same condition and subjected to bioassay, which showed that it was active to the same extent as the original fraction. We therefore tried to isolate each compound which showed a major peak in the gas chromatogram.

Table 1. Compounds identified in the active fraction of rice plant

Peak No.	Compound	Amount[a]
1	2-Heptanol (A)	1.6
2	3-*Z*-Hexen-1-ol (A)	1.5
3	1-Octen-3-ol (A)	tr.
4	Benzaldehyde (C)	1.3
5	Acetophenone (C)	tr.
6	Linalool (A)	0.6
7	Methyl benzoate (E)	0.8
8	Ethyl benzoate (E)	0.7
9	Ethyl phenylacetate (E)	tr.
10	2-Tridecanone (C)	tr.
11	β-Ionone (C)	tr.
12	Methyl myristate (E)	tr.
13	2-Pentadecanone (C)	6.7
14	Methyl pentadecanoate (E)	0.6
15	6, 10, 14-Trimethyl-pentadecan-2-one (C)	1.9
16	Methyl palmitate (E)	48.2
17	2-Heptadecanone (C)	tr.
18	Ethyl palmitate (E)	1.2
19	Diethyl phthalate (E)	tr.
20	Triallyl isocyanurate (C)	13.1
21	Methyl stearate (E)	2.3
22	Methyl oleate (E)	18.3
23	Methyl linoleate (E)	17.2
24	Methyl linolenate (E)	24.4
25	Ethyl linolenate (E)	20.8
26	Phytol (A)	7.6
27	Di-*n*-butyl phthalate (E)	5.4

[a] μg per rice plant (10 g).
(E): Ester group 14. (A): Alcohol group 5.
(C): Carbonyl group 8.

Isolation of components in the active fraction

The isolation procedure is summarized in Fig. 3. The active fraction was subjected to column chromatography on silicic acid (Kieselgel 60, 50 g) using a mixture of pentane with a stepwise increasing mixing ratio of ether to separate into 8 fractions (A–H), as shown in Fig. 3. Each fraction was further purified by column chromatography on silicic acid (Wakogel C-200) impregnated with or without silver nitrate and/or by preparative GC to isolate 13 components as a single compound (indicated by underlining in Fig. 3) and the remaining 12 compounds as mixtures. The other two components (5 and 11) were identified by the following method using Girard's reagent T: To an aliquot of the active fraction were added anhydrous ethanol (80 m*l*), (carboxymethyl) trimethylammonium chrolide hydrazide (Girard's reagent T, 5 g) and Amberlite IRC-50 ion exchange resin (1 g). After refluxing the mixture for 1 hr, the solution was poured into 400 m*l* of distilled water and extracted with ether. The aqueous phase was added to 100 m*l* of formalin, allowed to stand overnight, and extracted with ether. The ether extract was analyzed with GC-MS.

Identification of 27 components in the active fraction

Every component in the active fraction was identified by comparing the GC-MS data and GC retention times with those of each authentic sample. The identity of the isolated components as a single compound was moreover established by comparison of their IR and PMR spectra with those of the authentic ones. Thus, each of 27 compounds was identified as shown in Table 1.

Esters

Peak No. 12, 14, 16 and 21 were identified as methyl myristate, methyl pentadecanoate, methyl palmitate and methyl stearate, respectively. They showed similar fragmentation patterns, especially a base peak at m/z 74 due to the McLAFFERTY rearrangement.

Peak No. 18 showed an ethyl ester fragment at m/z 88 and was identified as ethyl palmitate.

Peak No. 22, 23, 24 and 25 were identified by GC-MS and PMR data as methyl oleate, methyl linoleate, methyl linolenate, and ethyl linolenate, respectively.

Peak No. 7, 8 and 9 were identified as methyl benzoate, ethyl benzoate and ethyl phenylacetate, respectively.

Diethyl phthalate and di-*n*-butyl phthalate were assigned to peak No. 19 and 27 by comparing their GC-MS, IR and PMR data with those of authentic specimens.

Carbonyl compounds

2-Tridecanone, 2-pentadecanone and 2-heptadecanone corresponding to peak No. 10, 13 and 17 showed a base peak at m/z 58, together with fragment ions of M^+-15 and M^+-58 indicating the presence of a methyl ketone group. The PMR spectrum of peak No. 13 showed characteristic signals at δ0.9 (triplet, 3H, CH_3-CH_2-), δ2.10 (singlet, 3H, -CO-CH_3) and δ2.40 (triplet, 2H, CH_2-CH_2-CO-). The reduction product of peak No. 13 with $LiAlH_4$, as well as the starting material, had the same retention times as those of the authentic compounds.

Peak No. 15 showed a molecular ion peak at m/z 268 and a base peak at m/z 58, suggesting the presence of a methyl ketone group in combination with the signal at δ2.1 (singlet, 3H, -CO-CH_3) in its PMR spectrum. This peak, however, appeared on the GC between those of 2-pentadecanone (M^+, 226) and 2-heptadecanone (M^+, 254), indicating the branching of carbon chains which was confirmed by the signal at δ0.8–0.9 (doublet, 12H, CH_3-CH-) in the PMR spectrum due to four methyl groups bonding to methine groups. Considering these data and the biosynthetic pathway, this peak was estimated to be 6, 10, 14-trimethylpentadecan-2-one and was confirmed by comparing spectral data with those of authentic sample prepared by ozonolysis of vitamin K_1.

Benzaldehyde corresponding to peak No. 4 was identified by GC-MS and PMR spectra.

Peak No. 5 and 11 obtained by Girard T procedure were identified as acetophenone and β-ionone, respectively. Peak No. 11 showed a characteristic base peak at m/z 177, different from the isomeric α-ionone which showed a base peak at m/z 121.

Alcohols

2-Heptanol corresponding to peak No. 1 showed fragment ions at m/z 116 (M^+),

Table 2. Bioassay result of the identified compounds

Sample	Concentration[a]	Activity
Rice plant (10 g)	1	##
The active fraction	1	##
Total mixture	10	+
	1	+
Esters	10	±
	1	±
Carbonyl compounds & isocyanurate	10	+
	1	+
Alcohols	10	±
	1	±

[a] Shown with amount equivalent to 10 g fresh rice plant.

98 (M^+-H_2O) and a base peak at m/z 45, indicating the presence of a secondary alcohol group. The acetylated product, as well as the original one, showed the same retention times as those of the authentic samples.

Peak No. 2 and 6 were identified by GC-MS data and GC retention times as 3-*Z*-hexen-1-ol and linalool, respectively. The acetylated product of peak No. 2 and the hydrogenated product of peak No. 6 also showed the same retention times as those of authentic samples.

1-Octen-3-ol corresponding to peak No. 3 showed an ion at m/z 110 (M^+-H_2O) and a base peak at m/z 57, indicating allyl alcohol moiety. Other fragment ions were in agreement with those of the authentic one.

Phytol corresponding to peak No. 26 could not be analyzed by GC-MS, but the acetylated product showed the following characteristic fragment ions: m/z 295 (M^+-CH_3CO), 278 (M^+-CH_3COOH), 265 (M^+-CH_3COOCH_2). The PMR spectrum showed signals at δ 1.6 (singlet, 3H, $\overset{|}{C}=C\text{-}CH_3$), δ 1.9 (triplet, 2H, $CH_2\text{-}CH_2\text{-}\overset{|}{C}=C$) and δ 4.1 (doublet, 2H, $C=C\text{-}CH_2\text{-}OH$) coupled with olefinic proton at δ 5.4 (multiplet, 1H), indicating the moiety of $-CH_2-\overset{CH_3}{\overset{|}{C}}=CH-CH_2OH$. The PMR spectrum moreover showed the signals at δ0.8 (doublet, 12H, CH_3CH) due to four branched methyl groups. Taking these spectral data and the biosynthetic pathway into account, this peak was estimated to be phytol and identified with the authentic one by IR and PMR spectra.

Triallyl isocyanurate

The PMR spectrum of peak No. 20 showed signals at δ 5.8 (multiplet, 1H), δ 5.3 (triplet, 2H) and δ 4.5 (doublet, 2H), attributed to a moiety of $CH_2=CH\text{-}CH_2\text{-}X$. The MS spectrum showed a molecular ion peak at m/z 249 ($C_{12}H_{15}N_3O_3$) and a base peak at m/z 83 (C_4H_5NO), suggesting that it was a trimerized product of a moiety C_4H_5NO containing an allyl group. A characteristic absorption band at 1,690 cm^{-1} in its IR spectrum was indicated as probably a triallyl isocyanurate, which was confirmed by comparison of MS, IR and PMR spectra with those of the authentic sample.

As shown in Table 1, 14 esters, 7 carbonyl compounds, 5 alcohols and 1 isocyanurate were identified in the active fraction of rice plant. The contents of these compounds in 10 g of fresh rice plant were determined by gas chromatographic analysis and are

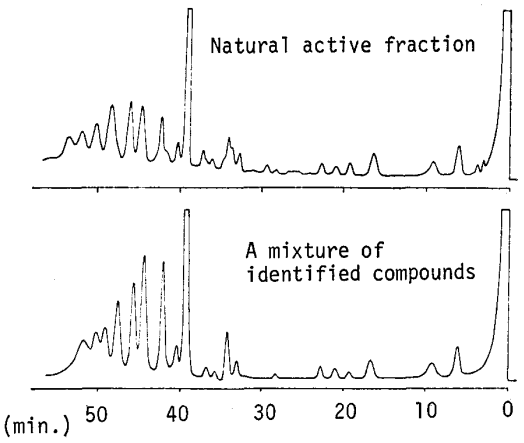

Fig. 4. Gas chromatograms of the active fraction (above) and a mixture of identified compounds (below).

listed in Table 1. Of these 27 compounds, diethyl and di-*n*-butyl phthalate, and triallyl isocyanurate (19, 27, and 20 in Table 1) were supposed to be likely artifacts or contaminants during isolation procedures.

Activity of the identified compounds

As described above, fresh rice plant (10 g) as well as the amount equivalent to 10 g rice plant of the active fraction exhibited potent attractancy against *N. lugens* on bioassay (Table 2). A mixture containing all 27 identified compounds in the same ratio as is present in rice plant (Table 1) showed attractancy to some extent on bioassay, but its activity was observed at a lower level than that of the active fraction even in a ten-fold amount (equivalent to 100 g of fresh rice plant), as shown in Table 2. The mixture gave a similar gas chromatogram to that of the active fraction with minor differences as shown in Fig. 4. The fact that the sample collected from the active fraction by preparative gas chromatographic technique through the entire range still showed a potent activity on bioassay suggested that some minor unidentified compounds played an important role in the attraction to the rice plant of *N. lugens*.

As indicated in Table 1, the identified compounds can be classified into three groups, the alcohol-(5 compounds), carbonyl-(including triallyl isocyanurate, 8 compounds), and ester-group (14 compounds). When each group mixed in the same ratio as in rice plant was bioassayed, the alcohol- and ester-group elicited no response from *N. lugens*, but the carbonyl-group showed the same level of attractancy as the mixture of all identified compounds shown in Table 2, suggesting that just the carbonyl-group may participate in the attractancy of rice plant to a certain extent.

In conclusion, the attractant factor for *N. lugens* is contained in the neutral fraction of the ether extract of rice plant and is fractionated by chromatography on a Florisil column to some extent. Further fractionation by column chromatography, however, results in distribution of the activity in a wide range of the eluates, suggesting that the attractant factor is not composed of a single compound but of multiple components in the neutral fraction. Gas chromatographic analysis indicates that the active fraction is composed of more than 27 compounds. A mixture of these 27 identified compounds,

however, showed the activity at a much lower level than the active fraction even in a ten-fold amount. The gas chromatogram of the mixture is superimposable on that of the active fraction with respect to 27 dominant peaks but differs in some minor peaks. These facts reveal that some minor components may play an important role in attractancy against the planthopper.

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