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HOST PLANT IRIDOID-BASED CHEMICAL DEFENSE OF AN APHID, *Acyrthosiphon nipponicus*, AGAINST LADYBIRD BEETLES

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Abstract—A Rubiaceae-feeding aphid, *Acyrthosiphon nipponicus*, is seldom attacked by the ladybird beetle, *Harmonia axyridis*. A potent deterrent against the beetle was isolated from the aphid and identified as paederoside, an iridoid glycoside originating in the aphid's host, *Paederia scandens*. The iridoid content was as high as 2% of the intact body weight, and a large portion was found in the cornicle secretion.

Key Words—Defense, deterrent, sequestration, iridoid glycoside, paederoside, *Acyrthosiphon nipponicus*, Homoptera, aphid, Aphididae, *Harmonia axyridis*, Coleoptera, Coccinellidae, *Paederia scandens*.

INTRODUCTION

A ladybird beetle, *Harmonia axyridis* (Pallas), feeds on a variety of aphids during its larval and adult stages. However, a monophagous aphid, *Acyrthosiphon nipponicus* (Essig et Kuwana) (synonym: *Aulacophora paederia* Takahashi), which feeds exclusively on *Paederia scandens* Merril (Rubiaceae), is seldom attacked by the ladybirds. When a hungry adult of *H. axyridis* bites into the aphid, it immediately drops the aphid, salivates, and quickly escapes from the aphid colony. Usually this is accompanied by a persistent grooming of its mouthparts. The aphid frequently secretes droplets from a pair of cornicles (Figure 1) when attacked by ladybirds and quickly smears the fluid onto the predator's mouthparts. The cornicle fluid strongly deters feeding of the ladybirds. Such feeding deterrence in the ladybirds was found to be due to a chemical

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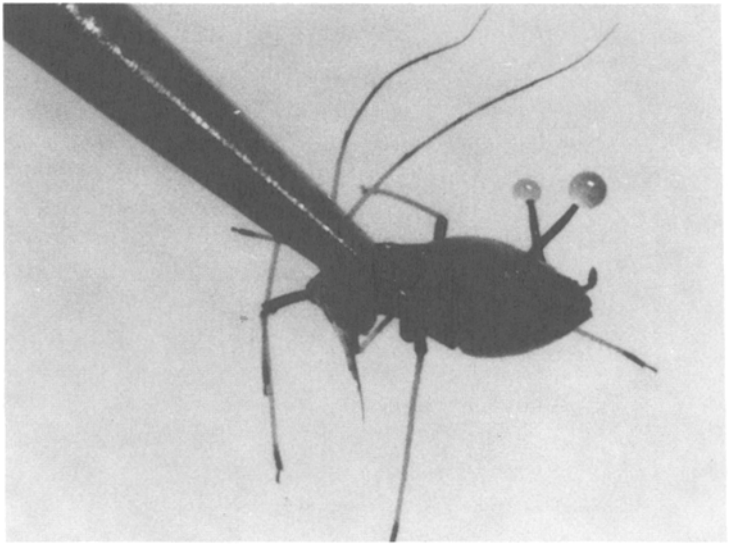

FIG. 1. *Acyrthosiphon nipponicus* secreting the cornicle fluid after disturbance. Usually the aphid quickly smears the fluid onto the forceps if picked up gently. The droplets contain paederoside in high concentration.

sequestered by the aphid from its host plant, *P. scandens*. We describe here the isolation and identification of the feeding deterrent of *A. nipponicus* against *H. axyridis*.

METHODS AND MATERIALS

Bioassay. Pupae of *H. axyridis* were collected from the field in Kyoto City. After adult ecdysis, they were regularly fed with drone honeybee powder (Matsuka and Niijima, 1985) and water for at least three days and then used for feeding bioassay. An aphid dummy similar in size to an aphid was made by dipping the round tip (2 mm id) of the glass rod in hot sucrose–agar solution (12% sucrose and 3% agar). *H. axyridis* adults usually feed on the dummy more than 5 sec (Figure 2). When the dummy surface was coated with an extract of *A. nipponicus*, the ladybirds stopped feeding immediately after the first bite. The test samples of known concentration were applied on the dummy surface as a 1-μl solution (in acetone or methanol) and brought into contact with the predator's mouthparts after evaporating the solvent in an air current. The bioassay was carried out at about 25°C (16:8 light–dark photoperiod) in a chamber (50 × 50 × 50 mm) which is open in front and illuminated by a fluorescent

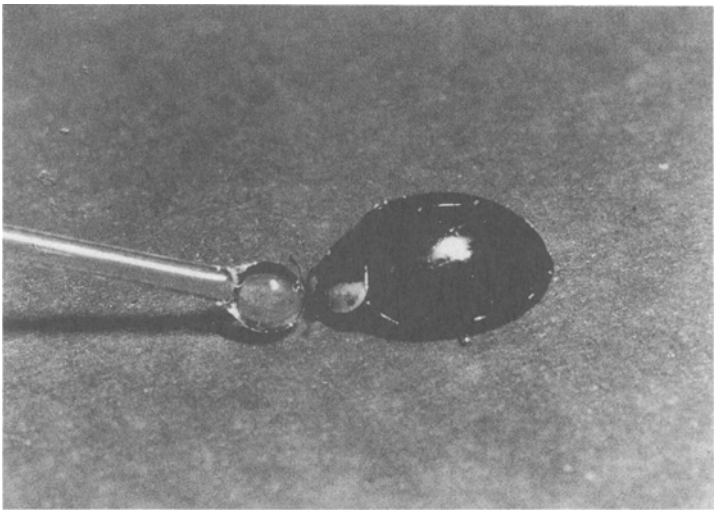

FIG. 2. Feeding deterrence bioassay against *Harmonia axyridis* using an aphid dummy.

lamp (15 W) placed behind a screen at the rear. The deterrency of each sample was obtained as a percentage of the number of ladybirds that stopped feeding on the sample dummy within 5 sec of exposure after the first bite was observed.

Instruments. Optical rotatory dispersion spectra were measured with a Jasco ORD J-5 spectropolarimeter at 30°C, and the ultraviolet (UV) spectrum was recorded with a Shimadzu UV-360 recording spectrophotometer. Field desorption mass spectrum was measured with a Hitachi M-80 mass spectrometer using a carbon emitter at 5.5 kV. Proton and carbon-13 magnetic resonance (PMR and CMR) spectra were measured with a JEOL JNM FX-200 spectrometer (200 MHz) using tetramethylsilane for CD_3OD or sodium 3-trimethylsilyl-1-propane sulfonate for D_2O solvent, respectively, as the internal standards. The letters s, d, t, q, and m represent singlet, doublet, triplet, quartet, and multiplet, respectively (J-values in Hz).

Isolation of Compound I (Paederoside). A. nipponicus feeding on the vine of *P. scandens* was collected in the campus of Kyoto University during July 1982. The aphids were homogenized in acetone (10 ml), and the residue was extracted twice with acetone (10 ml × 2). The combined extracts (0.20 g), after filtration (Toyo Filter Paper, qualitative No. 2) and evaporation, were fractionated into hexane, ethyl acetate, and water layers by solvent extraction (hexane layer = 47 mg, ethyl acetate layer = 4 mg, water layer = 143 mg). The deterrent activity was recovered from the water layer, which was chromatographed on a reverse-phase column (Sep-pak cartridge C_{18}, Waters Associates), by elut-

ing successively with each 4 ml of water, 20% methanol, 40% methanol, 60% methanol, and 100% methanol (Figure 3). The 20% methanol eluate (24 mg) was subjected in part to high-performance liquid chromatography (HPLC) with a reverse-phase column (Radial Pak liquid chromotography cartridge, μBondapak C_{18}, 10μm, 100 mm × 8 mm ID, Waters Associates) eluting with 30% methanol (2 ml/min). The eluate was fractionated into eight fractions, monitored by refractive index (differential refractometer, model R 401, Waters Associates) as shown in Figure 4A. Pure compound I, isolated from fraction 6 [retention time (*Rt*) = 12.0 min, yield = 20 mg from 1.3 g of the intact aphids], was then crystallized from acetone to yield colorless needles (mp 118°C). $[\alpha]_D = -44°$, $[\alpha]_{233} = -3720°$ (c = 0.36, methanol). UV: λ_{max} = 233 nm (ϵ = 11,500, methanol). PMR (CD_3OD): δ 7.30 (1H, d, J = 2.2), 5.94 (1H, d, J = 1.8), 5.73 (1H, m), 5.56 (1H, broad d, J = 7), 4.85 (2H, m), 4.68 (1H, d, J = 8.0), 3.92 (1H, double d, J = 12.0 and 1.9), 3.74 (2H, m), 3.19 (1H, double d, J = 8.0 and 8.5), 2.34 (3H, s).

Isolation of Compound III (Methyl Paederosidate). Compound III was isolated as an artifact when methanol was used for extraction instead of acetone. The compound was eluted with 40% methanol on a Sep-pak cartridge C_{18}, and purified by preparative HPLC (*Rt* = 39.6 min, under the same condition for compound I). $[\alpha]_D = +13°$, $[\alpha]_{271} = +680°$ (c = 1.67, methanol). PMR (CD_3OD): δ 7.66 (1H, d, J = 1.2), 6.02 (1H, m), 5.10 (1H, broad d, J = 15), 5.06 (1H, d, J = 8.4), 4.94 (1H, broad d, J = 15), 4.72 (1H, d, J = 7.6), 3.86 (1H, double d, J = 12.0 and 1.2), 3.74 (3H, s), 3.62 (1H, double d, J = 12.0 and 6.0), 3.04 (1H, m, J = 7.8, 6.0, and 1.2), 2.63 (1H, double d, J = 8.4 and 7.8), 2.34 (3H, s). CMR (D_2O): δ (off-resonance C–H splitting pattern) 15.64 (q), 42.81 (d), 47.24 (d), 54.61 (q), 63.61 (t), 68.09 (t), 72.27 (d), 72.58

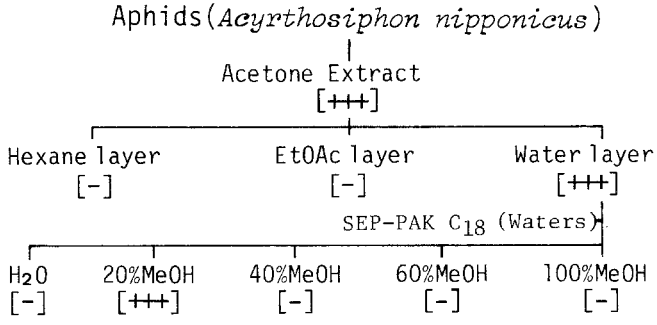

FIG. 3. Separation scheme of the aphid extract and the deterrent activity of each fraction; [−]: less than 10%, and [+++]: more than 90% deterrency were observed at a dose of 300 μg aphid equivalent/dummy (N = 20).

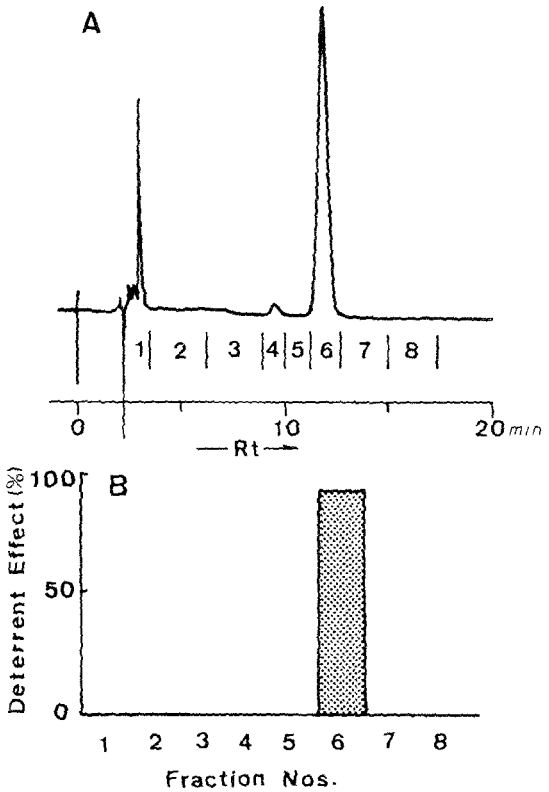

FIG. 4. (A) *Liquid chromatogram of the 20% methanol eluate using refractive index.* (B) Deterrent activity of each fraction against *Harmonia axyridis* at a dose of 300 μg aphid equivalent/dummy ($N = 20$).

(d), 76.74 (d), 78.40 (d), 78.89 (d), 101.70 (d), 103.02 (d), 109.27 (s), 134.39 (d), 146.31 (s), 157.72 (d), 172.37 (s), 176.53 (s).

RESULTS

Deterrency of Aphid Extracts. The deterrent component of *A. nipponicus* against the ladybirds was efficiently extracted with acetone. The crude acetone extract at various doses was tested on *H. axyridis* adults using the aphid dummy in the manner as shown in Figure 2. Figure 5 shows the dose–response curve of the crude extract. The mean deterrency at the 50% dose–response was approximately 50 μg aphid equivalent per dummy.

Isolation and Identification. An acetone extract of *A. nipponicus* was frac-

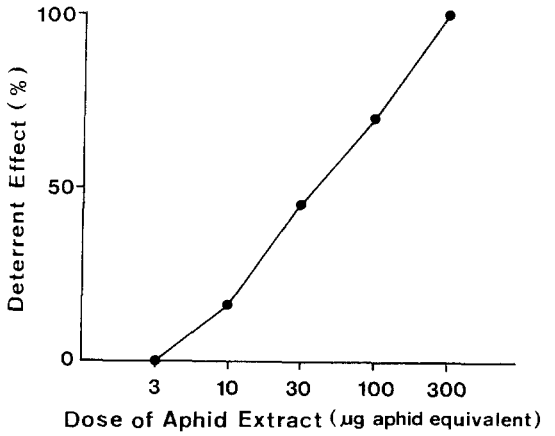

FIG. 5. Dose-response curve of the acetone extract of *Acyrthosiphon nipponicus* in the feeding test using *Harmonia axyridis* ($N = 30$).

tionated into hexane, ethyl acetate, and water layers by solvent extraction. The water layer showed the activity, which was chromatographed on a C_{18} reverse-phase cartridge column. The deterrent activity was recovered from a fraction eluted with 20% methanol (Figure 3). The active fraction was then subjected to preparative HPLC (Figure 4A), and the deterrent activity was found solely in fraction 6 (Figure 4B). The active compound I was isolated as colorless crystals (mp 118°C) from fraction 6. The yield of compound I was 15–20 mg from 1 g of intact aphids.

Compound I was characterized as paederoside, an iridoid glucoside known from aphid's host plant, *P. scandens* (Inouye et al., 1968, 1969), the structure of which has been revised by Kapadia et al. (1979). Field desorption mass spectrometry was employed in order to obtain an unequivocal assignment of the molecular weight (M: *m/z* 446), in which prominent peaks *m/z* 447 (M+H) and 469 (M+Na) were observed along with other fragment peaks, as shown in Figure 6. The dose-response of authentic paederoside provided by Dr. H. Inouye (from *P. scandens*) also coincided with that of compound I, as shown in Figure 7. The mean deterrency at the 50% dose-response was approximately 1 μg/dummy, comparable to deterrency of the original aphid extract (see Figure 4, a 50-μg aphid equivalent extract contains approximately 1 μg of paederoside).

Activity of Iridoid Analogs. Paederoside is a tricyclic iridoid with an unique thiocarbonate group, $OCOSCH_3$, which might be indispensable for deterrency. However, asperuloside (II), an acetate analog of the iridoid glusoside, also

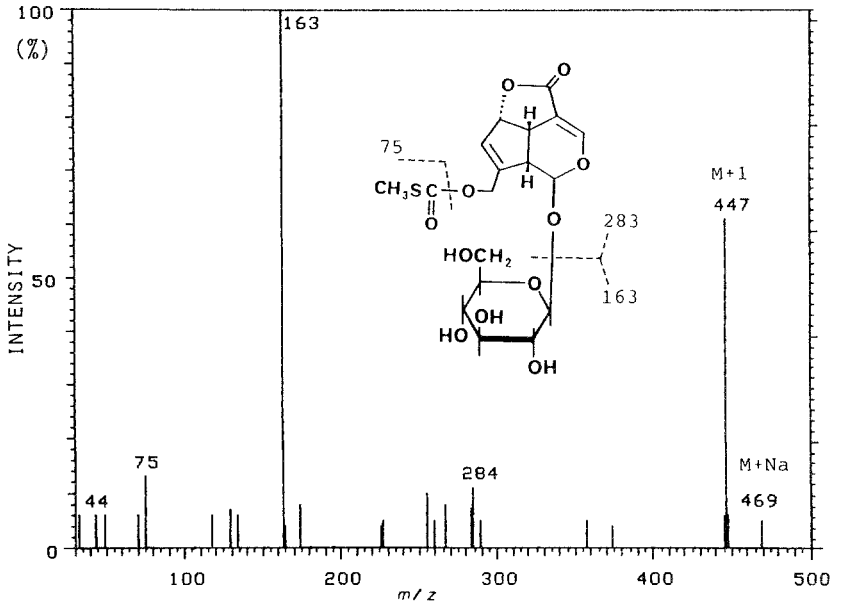

FIG. 6. Field desorption mass spectrum of paederoside (I).

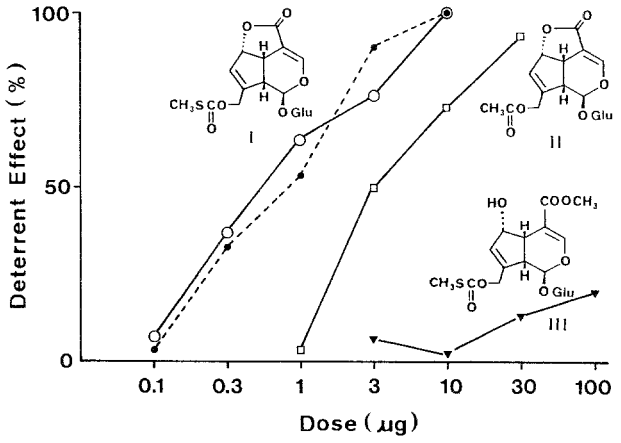

FIG. 7. Dose-response of paederoside (I) (solid line: from aphids, *Acyrthosiphon nipponicus;* broken line: from the host plant, *Paederia scandens*), asperuloside (II) and methyl paederosidate (III) in the feeding deterrence bioassay using *Harmonia axyridis* ($N = 30$).

deterred the ladybirds, while methyl paederosidate (III), a bicyclic analog with the sulfur-containing moiety, was shown to be inactive (Figure 7).

DISCUSSION

It has been clearly demonstrated that *A. nipponicus* is chemically protected from the predatory attack of a ladybird beetle, *H. axyridis,* by possessing a potent deterrent, paederoside (I), which originates from its host plant, *P. scandens.* Paederoside is an iridoid glucoside with a methyl-thiocarbonate moiety that is a very rare functional group in nature. It is suggested that methyl mercaptan may be formed through enzymatic hydrolysis of paederoside when the ladybirds contact the compound with their mouthparts. According to our preliminary observations, a dilute methanolic solution of methyl mercaptan repelled the ladybirds when applied as an air puff. However, an analogous compound III was inactive, whereas the acetyl derivative II was moderately deterrent against *H. axyridis,* suggesting the importance of the tricyclic structure rather than the thiocarbonate moiety. Although the host plant contains a few other iridoid glucosides, including asperuloside (II) (Inouye et al., 1968, 1969), the aphid appears to selectively sequester paederoside in the body tissues. The iridoid content was as high as 2% of the intact body weight of the aphid. Microanalysis of the cornicle fluid revealed that the water-soluble material was pure paederoside (approximately 1 μg/droplet). The aphid appears to promote its chances of surviving because of the deterrent effectiveness of the secreted droplets from the cornicles in response to predatory attacks. Similarly, a nymphalid butterfly, *Euphydryas anicia,* which feeds on *Besseya* and *Castilleja* plants (Scrophulariaceae), is known to sequester iridoid glycosides (catalpol, aucubin, and macfadienoside) in the body tissues (Stermitz et al., 1986). Catalpol was also shown to be sequestered by two *Penstemon*-feeding geometrid larvae (*Meris alticola* and *Nepterpes graefiaria*) (Stermitz et al., 1988).

Two other aphid species are known to accumulate host-plant products after feeding. *Aphis nerii* Fonscolombe sequesters cardiac glycosides from *Nerium oleander* (Rothschild et al., 1970), and *Aphis cytisorum* sequesters sparteine and some other analogous compounds from its host plant, *Cytisus scoparius* (L.) (Wink et al., 1982). Interestingly, nepetalactone and nepetalactol, two monoterpenes that are very stimulatory for cats and have been previously identified in *Nepeta* spp., have recently been characterized as the sex attractant pheromones of the vetch aphid, *Megoura viciae* (Dawson et al., 1987). However, in this case, the terpenes may not be directly derived from the aphid host plant, as is the case for other insects that produce iridoid pheromones and allomones (Blum and Hermann, 1978; Rowell-Rahier and Pasteels, 1986).

Iridoids are widely distributed secondary metabolites in the plant kingdom

and are considered to act as chemical barriers against herbivores. *A. nipponicus* seems to have gained an ecological advantage by selectively feeding on and sequestering the specific deterrent iridoid from its host plant, *P. scandens.*

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