Antifeeding Active Substances for Insect in Plant

Shigeki HOSOZAWA, Natsuki KATO, Katsura MUNAKATA
and Yuh-Lin CHEN*

Department of Agricultural Chemistry, Nagoya University, Nagoya, Japan

*Department of Agricultural Chemistry, National Taiwan University, Taipei, Republic of China

Received December 14, 1973

The survey of the presence of chemical resistant factors in plants against the larvae of *Spodoptera litura* F. was examined. In addition, the antifeeding diterpenes were surveyed from thirteen species of plants that belong to Verbenaceae family. Finally isolated thirteen antifeedants and one derivative were examined the antifeeding activity for the larvae by the leaf disk method.

In the investigations of host plant selection of phytophagous insects, three aspects of particular interest for host specificity studies on weed insect had been proposed: host plant feeding, host plant acceptance for feeding and oviposition, host plant suitability for development and reproduction.^{1a~e)} And also, it was suggested that the host plant acceptance for feeding in polyphagous species was governed by the botanical distribution of feeding stimulants and inhibitors.^{1a)} Recently many reports have dealt with the distribution and nature of chemical substances having the antifeeding activity for insect in plants.^{2a~f)}

The authors examined the survey of the presence of chemical resistant factors in various plants against the third instar of larvae of tobacco cut worm, *Spodoptera litura* F.^{2e, d, f)} This report consisted the isolation of thirteen antifeeding substances from five plants and the comparison of antifeeding activities of these compounds.

MATERIALS AND METHODS

Plants. Twenty three species of plants belonging to thirteen families were collected on Mt. Sanage, Aichi Prefecture, in Japan, Table I. Thirteen species of test plants that belong to Verbenaceae family were collected in Taiwan, Table II.

Preparation of sample solution for bioassay. The air-dried ground plant was refluxed with benzene for thirty minutes, and then the filtered benzene ex-

tracts were concentrated under reduced pressure to give the crude gum. The crude gum was dissolved in acetone for the sample solution of feeding test in 10, 5, 2.5, and 1% concentrations, respectively.

Bioassay. The feeding test was carried out by the leaf disk method which was described in the previous paper.³⁾ After 2 hr for feeding test, each sample and control disks were removed from the polyethylene cup, and the remaining areas were measured and compared with each other.

RESULTS AND DISCUSSION

The results of the antifeeding activities for the benzene extracts from the test plants were shown in Table I. The benzene extracts of four plants, *Caryopteris divaricata* Maxim. (leaf and stem), *Callicarpa japonica* Thunb. (leaf), *Boenninghausenia albiflora* Reichb. (whole plant), and *Ficus carica* L. (leaf), showed the strong antifeeding activity at 1% concentration for the feeding test. Therefore, the presence of some antifeeding substances was expected in these plants.

Antifeedants from B. albiflora Reichb

The benzene extract of air-dried ground whole plant was chromatographed over alumina (Brockmann grade II) by elution with MeOH—benzene gradient solvent system. The entomological test was used as a monitor for the fractionation. The antifeeding active fraction (1.5% MeOH—benzene) was chromatographed over silicagel by elution with

TABLE I. ANTIFEEDING ACTIVITY OF BENZENE EXTRACT OF PLANT

Family	Species	Activity ^{a)}
Magnoliaceae	<i>Magnolia obovata</i>	—
Caprifoliaceae	<i>Sambucus sieboldiana</i>	—
Rosaceae	<i>Agrimonia eupatoria</i>	—
	var. <i>pilosa</i>	++
Chloranthaceae	<i>Chloranthus serratus</i>	—
Verbenaceae	<i>Caryopteris divaricata</i>	++++
	<i>Callicarpa japonica</i>	++++
	<i>Lantana camara</i>	—
Saxifragaceae	<i>Hydrangea macrophylla</i> Seringe	—
	subsp. <i>serrata</i> var.	—
	<i>acuminata</i>	—
Compositae	<i>Achillea sibirica</i>	++
Leguminosae	<i>Pisum sativum</i> L. var.	—
	<i>arvense</i>	+
	<i>Pueraria thunbergiana</i>	—
Phytolaccaceae	<i>Phytolacca americana</i>	—
Vitaceae	<i>Ampelopsis</i>	—
	<i>brevipedunculata</i>	++
Rutaceae	<i>Boenninghausenia</i>	—
	<i>albiflora</i>	+++
Ericaceae	<i>Enkianthus perulatus</i>	+
Araceae	<i>Symplocarpus foetidus</i>	—
	Nutt. forma <i>latissimus</i>	—
Moraceae	<i>Ficus carica</i>	++++
Celastraceae	<i>Euonymus japonica</i>	++
Osmundaceae	<i>Osmunda japonica</i>	—
Parkeriaceae	<i>Coniogramme intermedia</i>	—
	<i>Pteridium aquilinum</i>	—
	<i>Cytomium fortunei</i>	—
	<i>Polypodium thunbergianum</i>	—

^{a)} +++++, +++++, ++, and + show 80~100% antifeeding activity at 1, 2.5, 5, and 10% concentrations, respectively. — shows no effect at 10% concentration.

EtOAc—*n*-hexane (1:1) and rechromatographed on silicagel eluted by 2% EtOH—CHCl₃ to yield two crystalline compounds, **1** and **2**, which were recrystallized from light petroleum to give mp 188~189°C and 127~129°C, respectively. The each compounds of **1** and **2** was identified as bergaptene and xanthyletine, respectively, by the comparison with the reported values of spectroscopic data and mp.

Antifeedant from *C. japonica* Thunb

The benzene extract of air-dried ground leaves was separated on alumina (Brockmann

grade II) and silicagel by elution with same solvent system as using in *B. albiflora*. The strong antifeeding activity was observed in less polar fraction (0.5% MeOH—benzene eluate of alumina chromatography). In spite of the purification of active fraction (100% antifeeding activity at 1000 ppm) using many isolation technique, strong antifeedant has not been obtained. However, the weak antifeedant **3** in the high content (0.2% yield on dried basis) was isolated from the active fractions, and identified as phytol by comparison with an authentic sample using spectroscopic data and GLC.

Antifeedants from *C. divaricata* Maxim

In our earlier works, it had been pointed out that the active substances in *C. divaricata* were unstable in the extraction with benzene under reflux condition rather than with ether, and very sensitive to acidic and basic conditions. Therefore, air-dried ground leaves and stems were extracted with ether at room temperature. Recently we reported the isolation and antifeeding activities of six diterpenes, **4**~**9**.⁴⁾ Then, in further scrutiny of the minor antifeeding diterpene components in this plant, we had isolated two new diterpenes, caryoptinol **10** and dihydrocaryoptinol **11**, which were recrystallized from EtOAc—ether—light petroleum to give mp 219~220°C and 204~205°C, respectively.⁵⁾ Above eight antifeeding diterpenes had the clerodane skeleton as a common structure, and showed a bitter taste.

Survey of antifeeding diterpenes from Verbenaceae plants

Recently N. Kato *et al.*⁶⁾ isolated the two antifeeding diterpenes, clerodendrin A and B, having the same skeleton as in clerodin from *Clerodendron tricotomum* Thunb. that belong to Verbenaceae family.

Air-dried test plants were extracted with ether at room temperature. The antifeeding activity of each ether extracts was examined by the leaf disk method. These results were shown in Table II. The ether extracts of leaves of *Clerodendron fragrans*, *C. calamitosum*, and

TABLE II. ANTIFEEDING ACTIVITY OF ETHER EXTRACT OF VERBENACEAE PLANT

Species	Activity ^{a)}
<i>Clerodendron fragrans</i> (leaf)	++++
(stem)	+
<i>Clerodendron inerme</i> (stem)	+
<i>Clerodendron paniculatum</i> (leaf)	+
(stem)	+
<i>Clerodendron calamitosum</i> (leaf)	++++
(stem)	+++
<i>Clerodendron cryptophyllum</i> (leaf)	++++
(stem)	+
<i>Clerodendron paniculatum</i> var. <i>albiflora</i> (stem)	—
<i>Callicarpa formosana</i> (leaf)	+
(stem)	+
<i>Duranta repens</i> (leaf)	—
(stem)	—
<i>Lippia nodiflora</i> (whole plant)	—
<i>Premna integrifolia</i> (leaf)	—
(stem)	—
<i>Vitex negundo</i> (stem)	—
<i>Vitex trifolia</i> (seed)	+++
<i>Verbena officinalis</i> (whole plant)	—

^{a)} +++++, +++, ++, +, —: as in Table I.

C. cryptophyllum exhibited the 100% antifeeding activity at 1% concentration. Consequently, the presence of one or more antifeeding substances was to be expected of these plants.

Antifeedants from *C. calamitosum* and *C. cryptophyllum*

The ether extract of *C. calamitosum* was chromatographed over neutralized alumina (Brockmann grade V) by elution with ether—benzene gradient solvent system. The new antifeeding diterpene compound, 3-epicaryoptin **12**, were obtained as colorless prisms, mp 171~172°C (0.01% yield on dried basis), by the chromatographic purification on silica-gel and recrystallization from ether—light petroleum.⁷⁾

The ether extract of *C. cryptophyllum* was separated on alumina column chromatography by elution with ether—benzene. The ether—benzene (1:5) fraction was chromatographed over alumina by elution with EtOAc—*n*-hexane (1:1). The antifeeding substance **13** was obtained as crystals after the recrystal-

FIG. 1.

lization from EtOAc—ether—light petroleum mixed solvent in yield 0.002% on a dried basis. **13** was identified as clerodendrin-A by comparison with an authentic sample using spectroscopic data, mp and specific rotation.

It is interesting that the compounds containing the clerodane skeleton are found both in *Clerodendron* and *Caryopteris* species. And also, it is interesting in view of biogenesis that caryoptin is not found but 3-epicaryoptin is observed in *C. calamitosum*.

Activity of natural occurring antifeedant

Finally obtained thirteen antifeeding substances and one derivative were examined the antifeeding activities against the larvae of

TABLE III. ACTIVITY OF NATURAL OCCURRING ANTIFEEDANT

	Compound	ppm ^{a)}
1	Bergaptene	500
2	Xanthyletine	2500
3	Phytol	5000
4	Caryoptin	200
5	Dihydrocaryoptin	80
6	Caryoptin hemiacetal	200
7	Clerodin	50
8	Dihydroclerodin-I	50
9	Clerodin hemiacetal	50
10	Caryoptinol	200
11	Dihydrocaryoptinol	100
12	3-Epicaryoptin	200
13	Clerodendrin-A	200
14	3-Epidihydrocaryoptin ^{b)}	100

^{a)} The concentration of the sample solution showing the 100% antifeeding activity within 2 hr.

^{b)} The catalytic reduction product of 12.

S. litura at the different concentrations. The antifeeding activity was embodied in the concentration of sample solution which exhibited the 100% antifeeding activity within the defined time (2 hr), Table III.

10, 12, and 13 showed the same 100% antifeeding activities at 200 ppm concentration as that of 4, which showed the weak antifeeding activity than clerodin. 11 and 14 showed the same 100% antifeeding activity at 100 ppm concentration. From these facts, the derivatives have the saturated D ring is more active for the larvae than those of the unsaturated D ring. Previously we have suggested that the difference of the activities between clerodin and caryoptin series may be attributed to the presence of 3 β -acetoxyl group on A ring.³⁾ However, the difference of the activities accompanying the change of the configuration and functional groups of C-3 position on A ring is not definitely observed in this feeding test.

In addition, the feeding test was allowed to continue for 6 hr and more at the concentration showing the 100% antifeeding activity. The test leaves treated with the antifeeding diterpenes, 4~14, were not bitten after 24 hr

and more, and then the larvae eventually starved to death.^{2f,3)} Therefore, the term of "absolute antifeedant"³⁾ is applicable to these diterpene compounds. And also the term of "relative antifeedant"³⁾ is used to 1, 2, and 3 which retarded the feeding of larvae only within the defined time. These three relative antifeedants did not exhibit the antifeeding activity after 6 hr.

If insect antifeeding active substances are classified as the concepts of "relative antifeedant" or "absolute antifeedant," it will shed light on novel and specific actions of the substances for insects.

REFERENCES

- 1) a. A. J. Thorsteinson, *Ann. Rev. Entomol.*, **5**, 193 (1960).
b. S. D. Beck, *ibid.*, **10**, 207 (1965).
c. J. S. Kennedy, *Ann. Appl. Biol.*, **56**, 317 (1965).
d. H. L. House, *Can. Entomol.*, **98**, 1121 (1966).
e. H. Zwölfer and P. Harris, *Ann. Rev. Entomol.*, **16**, 159 (1971).
- 2) a. H. Buhr, R. Toball, and K. Schreiber, *Ent. Exp. Appl.*, **1**, 209 (1958).
b. B. Stürckow and I. Löw, *ibid.*, **4**, 133 (1961).
c. K. Wada and K. Munakata, *J. Agr. Food Chem.*, **16**, 471 (1968).
d. K. Wada, K. Matsui, Y. Enomoto, O. Ogiso and K. Munakata, *Agr. Biol. Chem.*, **34**, 941 (1970).
e. J. H. Butterworth and E. D. Morgan, *J. Insect Physiol.*, **17**, 969 (1971).
f. N. Kato, M. Takahashi, M. Shibayama and K. Munakata, *Agr. Biol. Chem.*, **36**, 2579 (1972).
- 3) S. Hosozawa, N. Kato and K. Munakata, *Agr. Biol. Chem.*, **38**, 823 (1974).
- 4) S. Hosozawa, N. Kato and K. Munakata, *Phytochemistry*, **12**, 1833 (1973).
- 5) S. Hosozawa, N. Kato and K. Munakata, *ibid.*, **13** (1974).
- 6) a. N. Kato, S. Shibayama and K. Munakata, *Chem. Commun.*, **1971**, 1632; *J.C.S. Perkin I.* **1973**, 712.
b. N. Kato, K. Munakata and C. Katayama, *J.C.S. Perkin II*, **1973**, 69.
- 7) S. Hosozawa, N. Kato and K. Munakata, *Phytochemistry*, **13**, 308 (1974).