Metabolism of Ingested Auxins in the Bug *Lygus disponi*: Conversion of Several Indole Compounds¹

Koji HORI

Laboratory of Entomology, Obihiro University, Obihiro,
Hokkaido 080, Japan

(Received August 5, 1978)

The metabolism of several indole compounds in the gut of the bug *Lygus disponi* was investigated. All the indole compounds were more or less metabolized in the gut. The metabolic patterns of IAA, indole-3-propionic acid and indole-3-butyric acid with $-(\text{CH}_2)_n\text{-COOH}$ in the side chain were similar to one another. Also, the compounds with $-\text{CH}_2-$ in the side chain (indole-3-ethanol, tryptamine, serotonin, gramine, indole-3-acetonitrile and indole-3-acetaldehyde) were converted to several metabolites. The metabolites of indole-3-butyric acid had auxin activity. The metabolites that appeared in the excreta of the IAA-feeding bugs were probably the substances conjugated by attaching some molecules to the side chain of indole nucleus. The significance of the auxin metabolism by the bug is also discussed.

INTRODUCTION

The bug *Lygus disponi* LINNAVUORI (Hemiptera : Miridae) as well as other mirid bugs is fond of feeding on the reproductive organs of host plants (DEMPSTER, 1964; HORI, 1971; LEIGH, 1976), where auxins are contained in relatively high concentration. In general, aromatic compounds including indole-3-acetic acid (IAA) are toxic to organisms (TOWERS, 1964). Thus, some insects metabolize auxins to non-toxic substances, while others utilize them for the development and reproduction (SCHEURER, 1976). HORI and ENDO (1977) found that *L. disponi* converted IAA to some metabolites with auxin activity in the gut, though these could not be identified. The greater part of the metabolites is excreted without being absorbed into the haemolymph, although a small amount passes through the haemolymph to the salivary gland (MILES and HORI, 1977). The present paper deals with the metabolism of several indole compounds in the gut of *L. disponi* in order to estimate the feature of the IAA metabolites in the excreta as clearly as possible.

MATERIALS AND METHODS

Insects used. Adults collected from the fields or reared from eggs in bottles containing cruciferous plants as food were used for the experiments.

Collection of excreta. The kinds and concentrations of indole compounds used

¹ Contribution No. 87 from the Laboratory of Entomology, Obihiro University.

Table 1. KINDS AND CONCENTRATIONS OF INDOLE COMPOUNDS
USED FOR FEEDING OF *L. disponsi*

Kind	Abbreviation	Concentration (%)
Indole-3-acetic acid	IAA	0.1
Indole-3-propionic acid	IPA	0.1
Indole-3-butyric acid	IBA	0.1
Indole		0.1
Tryptamine	TyrAm	0.1
Indole-3-aldehyde	Iald	0.02
Indole-5-carboxylic acid	5-ICA	0.05
Indole-3-carboxylic acid	3-ICA	0.05
Indole-3-acetonitrile	IAN	0.05
Indole-3-ethanol	IEt	0.05
Indole-3-glycollic acid	IGA	0.05
Indole-3-acetaldehyde	IAald	0.05
Acetoxy indole	AceI	0.05
Gramine	Gram	0.05
Indoline	Indo	0.05
Serotonine	Sero	0.05
Tryptophan	Try	0.05
Anthranilic acid	Ant	0.05

for the experiment are shown in Table 1. Twenty bugs were fed with each compound in 10% sucrose solution containing 0.01% amaranth (indole compound-feeding bugs) by the modified method of MILES and HORI (1977). For controls, another batch of 20 bugs was fed with the same diet but containing no indole compound. The excreta were adsorbed on filter paper laid in the feeding cages. The collection of excreta was repeated a few times to get a sufficient volume for experimentation.

Preparation of test excreta solution. The excreta on the filter paper were eluted by water in a chromato-chamber for 5 hr in the dark. The extracts were diluted up to 5 ml to determine the quantity of the excretion by measuring absorbancy at 520 nm. They were then lyophilized in the dark and kept at -18°C before use.

Thin-layer chromatographic (TLC) analysis of indole compound metabolites in excreta. The stocked dry excreta of indole compound-feeding and control bugs were dissolved in 1.0 ml 50% ethanol. Ten to 30 μl of the solutions, 5 μl of standard solution and 1 μl of the diet which the bugs fed on were spotted on Eastman Chromatogram sheets and developed up to 10 cm in the dark. The solvent systems used are shown in Table 2. The spray reagents were (a) Ehrlich reagent for detection of indole nucleus and (b) Fe-reagent (Hori, unpublished) for detection of -COOR or OR of the side

Table 2. SOLVENT SYSTEMS USED FOR TLC ANALYSIS OF INDOLE COMPOUND-METABOLITES

No.	Solvent composition (v/v)
I	Butanol-Ethanol-Water (76+19+5)
II	Propanol-Methyl acetate-7N Ammonium hydroxide (45+35+20)
III	Chloroform-Acetic acid (96%) (95+5)
IV	Chloroform-Ethylacetate-Formic acid (35+55+10)

chain. In the case of Ehrlich reagent, the developed sheets were put into a glass chamber with HCl vapour and then heated at 110 °C for color development.

Avena assay of auxin activity of excreta. The assay was carried out only for the IBA excreta. Each 60 μ l (15 μ l $\times$ 4 spots) of the solutions of IBA and control excreta for Avena assay, and 10 μ l of IBA excreta for determining Rf values of IBA metabolites were spotted on a TLC sheet and developed by solvent system I. The developed sheet was cut into three strips (two of IBA and control excreta for Avena assay, and one of IBA excreta for color development by Ehrlich reagent). The former two strips were each cut into ten pieces of Rf fractions, and the 2nd fraction was further cut into two sub-pieces corresponding to metabolites C+B and A. Each cut piece was put into a petri dish containing 5 ml distilled water and 15 Avena coleoptiles. Also, each 60 μ l of the crude solutions of IBA and control excreta was put into a petri dish containing 5 ml distilled water, 15 Avena coleoptiles and a TLC piece of the lower side of the original spot. Water containing no excreta solution was set as a control. The Avena assay followed the same procedure employed by HORI (1974).

RESULTS AND DISCUSSION

Quantity of excretion and mortality of L. disponi

The mortality did not vary among the bugs fed with various indole compounds

Table 3. EFFECT OF INDOLE COMPOUNDS ON QUANTITY OF FEEDING OF *L. disponi*

Iodole compound	Mortality (%)	Quantity of feeding μ l/bug/day
Control	0	7.8
5-ICA	10	10.2
IAald	10	10.8
Indole		0
Control	25	9.7
IAN	12	2.7
3-ICA	15	10.6
Gram	20	4.5
Control	10	6.0
IBA	20	5.6
TryAm	5	5.8
Control	25	8.0
Try	30	11.2
IEt	20	9.0
IGA	15	5.0
Control	10	5.1
AceI	20	7.7
Control	15	7.6
IPA	10	6.6
Control	40	6.8
Ant	20	9.3
Sero	5	8.0
Indo	20	8.0
Control	25	4.7
Iald	30	5.2
Control	15	12.4
IAA	15	15.3

(Table 3). The quantity of feeding (corresponding to the quantity of excretion) differed with the kind of indole compounds fed (Table 3). The bugs never fed on the diet including 0.1% indole and only a little on the diet with IAN, whereas some indole compounds seemed to stimulate feeding. Nevertheless, the effect of indole compounds on feeding should be investigated further by changing their concentration in the diet.

Indole compound metabolites in excreta

Figures 1, 2, 3 and 4 show the TLC of excreta developed by solvents I, II, III and IV, respectively. All the indole compounds used were more or less metabolized

Fig. 1. TLC of indole compound-metabolites in the excreta of compound feeding bugs (solvent I); ○-trace.

Fig. 2. TLC of indole compound-metabolites in the excreta of compound feeding bugs (solvent II).

Fig. 3. TLC of indole compound-metabolites in the excreta of compound feeding bugs (solvent III).

Fig. 4. TLC of indole compound-metabolites in the excreta of compound feeding bugs (solvent IV).

in the gut. The metabolic patterns of IPA and IBA were very similar to that of IAA, which was converted to more than four metabolites. This similarity seemed to be due to the presence of $(\text{CH}_2)_n\text{COOH}$ in the side chain of these compounds. Also, the compounds with $-\text{CH}_2-$ in the side chain (IET, TryAm, Sero, Gram, IAN and IAald) were converted to several metabolites, whereas the compounds without $-\text{CH}_2-$ in that position (IGA, AceI, 3-ICA, Indo and Iald), except 5-ICA which was distinctly converted to several metabolites, produced only weak or few spots. The greater part of tryptophan ingested seemed to be absorbed in the haemolymph, although some was

decomposed in the gut to be excreted. Anthranilic acid having no indole nucleus was metabolized as well as several indole compounds. All the metabolites of the indole compounds could not be identified by means of comparison of the results of the present study with those shown in Tables 88 and 89 given by KALDEWEY (1969).

The number of electrons which pass to $-\text{COOH}$ of the side chain in indole compounds decreases as the number of $-\text{CH}_2-$ present between $-\text{COOH}$ and indole nucleus increases, and consequently the electrons which stay at the nucleus increase (screen effect). The color developed by Ehrlich reagent turns from pink to blue with the increase in the number of $-\text{CH}_2-$ (MINO, personal communication). In the present study, it was found that the color of the metabolites in ICA, IAA, IPA and IBA ($(\text{CH}_2)_n$; $n=0, 1, 2$ and 3 , respectively) was very similar to that of the respective indole compounds (pink, red-purple, purple and blue-purple, respectively), and that the R_f values of the metabolites in IAA, IPA and IBA were proportional to those of respective compounds. This suggests that all the metabolites not only have an indole nucleus but also an original side chain ($(\text{CH}_2)_n\text{COOR}$). This indicates that the metabolites are probably the substances conjugated by attaching amino acids, sugars, proteins or some other substances to $-\text{COOR}$. That the metabolites were coloured by Fereagent (Fig. 5) may support this idea, because color development by the reagent suggests chelation between $>\text{NH}$ of the indole nucleus and $-\text{COOH}$ of the side chain (MINO, personal communication).

Auxin activity of IBA metabolites in excreta

All the metabolites had a high auxin activity, which seemed to differ little among them as judged by comparing the concentrations of the metabolites with their auxin activities (Fig. 6).

Indole compounds seem to be so toxic to organisms (TOWERS, 1964) that the insects which fed on them have to metabolize to non-toxic substances for their development. SCHEURER (1976) has reported that auxins are toxic to some insects but useful

Fig. 5. TLC of indole compound-metabolites in the excreta of compound feeding bugs: Comparison between two color-development methods.

Fig. 6. Auxin activity of IBA metabolites in the excreta of IBA feeding bugs;

⊖-high concentration, ⊙-medium, ○-low, ⊖-trace.

for development and reproduction in other insects. In the present study, it was found that *L. disponsi* metabolized various indole compounds, leading to the assumption that the bug conjugated IAA in the gut by attaching such substances as glucose, some amino acids or proteins to the side chain ($-\text{CH}_2\text{COOR}$) of the indole nucleus. This may be a detoxication mechanism and a mechanism preventing the ingested IAA from being absorbed into the haemolymph, which is important for the bug which is fond of feeding on the reproductive organs of plants where auxins are contained in higher concentration. However, a small amount of IAA or IAA metabolites enter the haemolymph and then transfer to the salivary gland (MILES and HORI, 1977). Some aphids have IAA in their salivary gland which may be one of the important factors making galls on the host plant (SCHÄLLER, 1968a, b). The IAA in the salivary gland comes from the diet (NUORTEVA, 1956) or is synthesized in it (MILES, 1968). If the IAA metabolites with auxin activity that appear in the excreta were transferred intact to the salivary gland through the haemolymph, they should be phytotoxic. Therefore, the metabolism of IAA in the haemolymph and the salivary gland should be investigated in the future.

ACKNOWLEDGEMENTS

The author is thankful to Prof. Y. NISHIJIMA of the Obihiro University for reading the manuscript. Thanks are also due to Dr. Y. MINO of the same University for his valuable suggestions.

REFERENCES

- DEMPSTER, J. P. (1964) The feeding habits of the Miridae (Heteroptera) living on broom (*Scorothamnus scoparius* (L.) WIMM.). *Ent. exp. appl.* **7**: 149-154.
- HORI, K. (1971) Studies on the feeding habits of *Lygus disponsi* LINNAVUORI (Hemiptera: Miridae) and the injury to its host plants-I. Histological observations of the injury. *Appl. Ent. Zool.* **6**: 84-90.
- HORI, K. (1974) Plant growth-promoting factor in the salivary gland of the bug, *Lygus disponsi*. *J. Insect Physiol.* **20**: 1623-1627.
- HORI, K. and M. ENDO (1977) Metabolism of ingested auxins in the bug *Lygus disponsi*: Conversion of indole-3-acetic acid and gibberellin. *J. Insect Physiol.* **23**: 1075-1080.
- KALDEWEY, H. (1969) Simple indole derivatives and plant growth regulators. In *Thin-layer Chromatography*

- (ed. STAHL, E.), pp. 471-493. Springer, Berlin.
- LEIGH, T. F. (1976) Detrimental effect of lygus feeding on plants. In *Lygus bug: Host plant interaction. Proc. XV Int. Congr. Ent. 1976 Washington, DC, USA* (ed. SCOTT, D. R. and O'KEEFE, L. E.), pp. 1-2. University Press of Idaho.
- MILES, P. W. (1968) Insect secretions in plants. *Ann. Rev. Phytopathol.* **6**: 137-163.
- MILES, P. W. and K. HORI (1977) Fate of ingested β -indolyl acetic acid in *Creontiades dilutus*. *J. Insect Physiol.* **23**: 221-226.
- NUORTEVA, P. (1956) Studies on the effect of the salivary secretions of some Heteroptera and Homoptera on plant growth. *Suom. Hyont. Aikak* **22**: 108-117.
- SCHÄLLER, G. (1968a) Biochemische Analyse des Aphidenspeichels und seine Bedeutung für die Gallenbildung. *Zool. Jb. Physiol.* **74**: 54-87.
- SCHÄLLER, G. (1968b) Untersuchung zur Erzeugung künstlicher Pflanzengallen. *Marcellia* **35**: 131-153.
- SCHEURER, S. (1976) The influence of phytohormones and growth regulating substances on insect development processes. *Symp. Biol. Hung.* **16**: 255-259.
- TOWERS, G. H. N. (1964) Metabolism of phenol in higher plants and micro-organisms. In *Biochemistry of Phenolic Compounds* (ed. HARBORNE, J. B.), pp. 249-294. Academic Press, London.