INSECT ANTIFEEDING SUBSTANCES IN PLANT LEAVES

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ABSTRACT

Insect antifeeding substances contained in plant leaves were widely surveyed by monitoring with 'leaf-disc test'. Larvae of tobacco cutworm, *Spodoptera litura* F., were mainly the test insects and leaves of sweet potato, *Ipomoea batatas* Lamark var. *edulis* Makino, the insect baits. Leaves of *Cocculus trilobus* DC, *Parabenzoin trilobum* Nakai (= *Lindera triloba* Blume), *Orixa japonica* Thunberg, *Clerodendron tricotomum, Caryopteris divaricata* Maxim and other *Verbenaceae* plants have been systematically examined. Many kinds of insect antifeeding substances, i.e. alkaloids, sesquiterpenes, coumarines and diterpenes, were isolated and identified.

These chemical structures have contributed to the knowledge of natural products chemistry and suggested the parent structure of insect antifeeding substances. The methodology for insect antifeeding substance research is discussed.

I. INTRODUCTION

Pesticide chemicals have played a significant part in increasing agricultural production and productivity. However, the continuous use of insecticides, in particular, has not only caused death through poisoning, accumulated in man, concentrated in food chains, but also caused resistance in pest populations and destroyed parasites, predators and pollinators. In this circumstance, research needs are aimed at establishing alternative means of insect control, i.e. non-insecticidal approaches.

One of the wisest approaches to alternative insect controls would come from the use of the knowledge on natural products able to influence insect chemosensory behaviour as attractants, repellents, stimulants, antifeedants and arrestants.

The feeding behaviour of insects can be divided into four steps: (1) host plant recognition and orientation, (2) initiation of feeding, (3) maintenance of feeding and (4) cessation of feeding. Antifeedant is concerned with steps (2) and (3). The term antifeedant is defined as a chemical that inhibits feeding but does not kill the insect directly, the insect remaining near the treated leaves and dying through starvation. Gustatory repellent, feeding deterrent

and rejectant are synonymous with antifeedant. Studies of the natural phenomenon of antifeeding (no consumption of plant material by insect) could reveal the presence of new antifeeding substances in plants, and provide correlations between chemical structure and antifeeding activities. Antifeeding could be of great value in protecting crops from noxious insects as an alternative insect control means to insecticides.

There are many investigations on synthetic chemicals which have insect antifeeding activity, but in this lecture the author would like to discuss the natural antifeeding substances contained in plants.

The host selection in phytophagous insects is governed by the presence or absence of attractants and repellents in plants[1]. For example, Buhr, Toball and Schreiber[2] reported that some of the alkaloid glycosides implants of *Solanaceae* acted as repellents to the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say. 2-Phenylethyl-isothiocyanate from edible parts of turnip (*Brassica rapa* L.) and 5-allyl-1-methoxy-2,3-methylenedioxybenzene or mytisticin from edible parts of parsnip (*Pastinaca sativa* L.) have been shown to act as naturally occurring antifeedants[3,4]. Moreover, one of the resistant factors in corn plants to the European corn borer, *Pyrausta nubilalis* (Hübner), was identified as 6-methoxybenzoxazolinone[5]. A waxy fraction from the extracts of wood of West Indian mahogany showed a high termite repellency[6]. Rudman and Gay[7] noted that 2-methyl-, 2-hydroxymethyl- and 2-formyl-anthraquinones present in the extracts of teak heart-wood were all effective in inhibiting termite activity. The alkaloidal glycosides, such as lepline II and III, demmissine and tomatine, inhibited feeding of tomato beetles[8].

Jermy[9] studied extensively the botanical distribution of antifeeding substances in plants of 43 families in relation to eight insect species, and suggested that there was a fundamental difference in the function of chemoreceptors reacting upon deterrents.

The seeds of neem, the Indian lilac, *Melia azadirachta* L. or *Melia indica*, have been shown to contain an antifeeding substance against locust[10]. The active principle was isolated and the chemical structure of the triterpenoid, named meliantriol, was established. However, the other antifeedant, named azadirachtin, from the same tree was isolated and its partial structure apart from meliantriol was reported by Butterworth and his group[11]. In this lecture the author would like to discuss the research results on antifeeding substances in plants achieved in the author's laboratory during the past 10 years.

II. SCREENING METHOD

In a preliminary experiment two leaf-discs, 16 mm dia., were punched out with a cork-borer from the leaves of food plants, usually sweet potato leaves, *Ipomoea batatas* Lamark var. *batatas* Makino. One disc was immersed in an acetone solution of the test sample for 2 min, and the other, the control, in pure acetone. After air drying, these discs were placed symmetrically in a polyethylene dish (100 mm dia. × 45 mm deep), the bottom of which was covered with a sheet of filter paper moistened with distilled water, and 10 test insect larvae, mainly the third instar larvae of tobacco cutworm, *Spodoptera litura* F., were introduced into the dish. About half the area of the control

disc was eaten usually within 2 h, and the consumed areas of the discs were measured by Dethier's method[12]. The consumed area of the sample disc expressed as a percentage of the consumed area of the control disc showed the feeding inhibitory activities of the samples. This 'leaf-disc test' was repeated twice. When consumption of the sample disc was less than 20% of the consumption of the control discs, the sample were considered to have feeding inhibitory activities.

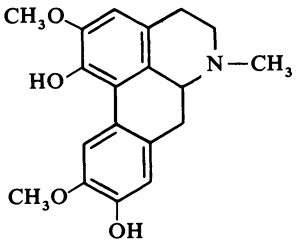
III. ANTIFEEDING SUBSTANCES IN PLANTS

A. Antifeedants from *Cocculus trilobus* DC

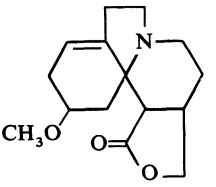
Cocculus trilobus DC, which is well known as the host plant of Japanese fruit-piercing moths, *Oraesia excavata* Butler and *O. emarginata* Fabricius, is scarcely attacked by other insects in nature. Therefore, it was assumed that *C. trilobus* contained toxins or feeding inhibitors against other insects.

Two alkaloids were isolated as crystalline forms from the fresh leaves of this plant. An insecticidal alkaloid was named cocculolidine (II), and an antifeedant alkaloid was identified as isoboldine (I) by the comparison of its physical properties with those of an authentic sample. The authors proposed structure II for cocculolidine.

To determine the threshold concentration of isoboldine for feeding inhibition, serial acetone dilutions of pure isoboldine were applied to leaves of *Euonymus japonicus* Thunberg, and the treated leaves were submitted to the leaf-disc test with *Abraxas miranda* Butler. The feeding ratios were near



(I) Isoboldine



(II) Cocculolidine

zero at concentrations of 200 p.p.m. or greater; at concentrations of 100 and 10 p.p.m. the feeding ratios were 40–59% and 48–126%, respectively (*Table 1*). The leaf-disc test with *Spodoptera litura* F. was conducted with 100 and 200 p.p.m. acetone solutions of pure isoboldine. Leaves of sweet potato, one of the many host plants of this insect, were used as feeding discs. This alkaloid showed feeding inhibitory activity at 200 p.p.m. (*Table 1*). The leaf-disc test with *O. excavata* eating leaves of *C. trilobus* was conducted using 500 and 1000 p.p.m. acetone solutions of pure isoboldine, but the insects ate up the treated leaf discs. These facts suggest an interesting host–insect interrelationship about the constituents in host plants[13].

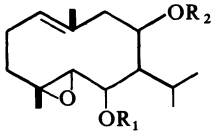
Table 1. The feeding inhibitory activity of isoboldine

Insect species	Concentration (p.p.m.)	Percentage consumed Control (*A*)	Treatment (*B*)	Feeding ratio (*B*)/(*A*) × 100
A. miranda	1000	15	0	0
		35	4	11·4
	500	28	3	10·7
		42	0	0
	200	23	0	0
		22	0	0
	100	39	23	59·0
		20	8	40·4
	10	23	11	47·8
		27	34	126·0
P. litura	200	33	2	6·1
		30	0	0
	100	37	10	26·4
		33	11	33 4
O. excavata	1000	38	38	100·0
		29	29	100·0
	500	65	35	54·0
		66	66	100·0

B. Antifeedants of *Parabenzoin trilobum* Nakai

(= *Lindera triloba* Blume)

Leaves of *P. trilobum* ('Shiromoji' in Japanese) are not attacked by the larvae of *Spodoptera litura.* The crude extract also showed antifeeding activity in the leaf-disc test. The active antifeedant material was benzene-extractable, neutral, and eluted by 30% ether in n-hexane from a silica-gel column. Two active compounds were isolated from the extract and named shiromodial diacetate (III) and shiromodiol monoacetate (IV) and their chemical structures were assigned as (III) and (IV)



(III) Shiromodiol diacetate. $R_1 = R_2 = Ac$
(IV) Shiromodiol monoacetate. $R_1 = H$; $R_2 = Ac$

The threshold concentration of IV was about 0·125% and 0·033% in acetone when tested with *S. litura* and *Abraxas miranda*, respectively[14].

C. Antifeedants from *Orixa japonica* Thunberg

This plant has long been used to insect-proof books in Japan, and after preliminary assessment of antifeeding substances for tobacco cutworm, *Spodoptera litura* F., the benzene extract of the leaves showed antifeeding activities. The active principles in the leaves were separated and identified

as isopimpinellin, bergapten and kokusagin. These and related compounds were assayed for antifeeding activities, and it was revealed that isopimpinellin and bergapten are rather potent, as shown in *Table 2*[15].

Table 2. Insect antifeeding activity of naturally occurring furocoumarines

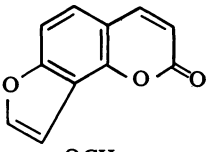
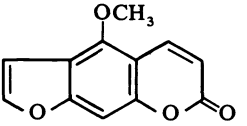
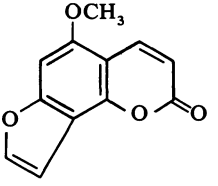
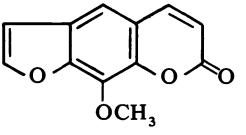
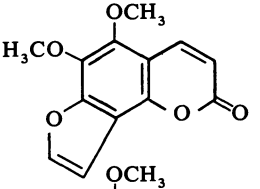
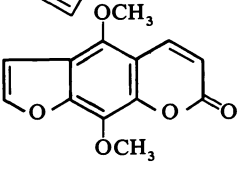
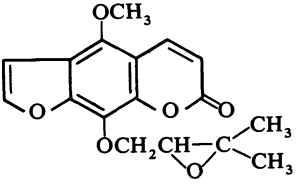
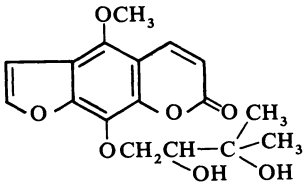
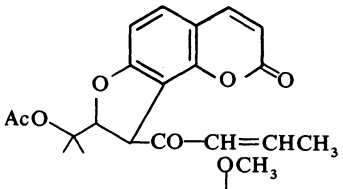
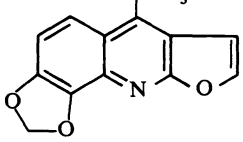
Compounds		Active conc. (p.p.m.)
Angelicin		(-)
Bergapten		500
Isobergapten		500
Xanthotoxin		(-)
Pimpinellin		1000
Isopimpinellin		300
Byakangelicol		(-)

Table 2—contd.

Byakangelicin		(–)
Edultin		(–)
Kokusagin		1000

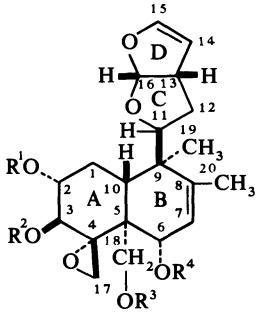
D. Antifeeding substances in *Clerodendron tricotomum* Thunberg

In 1962 T. Miyake investigated antifeeding activities of the hot water extracts from the fresh leaves of various plants. *Euproctis pseudoconspersa* Strand and *Cicadella viridis* Linn., and particularly the extracts of *Clerodendron tricotomun* Thunberg (*Verbenaceae*), showed a strong antifeeding activity[16]. We have reinvestigated the matter and found that benzene extracts from air-dried leaves of *C. tricotomum* Thunberg showed distinct antifeeding activity for the larvae of tobacco cutworm, *Spodoptera litura* F. In order to isolate and identify the active principle present in the species, a systematic chemical analysis monitored by leaf-disc test was undertaken.

Purification by column chromatagraphy over alumina and silica-gel gave two crystallines named clerodendrin A and B. Clerodendrin A and B were determined as (VI)[17] and (VII)[18], both having a clerodon skeleton[19], from the chemical and spectroscopic evidence. The absolute configuration of clerodendrin A was confirmed as (VI) by x-ray crystallography of the *p*-bromobenzoate chlorohydrin derivative[17]. The structure of clerodin (V), a bitter principle of the Indian bhat tree, *Clerodendron infortunatum*, had been established in 1961[19].

Clerodendrin A and B, having slightly bitter taste, showed 100% antifeeding activities at 300 and 200 p.p.m. concentrations, respectively[20]. Clerodin exhibited a 100% antifeeding activity at 80 p.p.m.

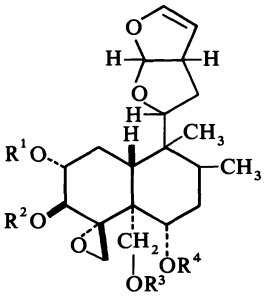
These diterpene derivatives having a clerodon skeleton showed bitter taste. However, there seems to be no linear relationship between the bitter taste and the antifeeding ability for the insect larvae, since the polyol derivatives with weak activities showed slightly bitter taste, whereas the methanol adduct derivative, which does not manifest a marked bitterness, showed the



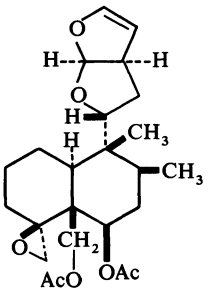
(VI) $R^1 = H$

$R^2 = C_2H_5C(CH_3)(OAc)CO-$

$R^3 = R^4 = Ac$



(VII) $R^1 \sim R^4$ = same groups as in (VI)



(V)

strongest antifeeding activity at 15 p.p.m. Furthermore, it was revealed that the larvae did not bite the treated leaves, and eventually starved to death, when the concentration of the test solution was raised above twice the concentrations showing the 100% antifeeding activities with the exception of the polyol and acetate derivatives. Although the exact mode of action has not been pinpointed, both 'by smell' and 'by taste' are possible.

Clerodendrin A and B did not inhibit feeding of the larvae of *Calospilos miranda* Butler at concentrations below 5000 p.p.m. and the larvae of the European corn borer, *Ostrinia mubilalis* Hubner, showed 100% antifeeding activities at the concentration of 5000 p.p.m. The principle also prevented feeding of the larvae of oriental tussock moth, *Euproctis subflava* Bremer, at the level of 1000 p.p.m. These limited results suggest that these compounds may act as antifeedants for the larvae of polyphagous rather than monophagous insects.

E. Antifeeding substances in *Verbenaceae*

After the discovery of insect antifeeding substances from *Clerodendron* plants, constituents of *Verbenaceae* plants have interested us for screening of insect antifeedants.

Plants of the family *Verbenaceae*, three in Japan, 13 in Taiwan, were collected, and their benzene extracts were subjected to the leaf-disc test of tobacco cutworm. The antifeeding activities of the benzene extracts are shown in *Table 3. Caryopteris divaricata, Callicarpa japonica, Clerodendron fragrans, Clerodendron calamitosum, Clerodendron cryptophyllum* showed strong antifeeding activities at one percent concentration in the leaf-disc tests.

From these plants many clerodon compounds were isolated and their chemical structures were investigated.

The extract of *Caryopteris divaricata* Maxim showed a strong antifeeding activity for the larvae, and eight antifeeding diterpenes having a clerodon skeleton—clerodin (I)[19], caryoptin (II), dihydroclerodin-I(V), dihydrocaryoptin

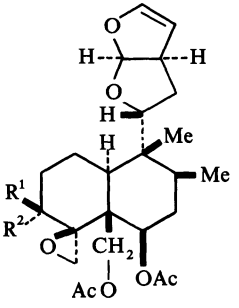
Table 3. Antifeeding activity of *Verbenaceae* plants

Species		Activity[a]
Caryopteris divaricata	(leaf)	++++
Callicarpa japonica	(leaf)	++++
Clerodendron fragrans	(leaf)	++++
	(stem)	+
C. inerme	(stem)	+
C. paniculatum	(leaf)	+
	(stem)	+
C. calamitosum	(leaf)	++++
	(stem)	++
C. cryptophyllum	(leaf)	++++
	(stem)	+
C. paniculatum var. *albiflora*	(stem)	−
Callicarpa formosana	(leaf)	+
	(stem)	+
Duranta repens	(leaf)	−
	(stem)	−
Lantana camara	(leaf)	−
Lippia nodiflora	(whole plant)	−
Premna integrifolia	(leaf)	−
	(stem)	−
Vitex negundo	(stem)	−
V. trifolia	(seed)	+++
Verbena officinalis	(whole plant)	−

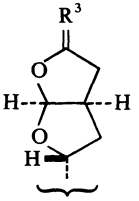
[a] ++++, +++, ++ and +, showed 80–100% antifeeding activity at 1, 2·5, 5 and 10% concentration, respectively. −, showed no effect at 10% concentration.

(VI), clerodin hemiacetal (VII), caryoptin hemiacetal (VIII), caryoptinol (IX) and dihydrocaryoptinol (X)—were isolated and identified[21, 22].

Clerodendron fragrans gave no strong antifeedants, but a weak antifeedant phytol in a high concentration (0·2% yield on dried basis) was isolated from the active fractions, and identified as phytol. *C. calamitosum* gave a new



I $R^1 = R^2 = H$
II $R^1 = OAc, R^2 = H$
IX $R^1 = OH, R^2 = H$
XI $R^1 = H, R^2 = OAc$



V $R^1 = R^2 = H, R^3 = H_2$
VI $R^1 = OAc, R^2 = H, R^3 = H_2$
VII $R^1 = R^2 = H, R^3 =$, OH
VIII $R^1 = OAc, R^2 = H, R^3 = H,OH$
X $R^1 = OH, R^2 = H, R^3 = H_2$

antifeedant named 3-epicaryoptin (XI), and from *C. cryptophyllum* clerodendrin-A (XII)[17] was isolated and identified to be the antifeeding substance[23].

It is interesting that the compounds containing the clerodon skeleton are found in both *Clerodendron* and *Caryopteris* species. Moreover, it is interesting in view of biogenesis that 3-epicaryoptin rather than caryoptin is observed in *C. calamitosum.*

The 11 antifeeding substances and one derivative obtained were tested against the larvae of *S. litura* at different concentrations. The antifeeding activity was embodied as the concentration of sample solution which exhibited the 100% antifeeding activity within the defined time (2 h) (*Table* 4).

Table 4. Activities of *Verbenaceae* antifeedants against *S. litura*

Compound	p.p.m.[a]	Compound	p.p.m.[a]
Phytol	5000	Clerodin hemiacetal	50
Caryoptin	200	Caryoptinol	200
Dihydrocaryoptin	80	Dihydrocaryoptinol	100
Caryoptin hemiacetal	200	3-Epicaryoptin	200
Clerodin	50	Clerodendrin-A	200
Dihydroclerodin-I	50	3-Epidihydrocaryoptin[b]	100

[a] The concentration of the sample solution showing the 100% antifeeding activity within 2 h.
[b] Catalytic reduction product of *X*.

The antifeeding activities of a series of clerodin derivatives are much stronger than those of the caryoptin derivatives. The difference in the activities may be attributed to the presence of 3β-acetoxyl group in ring A. It can be concluded that the functional groups in ring A contribute greatly to the antifeeding activity of these diterpenes.

In addition, the feeding test was allowed to continue for 6 h and more at the concentration showing the 100% antifeeding activity within 2 h. The test leaves treated with the antifeeding diterpenes were not bitten after 24 h and more, and the larvae eventually starved to death. Therefore, the term absolute antifeedant is applicable to these diterpene compounds. The term relative antifeedant is used of compounds retarding the feeding of larvae only for the defined time.

IV. CONCLUSIONS

We have isolated and identified many natural products having insect antifeeding activities from plant leaves. In nature these compounds seem to have a role as resistant factors against insect attacks. Based on our experience of the isolation and identification of insect antifeedants from plant leaves, the following methodology could be suggested for insect antifeedant studies:

(1) development of a rearing method for the test insect larvae for the whole year;

(2) survey for a plant which is not eaten by test insect larvae among many kinds of plant leaves (in this step one or several leaves are sufficient for testing);

(3) preparation of the leaf-disc test for monitoring the isolation experiment;
(4) preliminary extraction of leaves with several solvents monitored by leaf-disc test (approximately 10 g of leaves would be usually used);
(5) collection of large amounts of leaves having antifeeding activity (up to 10 kg has been used);
(6) isolation experiments monitored by the leaf-disc test;
(7) identification of active compounds, and if necessary chemical synthesis of them and their related compounds;
(8) field tests.

Up to now we have unfortunately been unable to develop practical antifeedants, but we have found many new natural products having antifeeding activities. If these natural products are resistance factors against insect attacks, the research interests should be directed to the problems of the host–parasite interrelationship and varietal resistance of crops to insects.

REFERENCES

[1] A. J. Thorsteinson, *Ann. Rev. Entomol.* **5**, 193 (1960).
[2] H. Buhr, R. Toball and K. Schreiber, *Ent. Exp. Appl.* **1**, 209 (1958).
[3] E. P. Lichtenstein, R. M. Strong and D. G. Morgan, *J. Agric. Fd. Chem.* **10**, 30 (1962).
[4] E. P. Lichtenstein and J. E. Casida, *J. Agric. Fd. Chem.* **11**, 410 (1963).
[5] E. E. Smissman, J. B. Lapidus and S. D. Beck, *J. Amer. Chem. Soc.* **79**, 4697 (1957).
[6] C. F. Asenjo, L. A. Marin, W. Torres and A. Campillo, *Chem. Abstr.* **53**, 22707 (1959).
[7] R. Rudman and F. J. Gay, *Hortzforschung*, **15**, 117 (1961).
[8] F. Strarchow and I. Loaw, *Ent. Exp. Appl.* **4**, 133 (1961).
[9] T. Jermy, *Ent. Exp. Appl.* **9**, 1 (1966).
[10] D. Lavie, M. K. Jain and S. R. Shpan-Gabrielit Gabrielith, *Chem. Comm.* **1967**, 910.
[11] J. H. Butterworth, E. D. Morgan and G. R. Percy, *J.C.S. Perkin I*, 2445 (1972).
[12] V. G. Dethier. *Chemical Insect Attractants and Repellents*, p. 210. Blakiston; Philadelphia (1947).
[13] (a) K. Wada and K. Munakata, *Agric. Biol. Chem.* **31**, 336 (1967);
(b) K. Wada, S. Marumo and K. Munakata, *Agric. Biol. Chem.* **31**, 452 (1967);
(c) K. Wada, S. Marumo and K. Munakata, *Agric. Bfol. Chem.* **32**, 1187 (1968);
(d) K. Wada and K. Munakata, *J. Agric. Fd. Chem.* **16**, 471 (1968).
[14] K. Wada, Y. Enomoto, K. Matsui and K. Munakata, *Tetrahedron Letters*, No. 45, 4673 (1968);
(b) K. Wada and K. Munakata, *Tetrahedron Letters*, No. 45, 4677 (1968);
(c) K. Wada, K. Matsui, Y. Enomoto, O. Ogiso and K. Munakata, *Agric. Biol. Chem.* **34**, 942 (1970).
(d) K. Wada, Y. Enomoto and K. Munakata, *Agric. Biol. Chem.* **34**, 947 (1970).
[15] N. Yajima, K. Tsuzuki, N. Kato and K. Munakata, Abstract of Papers, Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April, 1969, p. 145.
[16] T. Miyake, private communication.
[17] N. Kato, S. Shibayama, K. Munakata and C. Katayama, *Chem. Commun.* 1632 (1971).
[18] N. Kato and K. Munakata· unpublished results.
[19] D. H. R. Barton, H. T. Cheung, A. D. Cross, L. M. Jackman and M. Martin-Smith, *Proc. Chem. Soc.* 76 (1971); *J. Chem. Soc.* 5061 (1961); D. A. Sim, T. A. Hamor, I. C. Paul and J. M. Robertson, *J. Chem. Soc.* 75 (1961); I. C. Paul, D. R. Sim, T. R. Hamor and J. M. Robertson, *J. Chem. Soc.* 4133 (1962).
[20] N. Kato, M. Takahashi, M. Shibayama and K. Munakata, *Agric. Biol. Chem.* **36**, 2579 (1972).
[21] S. Hosozawa, N. Kato and K. Munakata, *Phytochemistry*, **12**, 1833 (1973).
[22] S. Hosozawa, N. Kato and K. Munakata, *Agric. Biol. Chem.* **38**, 823 (1974).
[23] S. Hosozawa, N. Kato, K. Munakata and Yuh-Lin Chen, *Agric. Biol. Chem.* **38** (5) (1974) in press.