Phloroglucinol-type Furocoumarins, a Group of Potent Naturally-Occurring Insect Antifeedants

Toshikazu YAJIMA and Katsura MUNAKATA

Laboratory of Pesticide Chemistry, Faculty of Agriculture,
Nagoya University, Nagoya, Japan

Received February 5, 1979

Phloroglucinol-type furocoumarins were found to have strong antifeeding activity against *Spodoptera litura* (tobacco cutworm), but the other furocoumarins belonging to pyrogallol- or resorcinol-type had only weak activity. Antifeeding activity of these compounds against the other insects was also reported.

We previously found that insect antifeeding substances in *Orixa japonica* (*Rutaceae*) are furocoumarins (isopimpinellin, bergapten and xanthotoxin), furoquinolines (kokusagine, evoxine, skimmianine and dictamnine) and phenyl quinoline (japonine).¹⁾ Insect antifeedants in *Angelica japonica* A. Gray (*Umbelliferae*) were also showed to be furocoumarins (isopimpinellin and xanthotoxin).²⁾ Among them, the furocoumarins exhibited the most potent antifeeding activity to *Spodoptera litura* in the leaf disc test.

Thus, we examined the antifeeding activity of typical naturally-occurring furocoumarins. We report here the insect antifeeding activity and insecticidal activity of furocoumarins.

MATERIALS AND METHODS

Bioassay. Insect antifeeding activity to *S. litura* was tested by the leaf disc method in which the 3rd instar larvae were fed enough on the leaves of sweet potato (*Ipomoea batatas* Lam. var. *edulis* Makino).³⁾ Insecticidal activity to *S. litura* was examined by the feeding method, in which the 3rd instar larvae were allowed to feed on artificial diets containing test chemicals for 6 days before larval mortality was determined.

The antifeeding activity against three species of cockroach, viz., *Periplaneta americana*, *Stylopyga rhombifolia* and *Blattella germanica* was measured by the modified schelter method, in which sugar pellets containing test chemicals were fed to the adult cockroach.⁴⁾ The antifeeding activity against *Musca domestica* (house fly; adult) was examined by the turning table method.⁵⁾ The insecticidal activity to *Culex pipiens pallens* (3rd instar larvae) was examined by immersion method.⁶⁾ The insecticidal activity to

Callosobruchus chinensis (adzuki-bean weevil; adult) was tested by dipping method.⁷⁾

Materials. Isopimpinellin, bergapten, xanthotoxin and kokusagine were isolated from *O. japonica*. Isobergapten, pimpinellin, angelicin and psoralen were supplied by Dr. K. Hata, Osaka College of Pharmacology and byakangelicol and byakangelicin were presented by Dr. H. Mitsuhashi, Faculty of Science, Hokkaido University. Peucedanin, khellin, umbelliferon and coumarin were purchased from commercial sources. Dihydroisopimpinellin, dihydroxanthotoxin and dihydrokokusagine were synthesized.

MS spectra were measured in a JEOL D-100 mass spectrometer. IR spectra were recorded with a JASCO IR-G spectrophotometer. PMR spectra were recorded on a JEOL MH-100 spectrometer. UV spectra were recorded with a Hitachi EPS-3T spectrophotometer. Melting points were uncorrected.

Dihydroisopimpinellin (XI). A suspension of 13.2 mg of 10% palladium on charcoal in 7 ml of ethyl acetate was placed in the reaction bottle of a catalytic reduction apparatus. The apparatus was evacuated and then filled with hydrogen. A solution of 29.2 mg of isopimpinellin in 3 ml of ethyl acetate was added to the reaction bottle and the mixture was shaken with hydrogen until the required volume of 2.84 ml was absorbed. The time required was about thirty minutes. The catalyst was filtered off and the solvent removed from the filtrate by distillation *in vacuo*. The dihydroisopimpinellin was recrystallized from aqueous methanol and the separated needles melted at 162~163°C. The yield was 21 mg (75%). MS *m/e*: 248 (M^+), 233 (base peak). PMR δ ^{CDCl_3} $_{Me_4Si}$: 3.41 (2H, d, $J=9.0$ Hz), 3.95 (3H, s), 4.72 (2H, t, $J=9.0$ Hz), 6.15 (1H, d, $J=10.0$ Hz), 7.89 (1H, t, $J=10.0$ Hz).

Dihydroxanthotoxin (XII). Xanthotoxin (80.2 mg) was hydrogenated over 10% palladium on charcoal (7.8 mg) suspended in ethyl acetate (10 ml). The

reaction mixture was purified with preparative TLC on silica gel (60PF₂₅₄) using a mixture of EtOAc, CHCl₃ and *n*-Hex. (1:1:1) as the developing solvent. The fraction possessing *R_f* value of 0.50 was collected, extracted and identified as dihydroxanthotoxin on the basis of the spectrometric data. The crude material was recrystallized from aqueous methanol to afford colorless needles (15.1 mg, 19%, mp 159~160°C, lit., 163°C).⁸⁾ IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1718, 1608, 1576, 1412, 1290, 1140, 1071, 1000, 881, 821. PMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 3.27 (2H, t, *J*=9.0 Hz), 4.04 (3H, s), 4.72 (2H, t, *J*=9.0 Hz), 6.19 (1H, d, *J*=9.0 Hz), 6.98 (1H, s), 7.98 (1H, d, *J*=9.0 Hz).

Dihydrokokusagine (XVIII). Kokusagine (19.6 mg) was hydrogenated over 30% palladium on charcoal (14 mg) suspended in ethanol (15 ml). The catalyst was filtered off and the solvent removed from the filtrate by distillation *in vacuo*. The resulting amorphous powder was crystallized from aqueous methanol to afford colorless needles of dihydrokokusagine (XVIII, 86 mg, 95%, mp 165~166°C, lit., 169°C).⁹⁾ MS *m/e*: 245 (M⁺, base peak), 220, 202, 174. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3110, 2980, 1620, 1578, 1529, 1441, 1369, 1278, 1230, 1088, 1067, 1045, 804, 754, 720, 490. PMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 3.62 (2H, t, *J*=9 Hz), 4.20 (3H, s), 4.62 (2H, t, *J*=9 Hz), 6.18 (2H, s), 6.97 (1H, d, *J*=8 Hz), 7.61 (1H, d, *J*=8 Hz). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 218 (28,700), 253 (46,600), 315 (4,100).

RESULTS AND DISCUSSION

Table I shows the insect antifeeding activity of furocoumarins against *S. litura*. The phloroglucinol-type furocoumarins which bear 1,3,5-trihydroxybenzene moiety in the molecules, *viz.*, isopimpinellin (I), bergapten (II), isobergapten (V) and pimpinellin (VI) showed insect antifeeding activity against *S. litura* with the low threshold concentrations over the range of 5~100 ppm, but byakangelicol (III) and byakangelicin (IV) having a bulky substituent in the phloroglucinol-type molecule had no antifeeding activity. Pyrogallol-type furocoumarin (1,2,3-trihydroxybenzene-type) *viz.*, xanthotoxin (VII) and resorcinol-type furocoumarin (1,3-dihydroxybenzene-type), *viz.*, angelicin (VIII), psoralen (IX) and peucedanin (X), exhibited antifeeding activity to *S. litura* only at the high concentrations over the range of 500~1000 ppm.

It has been reported that many examples of

TABLE I. FEEDING INHIBITORY ACTIVITY OF FUROCOUMARINS AGAINST *Spodoptera litura*^a

Compounds	Concentrations (ppm)	Per cent consumed		
		Control (A)	Treatment (B)	Feeding ratio ^b (B)/(A) × 100
Isopimpinellin (I)	1000	96	0	0
	100	90	1	0
	10	74	5	7
	5	20	7	35
	1	80	15	19
Bergapten (II)	1000	100	0	0
	100	45	3	7
	20	42	5	12
	10	33	9	26
	5	7	50	>100
Byakangelicin (III)	1000	25	25	100
Byakangelicol (IV)	1000	60	31	52
	100	15	20	>100
Isobergapten (V)	100	46	3	7
	20	5	17	>100
	10	60	2	3
	5	49	2	4
Pimpinellin (VI)	1000	57	1	2
	100	74	1	1
Xanthotoxin (VII)	1000	99	2	2
	500	70	12	17
	100	9	8	>100
Angelicin (VIII)	1000	90	4	4
	100	30	95	>100
Psoralen (IX)	1000	65	35	54
	100	70	81	>100
Peucedanin (X)	1000	100	12	12
	100	26	38	>100

^a The results are averages of two examinations. For each examination, 10 larvae of test insect were used. The test larvae were allowed to feed on the leaf discs of both control and sample for 90 min before the antifeeding activity was determined.

^b Strong feeding inhibitory activity, 0~20%: slight, 20~50%: none, 50% and greater.

insect antifeeding substances that carry furan or saturated furan group, for example, clerodendrin A and B, clerodin, caryoptin, piperene, meliantriol, azadirachtin and pinguison besides furocoumarins, furoquinolines and khellin.*^{10~13)} Therefore, it is interesting to

* Khellin (5,8-dimethoxy-2-methyl-6,7-furanochromone (XIX), showed insect antifeeding activity. See Table III.

Thus, antifeeding activity of simple coumarins was examined as shown in Table II. 5-Methoxy-7-hydroxycoumarin (XIII), 5-methoxy-7-acetoxycoumarin (XIV), umbelliferon (XV) and coumarin (XVI), all showed no remarkable insect antifeeding activity, namely, removal of fused furan moiety in the furocoumarin molecule caused loss of insect antifeeding activity in our limited instances. The fused furan moiety in the furocoumarin molecule is possibly requisite for having the insect antifeeding activity of furocoumarins.

^b Strong feeding inhibitory activity, 0~20%: slight, 20~50%: none, 50% and greater.

TABLE III. FEEDING INHIBITORY ACTIVITY OF KOKUSAGINE, DIHYDROKOKUSAGINE AND KHELLIN AGAINST *Spodoptera litura*^a

Compounds	Concentrations (ppm)	Per cent consumed		
		Control (A)	Treat- ment (B)	Feeding ratio ^b (B)/(A) × 100
Kokusagine	100	38	14	37
(XVII)	10	23	17	74
Dihydrokokusagine	300	37	8	22
(XVIII)	100	71	51	72
Khellin	1000	25	5	20
(XIX)	100	55	31	56

^a The results are averages of two examinations. For each examination, 10 larvae of test insect were used. The larvae were allowed to feed on the leaf discs of both control and sample for 90 min before the antifeeding activity was determined.

^b Strong feeding inhibitory activity, 0~20%: slight, 20~50%: none, 50% and greater.

xanthotoxin (VII), bergapten (II) and isopimpinellin (I) exhibited insect antifeeding activity to three species of cockroach, viz., *P. americana*, *S. rhombifolia* and *B. germanica* at ca. 67 ppm concentrations by sugar pellet method. Furocoumarins, however, exhibited only weak antifeeding activity against house fly, *Musca domestica*.

Xanthotoxin has been reported to be toxic to southern army-worm, *Spodoptera eridania* at the concentrations of 0.1 and 1.0%. Its toxicity was enhanced by the presence of UV light.¹⁴⁾ Pachyrrhizin and crosnin belonging to 3-phenyl-furocoumarin have been reported to possess insecticidal activity.¹⁵⁾ Therefore, the insecticidal activity of furocoumarins having antifeeding activity was examined.

As shown in Table V, three species of noxious insects, viz., *C. pipiens pallens*, *S. litura* and *C. chinensis* were examined for insecticidal ac-

TABLE IV. FEEDING INHIBITORY ACTIVITY OF FUROCUMARINS, FUROQUINOLINES AND FUROCHROMONE AGAINST VARIOUS INSECTS^a

Compounds		Per cent inhibition			
		<i>Periplaneta americana</i>	<i>Stylopyga rhombifolia</i>	<i>Blattella germanica</i>	<i>Musca domestica</i>
(Furocoumarins)					
Isopimpinellin	(I)	57%	13%	31%	0%
Bergapten	(II)	3	89	54	8
Xanthotoxin	(VII)	54	88	43	7
(Furoquinolines)					
Kokusagine	(XVII)	91	91	70	20
Dihydrokokusagine	(XVIII)	100	85	51	1
(Furochromone)					
Khellin	(XIX)	33	34	87	17

^a Sugar pellet method: 0.1 mg/a pellet (1.5 g); Feeding inhibitory activity=(Sugar consumed by untreated insects - Sugar consumed by treated insects) × 100/(Sugar consumed by untreated insects).

TABLE V. INSECTICIDAL ACTIVITY OF FUROCUMARINS AGAINST VARIOUS INSECTS

Furocoumarins		Per cent kill			
		<i>Culex pipiens</i> ^a <i>pallens</i> (larvae)		<i>Spodoptera</i> ^b <i>litura</i> (larvae)	<i>Callosobruchus</i> ^c <i>chinensis</i> (adult)
		1 ppm	10 ppm	500 ppm	2000 ppm
Isopimpinellin	(I)	0	1.7	0	0
Bergapten	(II)	0	15.0	0	0
Xanthotoxin	(VII)	0	1.7	0	0

^a Immersion method (after 24 hr)

^b Feeding method (after 6 days)

^c Dipping method (after 24 hr).

tivity of furocoumarins (isopimpinellin (I), bergapten (II) and xanthotoxin (VII)). All of the furocoumarins had no insecticidal activity at the tested concentrations except for the case in which bergapten had weak insecticidal activity against *C. pipiens pallens* at the concentration of 10 ppm by immersion method.

In this insecticidal test, feeding consumption rates exhibited by larval *S. litura* on artificial diet containing 500 ppm of furocoumarin were comparable to those displayed on control diet. While, isopimpinellin showed insect antifeeding activity even at a low concentration of 5 ppm by the leaf disc method as shown in Table I.

Furocoumarin in general originates from umbelliferon *in vivo*. The successive biosynthetic reaction of umbelliferon, that is, prenylation, epoxidation, oxidative cyclisation and oxidative splitting of C₃ unit, lead to the formation of psoralen and angelicin which are intermediate furocoumarins.⁶⁾ Bergapten, xanthotoxin and isopimpinellin are further oxidation products of the former and isobergapten and pimpinellin are those of the latter *in vivo*. Both the original umbelliferon and intermediate furocoumarins had a little or no insect antifeeding activity. Phloroglucinol-type, on the other hand, showed the marked biological activity. This finding lends experimental support to host plant selection mechanism of phytophagous insects and insect—host plant coevolution. Furocoumarins occur with great regularity and diversity only in *Rutaceae* and *Umbelliferae*.¹⁷⁾ Up to almost seventy kinds of isopimpinellin-bearing plants are registered in Chemical Abstracts and over hundred kinds of plants containing bergapten are recorded for last two decades. Plants containing phloroglucinol-type furocoumarin might escape from attack by many kinds of phytophagous insects in nature because of their potent insect antifeeding activity. Phloroglucinol-type furocoumarin enables us to make use of it as a guiding genetic index of selective breeding programs in order to seek for more insect-resistant strain of useful vegetables and fruit trees which belong to *Umbelliferae* and *Rutaceae*.¹⁸⁾

Acknowledgment. The authors wish to express their thanks to Dr. Saito and Mr. H. Honda, Laboratory of Applied Entomology and Nematology, Nagoya University, for their kindness in supplying the test insects. They are also grateful to Dr. A. Hayashi, Taisho Pharmaceutical Co., Ltd., for measuring insect antifeeding activity against cockroach and house fly; Members of Laboratory of Pesticide, Research Department, Sumitomo Chemical Co. Ltd., kindly measured insecticidal activity. They are also indebted to Dr. K. Hata, Osaka College of Pharmacology and Dr. H. Mitsuhashi, Faculty of Sciences, Hokkaido University for gift samples. They also wish to express their thanks to Dr. S. Marumo and Dr. K. Wada in their Laboratory for helpful discussion and valuable comments on the manuscript.

REFERENCES

- 1) T. Yajima, N. Kato and K. Munakata, *Agric. Biol. Chem.*, **41**, 1263 (1977).
- 2) T. 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.
- 3) K. Wada and K. Munakata, *J. Agric. Food Chem.*, **16**, 471 (1968).
- 4) Y. Ikeda and I. Kondo, *Botyu-Kagaku* **26**, 112 (1961).
- 5) T. Saito, "Nōyaku no Seibutsu-Kenteihō," ed. by K. Huruyama and H. Sugawara, Nankō-do Co. Ltd., Tokyo and Kyoto, 1965, p. 219.
- 6) H. Sugawara, T. Kaneko and S. Otsuka, *Annual Report of The Agricultural Chemical Inspection Station*, **5**, 49 (1959); *ibid.*, **5**, 55 (1959).
- 7) S. Iwata and A. Sugimoto, "Nōyaku no Seibutsu-Kenteihō," ed. by K. Huruyama and S. Sugawara, Nankō-do Co. Ltd., Tokyo and Kyoto, 1965, p. 100.
- 8) M. E. Brokke and B. E. Christensen, *J. Org. Chem.*, **23**, 589 (1958).
- 9) M. Terasaka, *Chem. Pharm. Bull. (Tokyo)*, **8**, 523 (1960).
- 10) K. Munakata, "ACS Symposium Series, No. 62, Host plant resistance to pest," ed. by P. A. Hedin, the American Chemical Society, 1977, p. 185.
- 11) D. Lavie, M.K. Jain and S.R.S. Gabrilith, *Chem. Commun.*, 910 (1967).
- 12) P. R. Zanno, I. Miura, K. Nakanishi and D. L. Elder, *J. Am. Chem. Soc.*, **97**, 1975 (1975).
- 13) K. Wada and K. Munakata, *Agric. Biol. Chem.*, **35**, 115 (1971).
- 14) M. Berenbaum, *Science*, **201**, 532 (1978).
- 15) D. G. Crosby, "Naturally Occurring Insecticide," ed. by M. Jacobson and D. G. Crosby, Marcel Decker, Inc., New York, 1971, p. 177.

- 16) M. F. Grondon, *Tetrahedron*, **34**, 143 (1978).
17) M. A. Pathak, F. Daniels and T. B. Fitzpatrick, *J. Invest. Dermatol.*, **39**, 225 (1962); J. Mendez and S. A. Brown, *Can. J. Bot.*, **49**, 2097 (1971); L. Tikhomirova, L. Markova, K. Tumbaa and G. Kutznetsova, *Khim. Prir. Soedin*, **10**, 404 (1974); M. Miyakado, N. Ohno, H. Yoshioka and T. Mabry, *Phytochemistry*, **17**, 143 (1978).
18) R. F. Chapman, *Bull. Entomol. Res.*, **64**, 339 (1974).
-