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Halopyrethroids: Structure-Activity Relationships. S. J. NORTON*, O. F. BODENSTEIN** and D. G. BROWN* (*Department of Chemistry, North Texas State University, Denton, Texas 76203 and **Biological Evaluation of Chemicals Laboratory, Agricultural Environmental Quality Institute, Agricultural Research Service, USDA, Beltsville, Maryland 20705, U. S. A.) Received June 3, 1975. *Botyu-Kagaku* 41, 1, 1976.

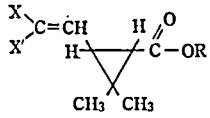
1. ハロピレスロイド類の構造活性相関 S. J. NORTON*, O. F. BODENSTEIN** and D. G. BROWN* (*North Texas 州立大学化学教室, **アメリカ合衆国農務省, 農業環境品質研究所) 50. 6. 3 受理

種々のハロゲンの置換された側鎖をもつ菊酸類縁体を合成し, 5-ベンジル 3-フリルメチルアルコールとのエステルとし, それらの活性を DDT 抵抗性イエバエとネッタイシマカを用いて測定した結果, ジハロビニル側鎖をもつものが, とくにイエバエに対し強力な落下仰転および殺虫活性を示すことがわかった. また Br, Cl, F 誘導体の順序に活性が低下すること, ハロゲンの側鎖二重結合に対する配置が活性にとって大きな影響を示すことが明らかとなった.

Introduction

The preparation and study of compounds related structurally to naturally occurring pyrethrins have been in progress in a number of laboratories[1,2,3]. Recently, in our laboratory, we have been interested in determining the effects of various halogen substituents in the acid moieties of synthetic pyrethroids on the knockdown and kill of certain insects[4]. Our goal also has included the correlation of structure of halogen-containing pyrethroids with insecticidal quality. The halogen

Series I

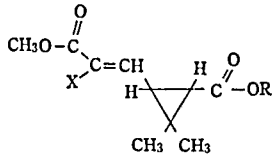


I X=F, X′=F
II X=F, X′=Cl (*cis, trans*)
III X=Br, X′=Cl (*cis, trans*)
IV X=Br, X′=Br
V X=Br, X′=Br

R=(5-Benzyl-3-furyl) methyl

R=from allethrolone

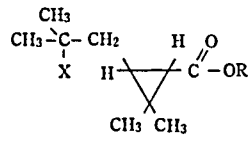
Series II



VI X=Cl
VII X=Br

R=(5-Benzyl-3-furyl) methyl

Series III



VIII X=Cl
IX X=Br
X X=I

R=(5-Benzyl-3-furyl) methyl

Fig. 1. Synthetic pyrethroids containing halogen substituents in the acid moiety

This paper reflects the results of research only. Mention of a pesticide or of a commercial or proprietary product in this paper does not constitute a recommendation by the U.S. Department of Agriculture. This study was supported in part by a grant from North Texas State University Faculty Research Fund and the Robert A. Welch Foundation of Texas, B-133.

atom substituents which have been employed in these studies include fluorine, chlorine, bromine, and iodine, and the halopyrethroids which we wish to discuss in this paper are presented in Fig. 1.

Biological testing

Insecticidal potencies of the halopyrethroids have been studied with DDT-susceptible house flies, *Musca domestica* L., and free-flying yellow-fever mosquitoes, *Aedes aegypti* (L.) with pyrethrins as a standard. The Peet-Grady method[5], involving spray formulations of the insecticides, was employed with house flies, and a variation of this method[6] was employed in studies with mosquitoes. Evaluation of the relative toxicity of the test compounds was made employing probit analysis to generate a dosetoxicity curve. A plot of the logarithm of concentration versus probits of percent toxicity was constructed using replicated tests at dose levels chosen to give percent toxicities between 25 and 75%[7]. Relative toxicities were then calculated from the ratio of the concentration of the test compound which would give 50% toxicity to that of standard pyrethrins giving the same toxicity. Pyrethrins standards levels were normalized to give values of unity.

Organic syntheses

The synthesis of the acid moiety of pyrethroid I, which has the difluorovinyl side chain, was accomplished by reaction of the ylide, (difluoromethylene) triphenylphosphorane, with the aldehyde-ester derived from ozonolysis of *tert*-butyl (±)-*trans*-chrysanthemate. The acid moieties of pyrethroids II and III which have the *cis, trans* chlorofluorovinyl and *cis, trans* bromochlorovinyl side chains respectively, were similarly prepared by reaction of the aldehyde-ester with the appropriate ylides. The synthesis of the acid moiety containing the dibromovinyl side chain of pyrethroids IV and V has been reported[4]; the methods reported are typical for the synthesis of all the dihalovinylpyrethroids prepared in this study. The synthesis of the acid moieties in compounds VI and VII was also by reaction of the appropriate ylide with the aldehyde-ester derived from *tert*-butyl-(±)-*trans*-chrysanthemate which yielded the desired halopyrethrates. Synthesis of the acid moieties of compounds VIII, IX and X was accomplished by HCl, HBr and HI additions respectively to(±)-*trans*-chrysanthemic acid. All of the final esters were prepared by condensation of the appropriate acid chloride with the desired alcohol (usually 5-benzyl-3-furyl carbinol, but in one instance (±)-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one, commonly called allethrolone). Partial separations of the *cis, trans*-halopyrethrates was accomplished by fractional crystallization. The separation of the *cis, trans* isomers of halopyrethroid II was accomplished by preparative gas chromatography employing diethylene-glycol succinate on chromosorb W.

Results

Relative house fly toxicity data for the halopyrethroids having dihalovinyl substituents in the acid moiety (Series I) are given in Table 1. It can be concluded that there is a general enhancement of toxicity in this series as the

Table 1. Relative effectiveness of pyrethroids of Series I against DDT-susceptible house flies.[a]

Dihalovinylpyrethroid	Relative Toxicity (24 hr)
I	71
II (*cis, trans*)	73
III (*cis, trans*)	65-70
IV	24
Pyrethrins	1[b]

[a] See the biological testing section for further details.

[b] Pyrethrins standard was assigned a value of unity. The $LC_{50} \cong 1.4$ mg/ml.

halogens of the dihalovinyl substituent become more electronegative (or decrease in covalent radii). The toxicities of the first three compounds of the series (I, II, III) are quite comparable, however. The toxicity of the dibromovinylpyrethroid (IV) is significantly less than that of the previous three, although it is still quite effective in insect kill when compared to the pyrethrins standard. From these data, and also from unpublished data in our laboratory, it

appears that the halogen substituent effectiveness in a variety of halogen-containing pyrethroids is (in increasing order of toxicity) Br < Cl≦F.

Much more striking differences in the activities of pyrethroids of Series I are seen in the relative knockdown qualities (Table 2). In this study a comparison was made of the 10-min knockdown

Table 2. Ten minute knockdown activity by halopyrethroids of Series I against DDT-resistant house flies[a]

Halopyrethroid	% Knockdown after ten min
I	70
II	50
III	28
IV	4

[a] Results obtained from parallel tests on the same lot of house flies at halopyrethroid concentrations of 0.0625 mg/m*l*. See the biological testing section for further details.

activity at constant halopyrethroid concentration. It is seen that as the electronegativities of the halogen substituents increase, the 10-min knockdown activity also increases. In another parallel study, pyrethroid I exhibited higher knockdown activity than 1-methyl 3-(benzyl-3-furyl) methyl *trans*-(+)-3-carboxy-α, 2, 2-trimethylcyclopropaneacrylic acid (NRDC 106), one of the more potent pyrethroids in knockdown activity[8]. Perhaps a more quantitative relationship between halogen substituent and knockdown activity is shown in Fig. 2, where a reverse trend between

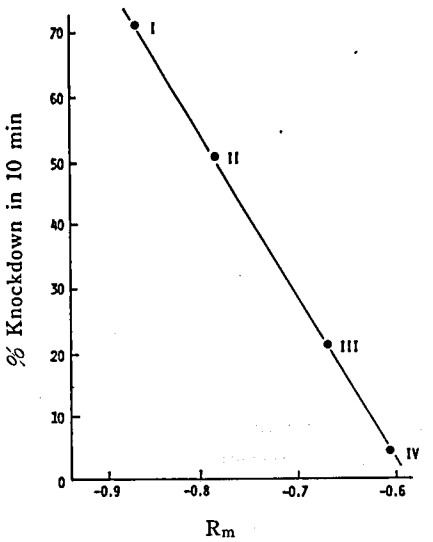
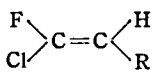
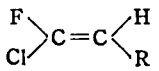
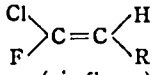


Fig. 2. Percent knockdown in 10 min by pyrethroids of Series I (0.0625 mg/m*l*) versus R_m values. R_m values were determined by use of silica gel plates with heavy mineral oil as the stationary phase and acetone: ethanol: water (60: 10: 30) as the mobile phase.

increasing lipophilic character (increasing R_m values) and knockdown activity is apparent.

While the toxicities of the difluorovinyl-and the chlorofluorovinylpyrethroids are almost identical (Tabl 1), there is a significant difference in knockdown activity. It was of interest to determine if the geometry of the halogen substituents in the *cis-trans* mixture of the latter

Table 3. Knockdown and kill activity of isomeric chlorofluorovinylpyrethroids (II) against DDT-susceptible house flies.[a]

Insecticide	Concentration, mg/m*l*	% Knockdown 10 min	% Kill 1 day
 II (*cis, trans* mixture)	0.0625	50	96
 II (*trans* fluoro)	0.0625	62	91
 II (*cis* fluoro)	0.0625	16	93
Pyrethrins	1.0	99	36

[a] Results were obtained from parallel tests on the same lot of house flies. Additional details on methods are given in the biological testing section.

compound could be involved in this difference in knockdown activity. The *cis* and *trans* isomers of the chlorofluorovinyl compound (II) were separated by liquid-gas chromatography and identified by NMR. Comparative toxicity and knockdown data are given in Table 3. The percent kill values are quite comparable for the isomeric mixture and for the separated geometric isomers; however, there are considerable differences in the 10-min knockdown values. The isomer in which the fluorine atom is in the *trans*-position (relative to the cyclopropane ring) has significantly greater knockdown activity than that of the *cis*-fluoro isomer.

The dihalovinylpyrethroids (I-V) were also tested against yellowfever mosquitoes (Table 4). While the enhancement of toxicity (relative to pyrethrins) was not as great as that of houseflies,

Table 4. Relative effectiveness of pyrethroids of Series I against yellowfever mosquitoes.[a]

Dihalovinylpyrethroid	Relative Toxicity (24 hr)
I	3
II (*cis, trans*)	6
III (*cis, trans*)	>6
IV	2
V	2
Allethrin	≪1
Pyrethrins	1

[a] Additional information is given in the biological testing section.

[b] Pyrethrins standard was assigned a value of unity. The $LC_{50} \cong 0.75$ mg/m*l*.

it was nevertheless, significant. In the case where allethrolone was the alcohol moiety esterified to the dibromovinyl acid moiety, the pyrethroid was much more toxic to mosquitoes than its analog, allethrin[9]. The halogen advantage is quite evident.

House fly toxicity data with pyrethroids VI and VII, in both their *trans* and *cis, trans* side chain geometries, are given in Table 5. These halopyrethrates exhibit kill effectiveness in the same concentration range as the pyrethrins standards. Knockdown activities were also found to be comparable to that of equivalent concentrations of pyrethrins standards. The insecticidal potency of these pyrethroids is also considerably less than that of NRDC 106, the acid moiety of which is pyrethric acid. It is interesting to note that the data in this table suggest that the ester groupings in the *cis* orientation (relative to the cyclopropane ring) of the methyl α-haloacrylate side chains have greater toxicity against house flies than do the *trans* ester groupings. On the basis of the ratios of *cis* to *trans* in the isomeric mixtures, the chloropyrethrate moiety is more potent than the bromopyrethrate moiety (VI, 40% *cis*, 60% *trans*; VII, 75% *cis*, 25%*trans*), in keeping with the general trend of chlorine substituents having greater effectiveness than bromo substituents.

Table 5. Relative effectiveness of pyrethroids of Series II against DDT-susceptible house flies.

Halopyrethrate	Relative toxicity (24 hr)
VI (*trans*. Carbomethoxy group is *trans* to remaining bulk of molecule)	1
VI (*cis, trans* mixture)	1.5
VII (*trans*. Carbomethoxy group is *trans* to remaining bulk of molecule)	0.8
VII (*cis, trans* mixtures)	1.8
Pyrethrins	1.0[a]

[a] Pyrethrins Standard was assigned a value of unity. The $LC_{50} \cong 1.4$ mg/m*l*.

Table 6 presents data obtained from toxicity studies with pyrethroids VIII, IX and X, the halodihydrochrysanthemates, against house flies. The toxicity of these pyrethroids is rather low when compared to the parent structure, resmethrin[1]. Interestingly, however, with the first two

Table 6. Relative toxicity of halodihydropyrethrates (Series III) against DDT-susceptible house flies.

Halodihydropyrethrate	Relative toxicity (24 hr)
VIII	10
IX	15
X	3
Pyrethrins	1[a]

[a] Pyrethrins standard was assigned a value of unity. The $LC_{50} \cong 1.4$ mg/m*l*.

members of this series, the reverse of the anticipated halo-substituent trend (Br<Cl), results upon saturation of the side chain of the acid moiety with hydrogen halides. In all instances these compounds exhibit higher toxicities than that of the pyrethrins standard. Indeed, the toxicity of the bromodihydrochrysanthemate (IX) is near that of halopyrethroid IV, which has the dibromovinyl side chain.

Discussion

It has been shown in this and other studies[3,4] that halogen substitutions (pyrethroids I-V) for the methyl groups of the isobutenyl side chain of the chrysanthemic acid moiety of resmethrin and of allethrin enhance both knockdown and kill in some insects. Knockdown activity can be rather well correlated with the covalent radii of halogen substituents attached to the vinyl group of those pyrethroids having structures analogous to resmethrin. Thus, as the covalent radii of substitutent halogens decrease (bromine→fluorine), the knockdown activity substantially increases. It may also be stated that the knockdown activities increase relative to the increase in electronegativities of the halogen substituents. Pyrethroid IV, which has the least house fly knockdown activity of Series I, has knockdown activity comparable to that of resmethrin[4].

One of the questions that can be asked concerning the high house fly knockdown activity of pyrethroid I is whether only one of the two fluorine atoms of the difluorovinyl side chain is involved in knockdown. It was therefore of interest to determine if the geometry of the halogen substituents in the *cis-trans* mixture of the chlorofluorovinylpyrethroid (II) was involved in determining relative knockdown activity. While the data of Table 1 indicate that halogen substituent geometry has little effect on toxicity, such geometry is apparently involved in determining knockdown efficiency. That the *trans*-fluoro isomer of pyrethroid II has knockdown activity comparable to that of the difluorovinyl-pyrethroid (I), suggests that it is the *trans*-fluoro substituent of the latter compound which is most responsible for high knockdown activity.

Numerous correlations have been made between physical measurements and biological activity. One physical characteristic that has been correlated with biological activity is the lipophilic character of a series of compounds[10]. This lipophilic character may be measured with reversed phase partition chromatography in terms of R_m, where $R_m = \log (1/R_f - 1)$. The correlation of knockdown effectiveness and lipophilicity, shown in Fig. 2, may be taken to indicate that rapid knockdown activity is dependent upon the rate of transfer across a lipid membrane barrier to the site of toxic action in the insect. Support for this correlation has been given by Burt and Goodchild[11], where they showed that pyrethrin I is at least ten times more effective in knockdown when injected than when applied topically to house flies.

The *trans* methyl group of the chrysanthemate side chain in pyrethrin I, resmethrin, and related esters has been shown to be a primary site of detoxification by an oxidative process in insects[12,13]. The replacement of the susceptible methyl group by halogens, with consequent negation of detoxification processes, may explain, in part, the enhanced insecticidal quality of the pyrethroids of Series I. Physical properties of the halopyrethroids which may lead to enhanced transport and disposition in insects should also be considered[14].

The greater house fly toxicity of the *cis*-oriented methyl ester group substituents (as compared with the *trans*-orientation) in pyrethrate ester-related pyrethroids of Series II may be partially[15] explained on the basis of an esterase detoxification process involving the pyrethric acid moiety. The esterase activity would thus be capable of attacking methyl ester groupings oriented in the *trans* position at a rate faster than that for ester groupings in the *cis* orientation.

The toxicity trend with pyrethroids VIII, IX and X is perhaps somewhat surprising. The saturation of the isobutenyl side chain of the chrysanthemic acid moiety by hydrogen halides does produce an expected decrease in insecticidal potency. Elliott *et al.*[16] report that dihydroresmethrin is only 10% as effective as resmethrin. (It can be calculated from that study that

dihydroresmethrin is about 2.5 fold more toxic than pyrethrin.) However, if one considers dihydroresmethrin as the parent compound for this series, all of the halo esters are better than or equal to, the parent compound in toxicity against house flies. This enhancement of toxicity may be due to increased membrane transport rates and/or due to the *in vivo* dehydrohalogenation of the tertiary halo esters into resmethrin, a potent pyrethroid. Bromopyrethroid IX was more toxic to house flies than chloropyrethroid VIII. These data may be interpreted as supporting the supposition that the tertiary halo esters do indeed undergo *in vivo* dehydrohalogenation, since tertiary chlorides are less susceptible than tertiary bromides to this form of decomposition. That the bromopyrethroid is more potent than the chloropyrethroid represents a trend opposite to that observed in Series I and II halopyrethroids. The iodopyrethroid X, however, does not follow this reverse trend. The decreased toxicity of the latter compound may result from a greatly decreased transport rate due to the great bulk and low polarity effects of the iodine atom substituent.

Summary

The high insecticidal quality, low persistency, and low mammalian toxicity of most pyrethroids offer an attractive alternative for insect control. The preparation and study of halogen-atom-containing pyrethroids was undertaken in an effort to correlate the structure of halopyrethroids with insecticidal quality, and to prepare new pyrethroids having enhanced insecticidal activity over parent pyrethroids. All modifications of structure presented in this investigation involve the acid moiety of the pyrethroid structure. The halogen atom substituents which have been employed include fluorine, chlorine, bromine and iodine.

Stereoisomeric cyclopropane acid moieties with halogen or halogen-containing functionalities attached to the three-membered ring were prepared and esterified with certain effective alcohols (usually 5-benzyl-3-furylmethyl alcohol). Carbene and ylide intermediates were employed in the synthesis of the acid moieties. The insecticidal quality of these compounds was tested on DDT-susceptible house flies and yellowfever mosquitoes. Several of the new pyrethroids, particularly those having the dihalovinyl side chain in the acid moiety (Series I), exhibited high potency in both knockdown and toxicity (kill) of house flies. The general trend of halogen substituent effectiveness found in this series was (in increasing order of effectiveness) $Br < Cl \leqq F$. Geometric orientations of halogen substituents were found to profoundly influence knockdown and toxicity with some of the new halopyrethroids.

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Development of Insecticide Resistance in *Dacus cucurbitae* Coq. Serajuddin KHAN and Nawab H. KHAN (Department of Zoology, Muslim University, Aligarh, India) Received July 5, 1975. *Botyu-Kagaku* 41, 7, 1976.

2. *Dacus cucurbitae* Coq. における殺虫剤抵抗性の発現

Serajuddin KHAN and Nawab H. KHAN (Dept. of Zoology, Muslim University, Aligarh, India) 50. 7. 5 受理

Melon fruit fly 成虫を γ-BHC および carbaryl によってそれぞれ11および13代にわたって実験室内で選抜した．その結果，原系と比較して，LC_{50} 値は BHC に対し11代目で16.36倍，carbaryl に対し13代目で1.51倍に達した．

Though detected as early as 1914, the phenomenon of insecticide resistance in insects as we know it today, came on the scene with the use of organic residual insecticides in 1946. Since then the number of resistant species is increasing continuously and at present we know of at least 232 species of insects and acarines which have developed tolerance to one or the other chemical and of these 130 species are of agricultural importance[1].

That the melon fruit fly, *Dacus cucurbitae*, can develop resistance to DDT and other chlorinated compounds was shown by Ten in 1959. He exposed the adults to filter papers treated with these chemicals for 15 successive generations and observed that while the species could develop significant resistance to DDT, it failed to achieve any significant tolerance to chlordane. During the present studies an attempt was made to find out if the species can develop any resistance to gamma BHC and carbaryl when subjected to insecticide pressure under laboratory conditions.

Materials and Methods

Adults of *Dacus cucurbitae* were obtained from the normal laboratory colonies maintained at 28±1°C and 60-70 percent relative humidity. Measured drops of acetone solutions of gamma BHC and carbaryl were applied topically to the dorsum of individual flies after the manner described by Abedi[2]. The treated flies were kept in 4×2 cages made up of rice paper and card board. Mortality counts were made after 24 hours of insecticides treatments and the survivors were bred to produce the next generation which was again subjected to insecticide pressure. In this way selection with gamma BHC and carbaryl was carried on for eleven and thirteen generations respectively.

The percentage mortalities of the normal and selected strains were plotted on a probit scale and LC_{50} values and slopes were derived from dosage mortality regression lines (Fig. 1 & 2). The slope of the lines was expressed as the change in probits per ten fold change in dosage[3].

Results

The results obtained (Table 1) show that while the species developed considerable resistance to gamma BHC it failed to show any significant tolerance to carbaryl. The initial LC_{50} values of 0.00022 and 0.00195 obtained with gamma BHC and carbaryl respectively for the normal strain, when compared with the corresponding values for the selected stock suggest that *D. cucurbitae* acquired 16.36 times tolerance to gamma BHC in 11 generations but only 1.51 times tolerance to carbaryl in 13 generations of selection (Table 2). The slight shift in the dosage mortality regression line of the 13th generation without any significant change in slope and its somewhat steeper position than the dosage mortality regression line for the