致落下仰転虫率と24時間後の致死率を求めた．これをBlissのprobit法にしたがって整理し，イエバエ1頭当りの中央致落下濃度及び中央致死濃度を求めた．

結果：実験の結果を表示すると Table 1, 及び2の如くである．

文　献

1) Chen, Y. L., Barthel, W. F. : *U. S. Dept. Agri.*, ARS 23 (1956).
2) Piquett, P. G., Gersdorff, W. A. : *J. Econ. Entomol.*, 51, 791 (1958).
3) Buei, K. : *Botyu-kagaku*, 30, 37 (1965).

Summary

Exploratory studies have been made with substituted benzyl esters of chrysanthemic acid in search for effective insecticides. Of many benzyl chrysanthemates synthesized, 4-allylbenzyl chrysanthemate was found to show a high insecticidal activity and was designated as "Benathrin". Benathrin was as 1.7 times toxic in knock-down and 9.2 times toxic in mortality as *α-dl-trans* allethrin to adult house fly, *Musca domestica vicina* Maq., when applied topically in acetone solution.

Studies on Saligenin Cyclic Phosphorus Esters with Insecticidal Activity. Part X. Synergism of Malathion against Susceptible and Resistant Insects. Morifusa Eto, Yasuyoshi Oshima, Setuo Kitakata*, Fumikazu Tanaka* and Ken'ichi Kojima* (Department of Agricultural Chemistry, Kyushu University, Fukuoka and *Institute of Agricultural Chemicals, Toa Noyaku Co. Ltd., Odawara) Received November 30, 1965. *Botyu-Kagaku*, 31, 33. 1965.

6. 殺虫性サリゲニン環状りん酸エステルの研究 (第10報) 感受性および抵抗性昆虫に対するマラチオンとの共力作用

江藤守総・大島康義・北方節夫*・田中文一*・小島建一* (九州大学農学部農芸化学科，*東亜農薬株式会社農薬研究所) 40. 11. 30 受理

マラチオンの毒力を増強させることで知られている tri-*o*-tolyl phosphate の主要活性代謝産物を含む7種のサリゲニン環状りん酸エステルについて，マラチオンとの共力作用を感受性および抵抗性のイエバエとツマグロヨコバイを用いて検討した．環状エステルのアリル誘導体は，ことに抵抗種に対し高い活性を示した．アルキル誘導体は抵抗種に対し弱い共力作用を示した．試験した化合物中，最有効な共力剤は 7-methyl-2-phenyl-4H-1, 3, 2-benzodioxaphosphorin-2-oxide であった．このものは抵抗性イエバエに対し，propyl paraoxon や Dibrom のような既知共力剤よりも有効であった．

Certain organophosphorus compounds increase the toxicity of malathion (*O, O*-dimethyl *S*-(1,2-biscarboethoxyethyl) phosphorodithioate) to mammals[1]. EPN (ethyl *p*-nitrophenyl phenylphosphonothionate)[2] and TOCP (tri-*o*-tolyl phosphate)[3] are well known examples. For insects, particularly resistant strains, several synergists of malathion have been reported : propyl paraoxon (dipropyl *p*-nitrophenyl phosphate)[4], EPN and its oxoanalog[5] for house fly ; tributyl phosphorotrithioate and its some related substances for house fly and mosquito[6,7] ; EPN for mosquito[8]; Dibrom (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) for green rice leafhopper[9]. All of these phosphorus compounds are known as inhibititors of ali-esterase or related hydrolases at least in vivo condition.

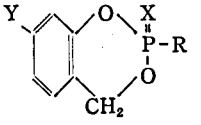
Although TOCP itself is not the inhibitor *in vitro*[10], it is metabolized *in vivo* to active anti-esterase agents, i.e., saligenin cyclic phosphates[11,12]. A series of saligenin cyclic phosphorus esters have been synthetically prepared[13,14] and examimed for biological activities. Aryl derivatives were selective inhibitors of ali-eaterase[15] and appeared to be synergistic with malathion[16], whereas small alkyl derivatives were highly insecticidal[17].

Results of investigations on the synergistic activities of some saligenin cyclic phosphorus esters against resistant insects are presented in this papar. It was observed that 7-methyl-2-phenyl-4H-1, 3, 2-benzodioxaphosphorin-2-oxide (VII) was a quite effective synergist of malathion.

Materials and Methods

Chemicals : Samples except Compound III were

Table 1. Joint action of saligenin cyclic phosphorus esters (I-VII) and some other phosphoric esters with malathion against susceptible house flies.



Compound No.	R	X	Y	LD_{50} (μg/♀) alone	with malathion (1 : 1)	Cotoxicity coefficient
Malathion				0.46		
I	OMe	O	H	0.02	0.06	0.6
II	OEt	O	H	0.16	0.14	1.7
III	OEt	S	H	0.11	0.22	0.8
IV	OPh	O	H	c. 10 (30%)	0.38	2.3
V	O-*o*-Tol	O	H	c. 10 (40%)	0.26	3.4
VI	Ph	O	H	c. 10 (60%)	0.36	2.5
VII	Ph	O	Me	c. 10 (70%)	0.26	3.4
TOCP				>10 (0%)	0.90	1.0
Dibrom				0.03	0.008	7.1
Propyl paraoxon				0.10	0.11	1.5
Isopropyl paraoxon				0.22	0.06	4.9

Abbreviations for designating structures : Me=methyl ; Et=ethyl ; Ph=phenyl ; Tol=tolyl.
Numbers in parentheses are mortality percentage at 10 μg dose per insect.

purified through column chromatography, distillation or recrystallization. Materials included were malathion, Dibrom, proply paraoxon, isopropyl paraoxon (diisopropyl *p*-nitrophenyl phosphate), TOCP and seven derivatives of saligenin cyclic phosphorus esters. The cyclic esters were prepared by a reported method.[13,14] Their structures are shown in Table 1 (I-VII).

Insects : Oriental house fly (*Musca domestica vicina* Macquart) and green rice leafhopper (*Nephotettix cincticeps* Uhler) were used. The strains of house fly were : (1) Sapporo, a susceptible strain and (2) Hokota, a diazinon-resistant strain showing cross-resistance to malathion. The strains of leafhopper were : (1) Odawara, a susceptible strain and (2) Koti, a malathion-resistant strain caught in a field of Koti prefecture[18]. In some experiments, this was used after further selection with malathion for several generations.

Test method : Acetone solution of test chemicals was topically applied to female adult insects. For green rice leafhopper, a previously reported micro technique[19] was used. Five replicate samples each of ten insects were used for each treatment. They were kept at 25°. Mortality counts were made 24 hours after treatment.

Joint toxic action was tested by applying the mixture of malathion and synergist at a 1:1 ratio. The joint action was evaluated by the cotoxicity coefficient[20,21] of the mixture. The coefficient was calculated by the following equation (1).

Cotoxicity coefficient=Actual toxicity index of a mixture/Theoretical toxicity index of a mixture ..... (1)

For some practically non-toxic compounds, a modified simple equation (2) could be applied.

Cotoxicity coefficient=LD_{50} of malathion alone/LD_{50} of malathion in a mixture..... (2)

The cotoxicity coefficient is the number of times of increase (or decrease) in toxicity.

Results

The activity of saligenin cyclic phosphorus esters in joint action with malathion was examined in comparison with some phosphoric esters which have been reported as synergists of malathion. Results with susceptible house flies are shown in Table 1. Aryl derivatives of saligenin cyclic esters

Table 2. Synergistic effect of saligenin cyclic phosphorus esters (I-VII) with malathion against resistant house flies in comparison with other phosphate synergists.

Compound	LD_{50} (μg/♀) alone	LD_{50} (μg/♀) with malathion (1 : 1)	Cotoxicity coefficient
Malathion	2.54		
I	0.15	0.06	4.7
II	0.40	0.22	3.1
III	0.20	0.10	3.6
IV	>10 (10%)	0.55	9.2
V	>10 (10%)	0.65	7.8
VI	>10 (25%)	0.57	8.0
VII	c. 10 (40%)	0.29	14.0
TOCP	>10 (0%)	3.44	1.5
Dibrom	0.07	0.07	2.0
Propyl paraoxon	0.73	0.46	2.5
Isopropyl paraoxon	0.43	0.36	2.2

Numbers in parentheses are mortality percentage at 10 μg dose per insect.

are synergistic with malathion. They increase the toxicity of malathion 2.3 to 3.4 times at a 1:1 mixing ratio. Their activities are more than propyl paraoxon and less than Dibrom and isopropyl paraoxon. The most active compounds among the tested cyclic esters are 2-(*o*-tolyloxy)-4H-1, 3, 2-benzodioxaphosphorin-2-oxide (V) which is the active metabolite of TOCP[11,12] and 7-methyl-2-phenyl-4H-1, 3, 2-benzodioxaphorin-2-oxide (VII). Phenyl phosphate (IV) and phenylphosphonate (VI) are almost same in the synergistic activity. Alkyl derivatives of saligenin cyclic phosphorus esters are much less synergistic (II) or even antagonistic (I, III). TOCP known to potentiate the toxicity of malathion in mammals[22] did not show any effect to house flies.

Synergism of saligenin cyclic phosphorus esters with malathion in a resistant strain of house fly

Table 3. Joint action of saligenin cyclic phosphorus esters (I-VII) with malathion against malathion susceptible green rice leafhoppers in comparison with some phosphate synergists.

Compound	LD_{50} (μg/♀) alone	LD_{50} (μg/♀) with malathion (1 : 1)	Cotoxicity coefficient
Malathion	0.003		
I	0.008	0.004	1.1
II	0.069	0.003	1.9
III	1.892	0.005	1.2
IV	0.240	0.003	2.0
V	0.464	0.008	0.8
VI	0.218	0.005	1.2
VII	3.120	0.003	2.0
TOCP	>10	0.005	1.2
Dibrom	0.208	0.005	1.2
Propyl poraoxon	0.006	0.002	2.0
Isopropyl paraoxon	0.211	0.003	2.0

Table 4. Synergistic effect of some organophosphorus esters with malathion against green rice leafhoppers

Compound	LD_{50} (μg/♀) alone	LD_{50} (μg/♀) with malathion (1 : 1)	Cotoxicity coefficient
Malathion	0.021 (0.048)*		
I	0.010	0.006	2.3
II	0.066	0.010	3.2
III	>2	0.019	2.2
V	(0.167)	0.015	2.2
VII	>3	0.011	3.8
TOCP	(>10)	(0.083)	1.1
Dibrom	0.533	(0.069)	1.3

* Data in parentheses were obtained by using insects of 12th generation of Koti strain which had been selected under the pressure of malathion at LD_{50}.

is demonstrated in Table 2. The strain, Hokota, was selected with diazinon. It showed more or less cross-resistance to all of tested phosphorus esters and was about 5 times resistant to malathion. For this strain synergistic activity of Dibrom and isopropyl paraoxon decreased and activity of propyl paraoxon slightly increased. On the contrary the synergistic effect of saligenin cyclic phosphorus esters remarkably increased. Even the alkyl derivatives acted as synergist of malathion. Thus, all of present cyclic esters are more active than other tested organophosphorus synergists. Aryl derivatives of cyclic esters are very effective synergists. Cyclic phenylphosphonate of methylsaligenin (VII) is the most effective synergist and increased the toxicity of malathion 14 times.

Results of tests on susceptible and resistant strains of green rice leafhopper are shown in Tables 3 and 4 respectively. The toxicity of malathion to the susceptible insects increased twice by the addition of cyclic ethyl phosphate (II) or phenyl phosphate (IV) of saligenin, cyclic phenylphosphonate of methylsaligenin (VII), propylparaoxon or isopropyl paraoxon. Synergistic effect of saligenin cyclic phosphorus esters increased in the resistant leafhopper but less than in the resistant house fly. They enhanced the toxicity of malathion 2.2 to 3.8 times. Difference in the synergistic activity against the leafhoppers between alkyl and aryl derivatives is not so distinctive as against house flies.

Discussion

The present results indicate that aryl derivatives of saligenin cyclic phosphorus esters synergize the insecticidal activity of malathion. The synergistic effect is remarkably enhanced in resistant strains. This is interesting to note in comparison with the fact that the effect of other phosphate synergists such as Dibrom and isopropyl paraoxon decreased in the resistant strain of house fly. Small alkyl derivatives of cyclic esters are generally not synergistic with malathion in susceptible strains but are synergistic in resistant strains.

The synergism appears to result primarily from the inhibition of detoxication of malathion. Malathion may be detoxified by either phosphatase action or esterase action. The low toxicity of malathion to mammals is explained by the latter mechanism[23]. It has been observed for a great number of organophosphorus compounds that synergism of malathion in mice and the degree of in vivo inhibition of ali-esterase are generally related[1,24]. For insects, high esterase activity hydrolysing malathion is considered to be responsible at least partly for malathion-resistance in some strains of mosquito (*Culex tersalis*)[8], house fly (*Musca domestica* L.)[5] and green rice leafhopper[9]. On the other hand, Oppenoorth and Asperen[4] proposed that the detoxication in some organophosphate-resistant strains of house fly was due to "mutant ali-esterases". All of these enzymes are inhibited by selective ali-esterase inhibitors

such as propyl paraoxon[4,5] and oxo analog of EPN[5]. Thus these inhibitors synergize malathion against the resistant insects.

The authors[15] have shown that aryl derivatives of saligenin cyclic phosphorus esters are the selective inhibitors of ali-esterase, whereas small alkyl derivatives are not so selective to ali-esterase inhibition. This appears to be responsible for their difference in synergistic properties. Although an active metabolite of TOCP, 2-*o*-tolyloxy-4H-1, 3, 2-benzodioxaphosphorin-2-oxide (V)[11,12], showed high synergistic activity with malathion, TOCP itself was not effective. TOCP may be slowly metabolized in house fly. Therefore, TOCP should be previously applied before treatment with malathion. Its homologous ester, di-*o*-tolyl phenyl phosphate synergized with malathion in house flies by its previous application 24 hours before malathion treatment[25].

Plapp[26] of U. S. D. A., Corvallis, Oregon tested our samples of saligenin cyclic phosphorus esters with his own susceptible and organophosphate-resistant strains of house fly (*Musca domestica*) and mosquito (*Culex tarsalis*). A strain of house fly was 120 times resistant to malathion and another strain was 10 times resistant to parathion. The resistant strain of *Culex tarsalis* was 100 times resistant to malathion. No cross-resistance to saligenin cyclic phosphorus esters was observed in both resistant strains of house fly. Thus, Salioxon (I)[17] and its thiono analog (Salithion)[17] were very effective insecticides against them. However, cross-resistance to them was present in the malathion-resistant mosquitoes. Cyclic butyl phosphate (VIII), phenyl phosphate (IV) and phenylphosphonate (VI) of saligenin and phenylphosphonate of methylsaligenin (VII) were only moderately effective as synergists for malathion against the malathion-resistant house flies. Against the resistant mosquitoes, Compounds VII and VIII were quite effective as malathion synergists. On the other hand, Salithion was found to be quite synergistic with parathion against parathion-resistant flies.

The recent development of malathion-resistance in green rice leafhopper in some regions of this country[18] requires the effective synergists of malathion. Their use, however, must be carefully controlled for possibilities to increase the toxicity of malathion to mammals and to cause ataxia[1].

Summary

Seven saligenin cyclic phosphorus esters including the principal active metabolite of tri-*o*-tolyl phosphate, which is known as a potentiator of malathion, were examined for synergism of malathion using susceptible and resistant strains of house fly and green rice leafhopper. Aryl derivatives of cyclic esters showed high activity, particularly against resistant strains. Alkyl derivatives showed low synergism against resistant strains. The most effective synergist among tested chemicals was 7-methyl-2-phenyl-4H-1,3,2-benzodioxaphosphorin-2-oxide. It was more effective against resistant house flies than some known phosphate synergists, such as propyl paraoxon and Dibrom.

References Cited

1) Casida, J. E., Baron, R. L., Eto, M. and Engel, J. L. : *Biochem. Pharmacol.*, 12, 73 (1963).
2) Frawley, J. P., Fuyat, H, N., Hagan, E. C., Blake, J. R. and Fitzhugh, O. G.: *J. Pharmacol. Exptl. Therap.*, 121, 96 (1957).
3) Murphy, S. D., Anderson, R. L. and DuBois, K. P. : *Proc. Soc. Exp. Biol. Med.*, 100, 483 (1959).
4) Oppenoorth, F. J. and Asperen, K. V. : *Ent. Exp. Appl.*, 4, 311 (1961).
5) Matsumura, F. and Hogendijk, C. J. : *ibid.* 7, 179 (1964).
6) Plapp, F. W. and Eddy, G. W. : *Science*, 134, 2043 (1961).
7) Plapp, F. W., Bigley, W. S., Chapman, G. A. and Eddy, G. W. : *J. Econ. Entomol.*, 56, 643 (1963).
8) Matsumura, F. and Brown, A. W. A. : *ibid.*, 54, 1176 (1961).
9) Kojima, K., Ishizuka, T. and Kitakata, S. : *Botyu-Kagaku*, 28, 17 (1963).
10) Aldridge, W. N. : *Biochem. J.*, 56, 185 (1954).
11) Casida, J. E., Eto, M. and Baron, R. L. : *Nature*, 191, 1396 (1961).
12) Eto, M., Casida, J. E. and Eto, T. : *Biochem. Pharmacol.*, 11, 337 (1962).
13) Eto, M. and Oshima, Y. : *Agr. Biol. Chem.*,

26, 452 (1963).
14) Eto, M., Kinoshita, Y., Kato, T. and Oshima, Y.: *ibid.*, 27, 789 (1963).
15) Eto, M., Hanada, K., Namazu, Y. and Oshima, Y.: *ibid.*, 27, 723 (1963).
16) Eto, M., Eto, T. and Oshima, Y.: *ibid.*, 26, 630 (1962).
17) Eto, M., Kinoshita, Y., Kato, T. and Oshima, Y.: *Nature*, 200, 171 (1963).
18) Kojima, K., Kitakata, S., Shiino, A. and Yoshii, T.: *Botyu-Kagaku*, 28, 13 (1963).
19) Kitakata S., Shiino, A. and Kojima, K.: *ibid.*, 28, 29 (1963).
20) Sun, Y. P. and Johnson, E. R.: *J. Econ. Entomol.*, 53, 887 (1960).
21) Sun, Y. P. and Johnson, E. R.: *J. Agr. Food Chem.*, 8, 261 (1960).
22) DuBois, K. P.: *Advanc. Pest Control Res.*, 4, 332 (1961).
23) Krueger, J. R. and O'Brien, R. D.: *J. Econ. Entomol.*, 52, 1063 (1959).
24) Casida, J. E.: *Biochem. Pharmacol.*, 5, 332 (1961).
25) Eto, M., Matsuo, S. and Oshima, Y.: *Agr. Biol. Chem.*, 12, 870 (1963).
26) Plapp, F. W.: Personal communication (1964).

A Case of Control of Sanitary Insect Pests by Bell 47-G2 Helicopter Dusting in Japan. Masayoshi Goida, Seiroku Sakai, Haruo Miura*, Hideo Koizumi* and Syōitu Nakagoshi. (Institute for Agricultural Chemicals, Yashima Chemical Industry Co., Ltd. Tomitake-Nagano, Nagano, and Hongo-Mura Public Health Section, Higashichikuma, Nagano*) Received December 8, 1965. *Botyu-Kagaku* 31, 38, 1966. (with English Summary, 47.)

7. ベル型ヘリコプター空中散布による衛生害虫防除の1例

合田昌義・酒井清六・三浦治夫*・小泉秀男*・中越省逸（八洲化学工業株式会社研究所，*本郷村保健課） 40. 12. 8 受理

都市の環境衛生の立場から，衛生害虫を対象に，3種の低毒性有機燐殺虫剤をベル 47-G2 型ヘリコプターで空中散布し，その防除効果を調査した．長野県松本市外浅間温泉の市街地とそれに隣接する水田に実験地を設定し，安全性，防除効果などの点から環境衛生の分野では稀薄多量散布が望ましいと考え，1.5% Sumithion, 1.5% ronnel および 1% trichlorfon の各粉剤を 6 kg/10a 散布した．直接殺虫効果，粉剤落下量とイエバエ成虫の時間—落下仰転率との関係，ハエ・カの個体群密度の変動，粉剤落下量分布型の測定および空中散布，効果調査実施中の気象などを散布前日から散布16日後まで調査した．実験地の主な害虫は，ハエの場合，屋内でヒメイエバエ〉イエバエ〉オオイエバエ，屋外でイエバエ〉オオイエバエ〉ヒメイエバエ〉センチニクバエであり，カの場合，コガタアカイエカ〉シナハマダラカ〉アカイエカであった．薬剤の効果は，速効性，殺虫性は Sumithion〉ronnel〉trichlorfon 残効性はハエ：ronnel〉Sumithion〉trichlorfon カ：Sumithion〉ronnel〉trichlorfon であった．粉剤落下量とイエバエの KT_{50} との間には高い相関関係が認められた．本実験結果から都市の衛生害虫防除を目的とした空中散布は，屋外のハエ，水田のカはもちろん，屋内のハエにも優れた防除効果を示すことが明らかになった．

近年，ハエとカをなくす生活実践運動の発展にともなって，公衆衛生事業は多大の成果をあげている．ヘリコプターや小型航空機を使って薬剤を空中散布しようと言う試みも各地で実施されるようになった．しかしその防除効果や実施上の基礎資料は少なく，長谷川ら[3]，鈴木ら[7]，Brown[2]，など2,3の報告があるにすぎない．

そこで筆者らは，都市の環境衛生の立場から，Sumithion, ronnel および trichlorfon などの低毒性有機燐殺虫剤の粉剤をベル47-G2型ヘリコプターで空中散布し，その防除効果を調査した．

本文を進めるに先きだち，ご懇篤なるご指導とご鞭撻を賜わった東京農業大学教授山本 亮博士に対し深謝する次第である．本文をご校閲いただいた京都大学農学部内田俊郎教授，石井象二郎教授に深謝する．本実験を行なうに当って，長野県松筑病害虫防除所 林清道氏，松本保健所主幹 伊藤 博氏に ご支援とご協力を賜わった．気象観測について松本測候所井村宇一郎所長，小川祐嗣氏のご協力を賜わった．長野県経済事業農業協同組合連合会小松辰男氏，青木長吉氏にご支援を賜わった．また，本郷村保健課岩渕世紀氏，八洲化学工業株式会社松石一樹，加藤輝男，東川征夫の3氏に熱心なご協力を賜わった．ここに銘記して感謝の意を表する．