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Résumé

The degradation of Malathion emulsifiable concentrate during storage comes up to 20 to 30% in a year; this is a serious matter for the manufacture and application of the concentrate.

In this report is given an outline of the study since 1955 upon the stability of Malathion e. c.

(1) The degration of Malathion e. c. depends mostly upon the kind of the emulsifiers and organic solvents, the moisture in the formulation, the storage temperature etc..

(2) Xylene and benzene gave good results as solvent.

(3) Moisture in the formulation is mostly derived from emulsifiers and has a very important effect upon the degradation of Malathion. pH of the emulsifiers (×100) is also important; optimum pH is 7.5 to 8.0 (Table 9)

(4) Polyoxyethylene glycol (PEG) in the emulsifiers affects the degradation of Malathion (Table 3, Table 5, X, XI, XII, XIII)

(5) Use of the molecular-destilled emulsifier, which contains very little quantity of PEG, brings forth low degradation. (Table 2, Table 5)

(6) It is recommendable that NaOH etc., which are used as catalysts, are not be neutralized in the process of synthesizing the emulsifiers for use in Malathion e. c., i. e., it is not necessary to adjust pH at 6 to 7; pH=7.5 to 8.0 is quite all right. Namely, the emulsifier No. 7 (Table 2) gave lower degradation of Malathion than the other.

(7) More than 4.0 is desirable for the pH of Malathion e. c. (×100).

(8) The essentials for preventing the degradation are written at above (1)—(7). It may be, however, preventative measures to use smaller quantity of emulsifiers, which have most important relations with the degradation of Malathion e. c.

Non-enzymatic Conversion of Dipterex into DDVP and Their Inhibitory Action on Enzymes. Studies on the Mode of Action of Dipterex I. Junshi MIYAMOTO (Research Laboratory, Osaka Works, Sumitomo Chemical Co.) Received July 24, 1959. *Botyu-Kagaku* **24**, 130, 1959.

26. Dipterex の DDVP への非酵素的変化およびその酵素阻害作用について Dipterex の作用機構に関する研究 第1報 宮本純之（住友化学工業株式会社大阪製造所研究部） 34. 7. 24 受理

Dipterex は弱アルカリ性の溶液においてのみならず，中性，弱酸性の条件においても，容易に1分子の塩酸脱離と転位をおこして DDVP となる．種々の pH 下における家蠅頭部の acetylcholinesterase, および chymotrypsin に対する Dipterex, DDVP の阻害実験をおこなった結果，Dipterex はそれ自身では，ほとんどあるいは全く阻害作用なく，Dipterex の *in vitro* における

阻害作用は，その分解生成物であるDDVPによることが明らかとなった．Dipterexの殺虫効果は，したがって，生体内で非酵素的に生成するDDVPに起因すると推定される．

Dipterex was proved to undergo dehydrochlorination and molecular rearrangement to form DDVP not only under neutral but even under slightly acidic conditions. By inhibitory experiment of Dipterex and DDVP on acetylcholinesterase and chymotrypsin at various pHs, it was revealed that Dipterex itself shows little or no inhibitory action. Inhibition caused by Dipterex, therefore, is attributed to DDVP derived from Dipterex.

The insecticidal action of Dipterex *in vivo* should be also due to DDVP which is expected to form spontaneously in the insect or plant body under physiological conditions.

Introduction

Dipterex or O,O-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate has attracted peculiar attention of biochemists as well as entomologists since discovesy of its excellent toxic effects to the house fly, *Musca domestica L.*, and of its weaker influence to mammals than other phosphorus containing chemicals. The author has observed recently that this compound undergoes dehydrochlorination and molecular rearrangement successively to form DDVP or O,O-dimethyl-dichlorovinyl phosphate in the neutral or even in the acidic media, similarly as under the alkaline condition[1-4].

This paper concerns with experimental results about spontaneous change of Dipterex and the inhibitory action of Dipterex and DDVP formed on acetylcholinesterase of the house-fly heads and also on chymotrypsin.

Experimental

Dipterex was synthesized from dimethyl hydrogen phosphite and chloral[5], and recrystallized from CCl_4 and then from ether. Its melting point and the results of elementary analysis are shown below :

mp. 78.5-79.5°C.

found : C18.34%, H3.27%, P12.84%, Cl40.26%

calculated: 18.65 3.10 12.03 41.50

Purity of this sample was proved to be 98.5% by polarographic method[6]. DDVP was prepared by dehydrochlorination of the Dipterex[1]. Its physical properties and the results of elementary analysis are shown below :

$n_D^{20°}$ 1.4590, $d_4^{20°}$ 1.414

found : C22.61%, H3.82%, P14.48%, Cl30.73%

calculated:21.70 3.16 14.00 32.20

Purity of both compounds were also examined by infrared absorption spectra. Acetylcholine perchlorate was prepared from commercial acetylcholine chloride[7], and white needle crystal of mp. 114-116°C was obtained. White crystal of benzoylcholine of mp. 241-243°C was prepared as iodide by the method described in "Organic Synthesis"[8]. L-Phenylalanine ethylester hydrochloride was prepared by the esterification of commercial L-phenylalanine in absolute ethanol[9] and recsystallized from absolute ethanol. Its melting point showed 147.5-149°C.

Enzyme preparation, assay of activity, and inhibition experiment. Chymotrypsin used was crystalline powder from Nutritional Biochemical Corp. Its activity was assayed at pH 6.5, 30°C according to the method of Parks and Plaut[10], L-phenylalanine ethylester hydrochloride being used as substrate. Inhibition experiments by Dipterex and DDVP were performed as follows: chymotrypsin and inhibitor were preincubated in 0.01M phosphate buffer at definite pH, at 4°C for 24 hrs.. After pH was readjusted to 6.5, enzyme activity was determined. Initial velocity observed was compared with that of the control, and inhibitory effect caused was determined. In the case of acetylcholinesterase, inhibitory action of Dipterex and DDVP *in vitro* were investigated manometrically by using supernatant from the homogenate of the adult house-fly heads by centrifugation at 6,000 rpm for 7 mins.. Before enzyme solutions were added, inhibitors

tested were preincubated at 37°C for appropriate time length in the buffer solution of each pH. The vapor toxicity of Dipterex and DDVP at various pHs was investigated as follows; in a large petri dish of 13cm in diameter and 7 cm in depth a smaller one containing a solution of Dipterex or DDVP was placed, which was covered with a wire screen to prevent the direct contact of insects to the insecticide. Twenty adult Azuki-bean weevil, *Callosobruchus chinensis* L. were confined in the larger petri dish. After standing overnight at 28°C, insects killed were counted.

Results and Discussion

1. Inhibition of chymotrypsin by Dipterex and DDVP

As shown in Fig. 1, DDVP was observed to inhibit chymotrypsin to nearly same extent at pH 6.5 and at pH 7.6, while inhibition by Dipterex was found to depend remarkably on pH of preincubation. Concentration of Dipterex and DDVP required to cause 50% inhibition

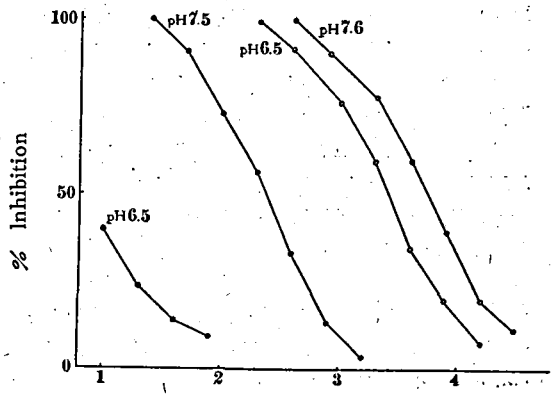


Fig. 1. Inhibition of chymotrypsin by Dipterex, DDVP. Inhibitor was preincubated with the enzyme (167γ/ml) in 0.01M phosphate buffer at each specified pH, 4°C for 24 hrs, after which pH was readjusted to 6.5 and enzyme activity was assayed. pH 6.5, 30°C. Reaction mixture (total volume 3 ml) contains; 75μM L-phenylalanine ethylester hydrochloride (pH adjusted to 6.5), 126 μM $NaHCO_3$, 50γ chymotrypsin and inhibitor. Gas phase 100% CO_2 •——• Dipterex, ○——○ DDVP

Table 1. IN_{50} of Dipterex and DDVP on chymotrypsin

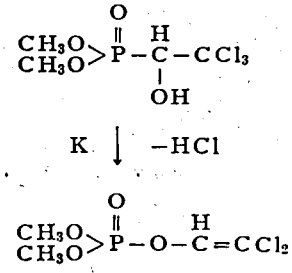
pH during preincubation	IN_{50} (M)	
	Dipterex	DDVP
6.5	$>1\times10^{-1}$	4.2×10^{-4}
7.6	4.5×10^{-3}	1.5×10^{-4}

(IN_{50}) under these experimental conditions were given in Table 1.

When Dipterex and chymotrypsin were preincubated at pH 5.0, 4°C for 24 hrs., no inhibition appeared, while under the same condition DDVP inhibited the action of enzyme to a certain extent; 5×10^{-3}M and 1.25×10^{-3} of DDVP caused 37% and 5% inhibition respectively when preincubated with the enzyme at pH 5.0, 6°C for 20 hrs.. As to the difference in inhibitory potency between these inhibitors, it might be considered as follows.

Phosphate bond P-O-C in DDVP molecule is presumed to be relatively stable with the change of pH, while phosphonate bond P-C in Dipterex molecule is very sensitive to pH, and Dipterex is presumed to be inhibitory only when it is converted into DDVP by preincubation.

The higher pH solutions may cause the more conversion of Dipterex and makes it the more effective.



2. Formation of DDVP from Dipterex under neutral and acidic conditions

The above presumption was tried to demonstrate by the following experiments. Liberation of hydrochloric acid concurrent with this transformation was ascertained by CO_2 evolution from CO_2-bicarbonate buffer. The results were shown in Fig. 2. Thus formation of acid from Dipterex in the neutral medium was demonstrated.

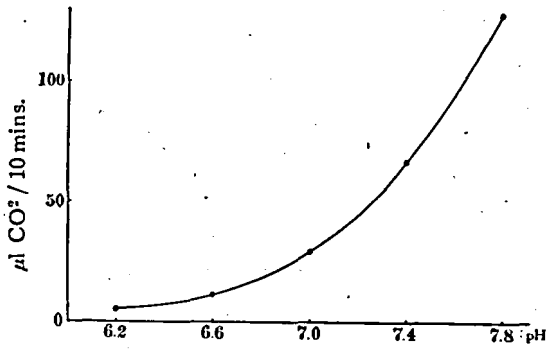


Fig 2. CO_2 evolution from CO_2-bicarbonate buffer at various pHs by dehydrochlorination of Dipterex at 37°C.
2×10^{-2}M of Dipterex was used. Reaction mixture, total volume 3 ml.

Even at pH 5.3 this reaction was observed to proceed very slowly (11 μl/3 ml reaction mixture/30 mins. when 6×10^{-2}M Dipterex was used). This acid production was proved to be neither accelerated by the addition of chemicals which were used in the inhibition studies of chymotrypsin and acetylcholinesterase, nor stimulated by fly head preparation. It seems to be evident, therefore, that the acid formation from Dipterex depends only on the pH. Formation of DDVP after liberation of hydrochlochloric acid was also identified by infrared absorption spectra of chloroform extract of the reaction mixture.

The results obtained were shown in Fig. 3. The absorption band at 6.07μ designates the presence of terminal C=C bond, and the band at 7.84μ coincides with that of P=O bond in phosphate. The further proof for the decomposition of Dipterex to DDVP was given by the following experiment. Dipterex was dissolved in 0.1M phosphate buffer of various pHs and vapor toxicity was investigated. The aqueous solution of Dipterex was fairly acidic and showed no vapor toxicity at all. Even at pH 5.0 vapor toxicity was not observed yet, while at pH 5.5 the toxicity appeared, and with the rising of pH, it was seen to become stronger. At pH 7.6 the toxicity was nearly same as that of DDVP (Table 2).

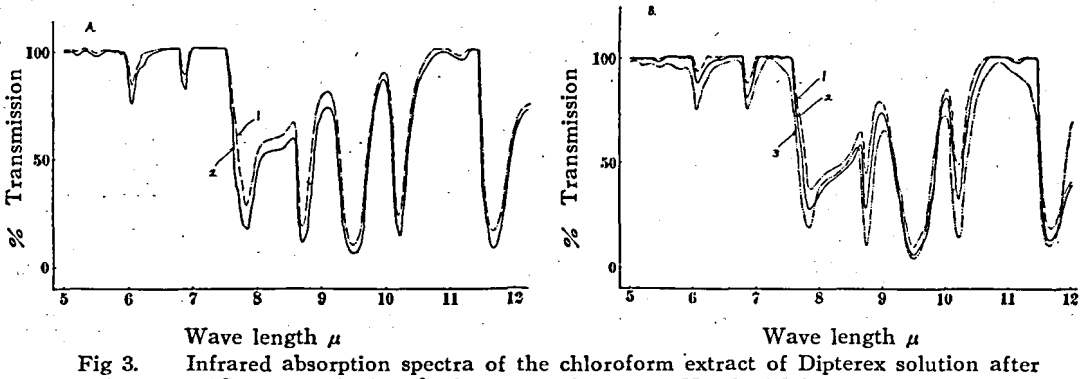


Fig 3. Infrared absorption spectra of the chloroform extract of Dipterex solution after standing at 37°C. A. 40 ml of 10^{-2}M Dipterex solution at pH 7.6, (1) for 30 min, (2) 90 min. B. 40 ml of 3×10^{-2}M Dipterex solution at pH 6.5 (1) for 80 min, (2) 160 min., and extracted with 4 ml of $CHCl_3$. This $CHCl_3$ layer was dehydrated with anhydrous Na_2SO_4. (3) in B; DDVP control (5×10^{-2}M of DDVP in $CHCl_3$)

Table 2. Vapor toxicity of Dipterex and DDVP

Experimental condition	Kill %*			
Concentration	1%	0.2	0.04	0.08
Dipterex (in H_2O)**	2.5	4.8	0	0
〃 (pH 5.0)***	5.9	2.1	0	0
〃 (pH 5.5)	93.0	8.3	10.3	0
〃 (pH 6.5)	100	100	22.2	0
〃 (pH 7.5)		100	100	59.9
DDVP****		100	100	94.6

*means of three replicates. **pH of the aqueous solution; 1% pH 3.3, 0.2%pH 4.3.
***diluted with the same buffer solution. ****dissolved in small amount of propylene glycol and diluted with H_2O.

The rate at which Dipterex converts into DDVP was determined at pH 6.5 and at pH 7.6 with the above method. The results obtained were shown in Fig. 4 and Fig. 5. Acid formation (DDVP formation) proceeded proportionally with the time during the period when decrease of Dipterex was not so much. Thus the process of DDVP formation may be represented by the first-order reaction $C=C_0(1-e^{-kt})$. The initial first-order rate constant K was determined as shown in Table 3. DDVP formation was 9 times slower at pH 6.5 than at pH 7.6. From these results in company with those in Fig. 2 and in Table 2, this reation seems to fall with the lowering of the pH of the medium, and at last cease at pH 5.0.

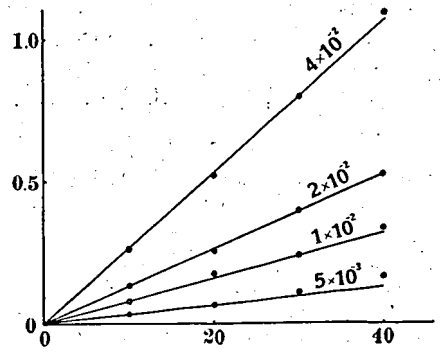


Fig 4. Acid formation from Dipterex at pH 6.5, 37°C. Number on each curve; concentration of Dipterex used (M)

Table 3. Determination of rate constant K for the reaction

Dipterex⟶DDVP (37°C)

pH	Initial concentration of Dipterex (C_0)M	Acid formation during 10mins. (CKt)M	Rate constant (K) min^{-1}
6.5	4.0×10^{-2}	0.265×10^{-3}	$6,6\times10^{-4}$
	2.0	0.140	7.0
	1.0	0.085	8.5
	0.5	0.035	7.0
	mean 7.3×10^{-4}		
7.6	3.0×10^{-2}	1.80×10^{-3}	6.0×10^{-3}
	1.0	0.78	7.8
	0.4	0.26	6.5
	0.2	0.135	6.5
	mean 6.7×10^{-3}		

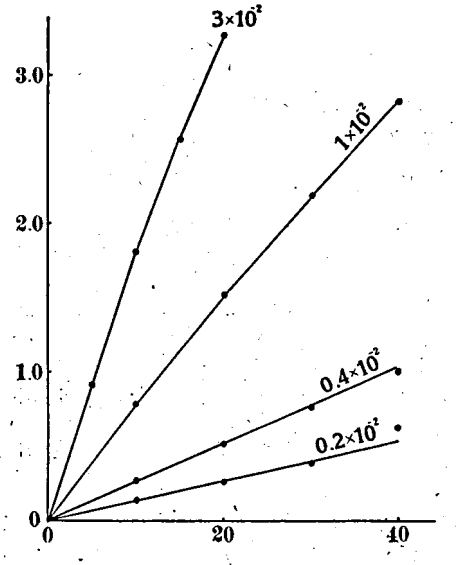


Fig 5. Acid formation from Dipterex at pH 7.6, 37°C. Number on each curve; concentration of Dipterex used (M)

3. Inhibition of fly head acetylcholinesterase by Dipterex, DDVP

Enzyme preparation from the adult house-fly head contains so called acetylcholinesterase as it hydrolyzes acetylcholine well and is inhibited by acetylcholine at high concentration and it shows little activity on to benzoylcholine (Fig. 6). Inhibition experiments by Dipterex and DDVP was carried out with this preparation. The results obtained were given in Fig. 7, 8 and Table 4. Preincubation at pH 7.6 and pH 6.5 makes Dipterex inhibitory to the markedly different extent, while by preincubation of DDVP at the same pHs any difference was scarcely observed between these two pHs.

DDVP considered to be formed from Dipterex during preincubation were calculated and shown in column 3 in Table 4. Its amount agreed well with that of DDVP necessary to cause 50% inhibition (last column in Table 4). The feeble inhibition observed by Dipterex without preincubation may be attributed also to the conversion of Dipterex in the buffer solution during the time for assay of enzyme activity.

The similar inhibition was also apparent in the experiment at pH 5.3 where DDVP

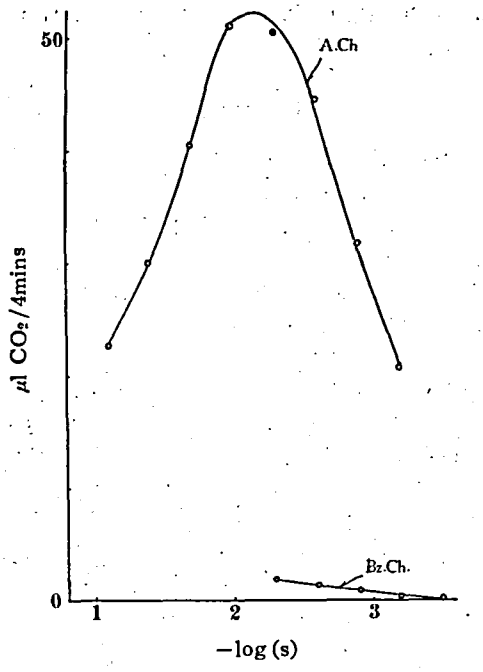


Fig 6. Substrate specificity of adult house fly head preparation. 40 heads / ml (containing 25 μM reduced glutathione, 300 μM NaCl, 80 μ M $MgCl_2$) were homogenized and centrifuged at 6,000 rpm for 7 mins. 0.3 ml of supernatant was used. Reaction mixture (total volume 3 ml) contains 450 μM NaCl, 120 μM $MgCl_2$, 7.5 μM reduced glutathione, 72 μM $NaHCO_3$, enzyme and substrate. Gas phase 5% CO_2, 95% N_2. pH 7.6, 37°C. A.Ch. ; acetylcholine. Bz.Ch. ; benzoylcholine.

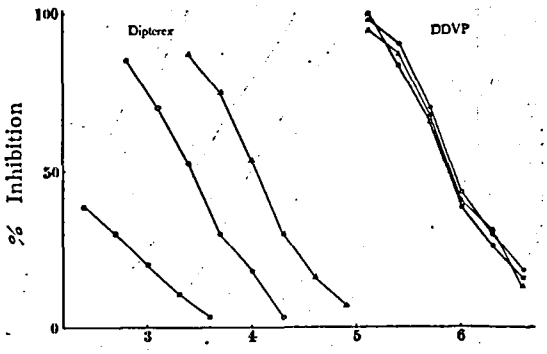


Fig 7. Inhibition of fly head acetylcholinesterase by Dipterex, DDVP at pH 6.5, 37°C after preincubation of inhibitors in the buffer solution for various time length. Reaction mixture, same as in Fig 6 except that inhibitor added and 15 μM acetylcholine, 126 μM $NaHCO_3$ used. Gas phase 100% CO_2. Preincubation of inhibitor in the buffer for 0(□——□), 5(○——○) and 20 mins. (△——△).

formation from Diterex occurs extremely slowly. At this pH it needs 10^{-6}M of DDVP to cause 50% inhibition, while IN_{50} for Dipterex is 2×10^{-2}M when without preincubation. From these facts, Dipterex is determined to possess no inhibitory potency by itself.

It has been assumed that when Dipterex is used as an insecticide, conversion of Dipterex into DDVP *in vivo* may be due to the action of a specific enzyme "dehydrochlorinase" present in the insect or plant body, but this presumption has not yet been demonstrated by experimental

Table 4. IN_{50} of Dipterex, DDVP on the fly head acetylcholinesterase (37°C)

Experimental Conditions		Dipterex M	DDVP formed from Dipterex M**	DDVP M	Dipterex/DDVP***
pH 6.5	t=0*	$>4.0\times10^{-3}$	—	1.4×10^{-6}	—
	5	3.7×10^{-4}	1.4×10^{-6}	1.2×10^{-6}	0.86
	20	9.0×10^{-5}	1.3×10^{-6}	1.3×10^{-6}	1.0
pH 7.6	t=0	4.3×10^{-4}	—	1.2×10^{-6}	—
	5	3.1×10^{-5}	1.0×10^{-6}	1.1×10^{-6}	1.1
	20	9.1×10^{-6}	1.2×10^{-6}	1.2×10^{-6}	1.0

*time length (min) of preincubation of inhibitors in the buffer solution before addition of enzyme. ** DDVP considered to form from IN_{50} of Dipterex under each experimental condition (calculated using rate constant $K(pH7.6)=6.7\times10^{-3}$ min^{-1}, $K(pH6.5)=7.3\times10^{-4}$ min^{-1}). ***ratio of potency (IN_{50} of DDVP calculated/found).

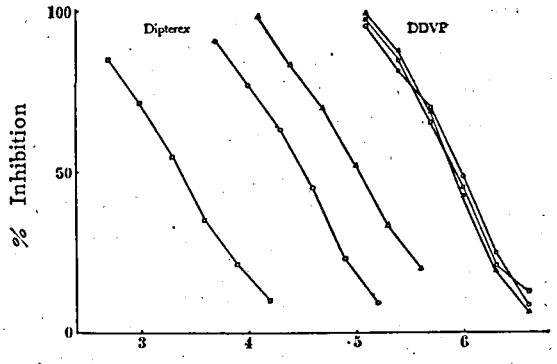


Fig 8. Inhibition of fly head acetylcholinesterase by Dipterex, DDVP at 7.6, 37°C after preincubation of inhibitors in the buffer solution for various time length.
Reaction mixture and gas phase, same as in Fig 6 except that inhibitor added and 15 μM acetylcholine used.
△——△, ○——○, □——□ same as in Fig 7.

results.[4),11),12)]. The conversion has been attained neither by the homogenates of adult house fly, cabbage and rice plant, nor with the plant tissues by vacuum penetration method in the author's laboratory. The presence of the above dehydrochlorinating enzyme is presumptive in *status quo*. From the experimental results which were shown, however, spontaneous decomposition of Dipterex to DDVP is expected to occur in the bodies of insect or plant. It is not always necessary, therefore, to assume the existence of the specific enzyme.

As to the low mammalian toxicity of Dipterex, it seems that the rate of this conversion may be slower than the rate of the action of some esterases[4)] which hydrolyze phosphonate bond of Dipterex and remove trichloroethyl moiety as the form of glucuronide.

Though the mechanism for spontaneous formation of DDVP from Dipterex has not been entirely elucidated, expulsion of proton from OH group of Dipterex seems to be essential. Acylated or phosphorylated derivatives of OH group in Dipterex are expected, therefore, to be innert to acetylcholinesterase and other enzymes susceptible to organophosphorus compounds. Inhibition of the enzymes by these deriveatives are now being investigated, and the results will be presented in the continuing papers.

Summary

1. Spontaneous conversion of Dipterex into DDVP was ascertained under neutral and acidic pH conditions. This conversion seems not to occur practically below pH 5.0, and with the rising of pH the rate increases proportionally.

2. Inhibition of Dipterex and DDVP on fly head acetylcholinesterase and on chymotrypsin was investigated, and it became clear that the apparent inhibitory action of Dipterex *in vitro* is due to the action of DDVP formed by conversion and Dipterex itself is not inhibitory.

3. The relation of this spontaneous conversion of Dipterex into DDVP to the insecticidal action of the former and to its lower mammalian toxicity was also discussed.

Acknowlegement

The author is indebted to Mr. K. Iwai, Mr. K. Sugiyama, Mr. Y. Okuno, and Dr. N. Ōi in this laboratory for their technical assistance in his work.

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Addendum

Just when the manuscript was almost completed, paper by R. L. Metcalf *et al* was published under the title of "Toxic action of Dipterex and DDVP to the house fly" (J. Econ. Ent. **52**, 44 1959), where they reported almost the same results as the author obtained which support spontaneous conversion of Dipterex into DDVP under similar conditions.

The Effect of Some Agricultural Chemicals on a Wasp, *Trichogramma japonicum* Ashmead, an Egg Parasite of the Rice Stem Borer, *Chilo suppressalis* Walker. Studies on the Influence of Agricultural Chemicals on Beneficial Insect. I. Yasushi Washizuka and Sadao Kuwana (Division of Entomology, Ihara Agr. Chem. Lab., Simizu, Sizuoka Pref.) Received July 28, 1959. *Bōyu-Kagaku*, **24**, 137—140, 1959 (with English résumé, 140).

27. ニカメイガの卵に寄生しているズイムシアカタマゴバチに及ぼす農薬の影響

農薬の益虫に及ぼす影響について 第1報 鷲塚 靖・桑名貞夫（庵原農薬研究所 昆虫研究室） 34. 7. 28 受理

ニカメイガの卵とそれに寄生しているズイムシアカタマゴバチに対する7種類の農薬の影響を浸漬法による室内実験によって調べた．一般にニカメイガ卵に対する作用の強い薬剤はズイムシアカタマゴバチに対しても強い作用を示すが，エンドリン，リンデン，ダイアジノン乳剤はとくにズイムシアカタマゴバチに強く作用し，PMA 乳剤はニカメイガ卵に強く作用するという結果を得た．

殺虫剤の散布が目的とする害虫のみならず，その天敵にも作用し，これが昆虫群集の生物的均衡を破壊する一因となり，害虫の異常発生を生じ，作物の被害を増加することが知られている[1-7]．水田における農薬の使用がニカメイガの卵に寄生しているズイムシアカタマゴバチにどのような影響を及ぼしているかを知る手がかりとして，室内実験によってこの両者に対する種々の農薬の殺虫力を比較検討した．

結果を報告するに先だち御批判および御助言を賜った京都大学農学部昆虫学研究室，内田俊郎教授，高橋史樹氏，ならびに庵原農薬研究所昆虫研究室，坪井武夫，篠原寛の両氏の各位に深甚な謝意を表する．

実験材料および方法

1958年6月15日静岡県清水市渋川附近の苗代でニカメイガの卵塊を多数採集し，ズイムシアカタマゴバチが寄生していると思われる変色卵の色の濃さを基準にして，これらを濃黒変色卵，黒色変色卵，灰色卵，白色卵の4段階に区分けし，実験に供した．4段階に区分けしたニカメイチュウの胚子の発育の程度をその孵化曲線より概算したところ，濃黒変色卵は孵化前2.1日，黒変色卵は3.6日，灰色卵は4.5日，白色卵は6.2日となった．またズイムシアカタマゴバチの発育程度についてもニカメイチュウで用いたと同じ概算法により推定することが出来る．すなわち濃黒変色卵は羽化前3.3日，黒変色卵は5.0日，灰色卵は7.4日，白色卵は9.0日となった．

用いた薬剤およびその濃度はエチルパラチオン，マラソン，リンデン，DDT，エンドリン，PMA の各乳剤の 0.025%，0.05% 溶液とダイアジノン乳剤の 0.02125%，0.0425% 水溶液である．これらの各濃度の薬液に1卵塊ずつ30秒間浸漬したのち，沪紙で薬液を吸いとり，卵塊周辺部の稲葉をつけたまゝ，ガラスチューブ（内径 1.5cm 深さ 6cm）に入れて綿栓を施し，温度 25°±1°C，関係湿度 70±5% の恒温恒湿下に放置した．その後，14日間にわたり羽化してくる卵寄生蜂と孵化してくるニカメイチュウの数を毎日一定時刻に記録した．

薬剤によって発育途中で死亡した卵寄生蜂の数と過寄生効果によって羽化しなかった卵寄生蜂の数を区別するため，調査期間後，4% 苛性加里煮沸液を用いて卵塊をばらばらにし，それぞれの数と卵粒総数とを調査した．

実 験 結 果

以上の実験処理によるズイムシアカタマゴバチおよびニカメイチュウの死亡率を示したのが第1表である．この結果により，ズイムシアカタマゴバチに高い死亡率を与えたのはエチルパラチオン，エンドリン，リンデン，ダイアジノンの各乳剤で，マラソン 0.025% 乳剤および DDT 乳剤はその作用が比較的少なかった．また，ニカメイチュウに対して比較的つよい殺卵