Studies on the Organophosphorus Insecticides

Part V. Synthesis and Toxicity of Phosphorates Containing Phenoxy- or Ethoxy-vinyl Groups

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During the course of an investigation of organophosphorus insecticides, the reactions of trialkyl phosphites*¹ with ethyl or phenyl trichloroacetate, diethyl monochloro- or dichloro-malonate and ethyl chlorocyanoacetate were attempted. It was cleared that the above reaction did not belong to the Arbuzov reaction but to the Perkow reaction and the products had the phosphate forms and the carbonyl group of the carboxylic acid linked with phosphorus atom by the enol-form.

The present author reported¹⁾ that 2-(*p*-nitrophenoxy)-vinyl phosphate (IX) have the toxic property and the activity towards some insects.

As the next step, it was attempted to determine whether the activity or the toxicity of the phosphorates changes or not, if the *p*-nitrophenyl group links to the 1-position of vinyl group instead of 2-position (VIII). Then the present author attempted the synthesis of the phosphorates having the 1-phenoxy- or 1-alkoxy-vinyl groups.

It was considered that the expected compounds may be prepared easily, if the carbonyl group of α -halo-carboxylates reacts with phosphites according to the Perkow's reaction²⁾ and links to phosphorus atom by enol form just as that of aldehydes and ketones.

When this investigation was attempted, it was not yet determined whether the reaction of trialkyl phosphite with α -halo-carboxylate

belongs to Michaelis-Arbuzov reaction³⁾ or to Perkow reaction.

Several investigations⁴⁾ have been attempted to use α -halo-carboxylate and phosphite, but in all cases the products have been assumed to be phosphonates of the type expected from normal Michaelis-Arbuzov reaction.

J. F. Allen et al.⁵⁾ reported at first that the reaction of ethyl trichloroacetate with triethyl phosphite produce the phosphate, but the proof of the structure of the product was not given in detail. Recently, F. Cramer et al.⁶⁾ showed that the structure should be of phosphate type using the same methods as that of the present author⁷⁾.

It had been shown⁷⁻⁹⁾ independently in

3) G.M. Kosolapoff, *Organophosphorus Compounds* 1950 p. 121 John Wiley.

4) a) P. Nylén, *Ber.*, **57**, 1023 (1924).

b) P. Muller, *Jap. Pat.*, Announced No. **8927** (1945).

c) G.M. Kosolapoff, *J. Am. Chem. Soc.*, **68**, 1103 (1946).

d) A.E. Arbuzov et al., *Chem. Abst.*, **42**, 4523 (1948).

5) J.F. Allen et al., *J. Am. Chem. Soc.*, **77**, 2871 (1955).

6) F. Cramer et al., *Ber.*, **91**, 704 (1958).

7) Y. Nishizawa and T. Mizutani, *Jap. Pat.*, Applied No. **31528**. (1957).

8) Y. Nishizawa and T. Mizutani, *Jap. Pat.*, Applied No. **25297**. (1957).

9) Y. Nishizawa and M. Nakagawa, *Jap. Pat.*, Applied No. **8195** (1957).

*1) The phosphorus compounds in this paper were named according to the Drake Committee's Reports (*Chem. Eng. News*, **30**, 4515 (1952)).

1) Y. Nishizawa, *Agr. Biol. Chem.*, **24**, 261 (1960).

2) W. Perkow, *Ber.*, **87**, 755 (1954).

(IIIa) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIIb) $\text{R}_1=\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIIc) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_6\text{H}_5$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIId) $\text{R}_1=\text{C}_2\text{H}_5$, $\text{R}_2=\text{C}_6\text{H}_5$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIIe) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_6\text{H}_4\text{-NO}_2\text{-}p$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIIIf) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_6\text{H}_4\text{-Cl-}p$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IIIg) $\text{R}_1=\text{C}_2\text{H}_5$, $\text{R}_2=\text{C}_6\text{H}_4\text{-Cl-}p$, $\text{R}_3=\text{R}_4=\text{Cl}$ (IVa) $\text{R}_1=\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{H}$, $\text{R}_4=\text{COOC}_2\text{H}_5$ (IVb) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{Cl}$, $\text{R}_4=\text{COOC}_2\text{H}_5$ (IVc) $\text{R}_1=\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{Cl}$, $\text{R}_4=\text{COOC}_2\text{H}_5$ (IVd) $\text{R}_1=\text{CH}_3$, $\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{H}$, $\text{R}_4=\text{CN}$ (IVe) $\text{R}_1=\text{R}_2=\text{C}_2\text{H}_5$, $\text{R}_3=\text{H}$, $\text{R}_4=\text{CN}$

this laboratory also that the reaction of phosphite with ethyl or phenyl trichloroacetate, diethyl chloro- or dichloro-malonate and ethyl chlorocynoacetate with phosphites produce the phosphorate and the carbonyl group of the carboxylates links with phosphorus atom by the enol-form.

All the products were liquid and purified by distillation under reduced pressure. In these reactions, it is possible to assign to the main product according to the microanalytical data, namely one of them was phosphorate form (III) and (IV), another was phosphonate form (V).

It was cleared by the following experimental

facts that the main products of the above reactions have phosphorate form (III) and (IV) and not phosphonate form (V).

In series (III), the products did not show the infrared absorption band of carbonyl group and showed the strong absorption band of ethylenic double bond at 1670 cm^{-1} ¹⁰ (see Fig. 1).

In series (IV), the products showed the strong absorption band of ethylenic double bond at 1645 cm^{-1} and the absorption band of carbonyl group shifted to 1720 cm^{-1} . It was considered¹⁰ that this shift is brought by the carbonyl group conjugated with some unsaturated group (see Fig. 2).

FIG. 1. Infrared Absorption Spectrum of O, O-Diethyl-O-(1-phenoxy-2, 2-dichloro-vinyl) phosphorate (IIId).

¹⁰) L.J. Bellamy, The Infrared Spectra of Complex Molecules (1958).

FIG. 2. Infrared Absorption Spectrum of O, O-Diethyl-O-(1-ethoxy-2-chloro-2-carboethoxy-vinyl)phosphorate (IVc).

The products absorbed one mole ratio of chlorine in carbon tetrachloride and yielded chloro-derivatives (VI) which does not show the absorption spectra of ethylenic double bond¹¹⁾.

The products reacted with acetic acid and yielded O,O-dialkyl-O-acetyl phosphate¹²⁾ (VII) and carboxylate^{6,7)} (VIII), though O,O-diethyl-(carboethoxy-methyl) phosphonate¹³⁾ did not react with acetic acid.

Alcoholysis of O,O-diethyl-O-(1-ethoxy-2-carboethoxy-vinyl) phosphorate (IVa) by sodium alcoholate in an alcoholic solution gave triethyl phosphorate and diethyl malonate. This decomposition is one of the proofs of the enol phosphorate¹⁴).

The following facts may be summarized from Table II showing the correlation between the toxicity and the activity of the new phosphorates and it's structures:

- 1) In series (III), the phosphorates having methyl group as R_1 have the same activity as that of ethyl derivatives but have lower toxicity towards mice, when the other groups were same.

- 2) In series (III), the phosphorates having phenyl group as R_2 have the same activity as that of ethyl derivatives but have lower toxicity towards mice, when the other groups were same.

- 3) The toxicity and the activity of 1-(*p*-nitrophenoxy)-2,2-dichlorovinyl phosphorate (IIIe) was as same as that of 2-(*p*-nitro-

R₁=alkyl, R₂=methyl or phenyl
R₃=COOC₂H₅ or CN

11) Y. Nishizawa et al., Jap. Pat. Applied No. **25298** (1957).

12) P. R. Whestone, *Chem. Abst.*, **48**, 8251 (1954).

13) B. Fisher et al., *Chem. Abst.*, **49**, 9493 (1955).

14) A.N. Pudovik, *J. Gen. Chem. USSR*, **26**, 1605 (1956).

phenoxy)-2-chlorovinyl phosphorate¹⁾ (IX), namely, the changing of the position of *p*-nitrophenoxy group on the vinyl group did not give the large variation in the toxicity and the activity of the phosphorates.

4) In series (IV), the phosphorate having the methyl group as R₁ did not show large difference from ethyl derivatives on the toxicity towards mice, while in series (III), the difference was large. Although the difference is not so large, methyl phosphorate in the series (IV) also showed lower toxicity than ethyl phosphorate.

5) In Phosdrin type phosphorates (X), the linkage of chlorine atom on vinyl group depressed the toxicity of the phosphorates¹⁾ but in series (IV) that effects were not found.

6) In Phosdrin type phosphorates (X), the phosphorates having cyano group as R₃ showed lower toxicity and activity than carboethoxy derivatives, when the other groups were same¹⁵⁾. But in series (IV), cyano phosphorates (IVd) and (IVe) showed higher toxicity than carboethoxy phosphorate (IVa) though the activity was lower.

7) Series (IV) showed higher toxicity than series (X) though the activity was lower.

8) Saturation of vinyl group by chlorine

in series (III) and (IV) give the lowering of the toxicity and the activity of the phosphorates just as Phosdrin type phosphorates¹⁾.

EXPERIMENTAL

1) Preparation of Vinyl Phosphorates (III) and (IV).

The following examples illustrate the preparation of vinyl phosphorates. Other examples are shown in Table I and their activities and toxicity are shown in Table II. *Caution: All of the phosphorates are powerful cholinesterase inhibitors and therefore highly toxic.*

2) Preparation of O,O-diethyl O-(1-phenoxy-2,2-dichloro-vinyl) Phosphorate (IIIId).

To a solution of 24.0 g (0.1 M) of phenyl trichloroacetate in 50 ml of ether was gradually added 16.6 g (0.1 M) of triethyl phosphite. The temperature was raised gradually to 40°C and mild cooling of the reaction mixture seemed necessary. After addition was complete, the reaction mixture was heated at 40°C for about 4 hours. Ether was distilled off and distillation of product gave 20.0 g of (IIIId), b.p. 154~156°C/0.9 mm. n_D^{33} 1.4966.

Anal. Found: P, 8.6; Cl, 20.71%

Calcd. for C₁₂H₁₅O₅Cl₂P: P, 9.1; Cl, 20.82%

3) Preparation of O,O-diethyl O-(1-ethoxy-2-chloro-2-carboethoxyvinyl) Phosphorate (IVc).

To a solution of 31.8 g (0.2 M) of diethyl dichloromalonate, in 100 ml of ether, there was added gradually 33.2 g (0.2 M) of triethyl phosphite. The temperature

TABLE I. THE YIELD AND THE PHYSICAL CONSTANTS AND THE MICROANALYTICAL DATA OF PHOSPHERUS COMPOUNDS

No.	Formula	Yield (%)	B.P.,		n_D^{+25}	Phosphorus, (%)		Chlorine, (%)	
			°C	mmHg		Calcd.	Found.	Calcd.	Found.
IIIa	(CH ₃ O) ₂ P(O)OC(OC ₂ H ₅) : CCl ₂	41.6	104~105	0.4	1.4479 ⁽²⁴⁾	11.7	11.5	26.79	26.83
IIIb	(C ₂ H ₅ O) ₂ P(O)OC(OC ₂ H ₅) : CCl ₂ ⁵⁾	51.5	106~109	0.3	1.4438 ⁽²⁷⁾	10.6	10.8	24.23	24.01
IIIc	(CH ₃ O) ₂ P(O)OC(OC ₆ H ₅) : CCl ₂	44.6	144~146	0.5	1.4932 ⁽³²⁾	9.9	9.6	22.68	22.29
IIId	(C ₂ H ₅ O) ₂ P(O)OC(OC ₆ H ₅) : CCl ₂	58.8	154~156	0.9	1.4966 ⁽³³⁾	9.1	8.6	20.82	20.71
IIIe	(CH ₃ O) ₂ P(O)OC(OC ₆ H ₄ NO ₂) : CCl ₂	36.1	150~155	0.3	1.5542 ⁽²⁸⁾	8.7	8.3	19.83	19.35
IIIf	(CH ₃ O) ₂ P(O)OC(OC ₆ H ₄ Cl) : CCl ₂	45.1	123~125	0.5	1.4833 ⁽³⁰⁾	8.9	9.3	30.65	30.18
IIIg	(C ₂ H ₅ O) ₂ P(O)OC(OC ₆ H ₄ Cl) : CCl ₂	56.3	149~153	0.4	1.5027 ⁽³⁰⁾	8.2	8.7	28.29	28.44
IVa	(C ₂ H ₅ O) ₂ P(O)OC(OC ₂ H ₅) : CHCOOC ₂ H ₅ ⁵⁾	70.3	137~140	0.3	1.4430 ⁽²⁷⁾	10.5	10.8	—	—
IVb	(CH ₃ O) ₂ P(O)OC(OC ₂ H ₅) : OCICOOC ₂ H ₅	61.6	135~138	0.4	1.4381 ⁽²¹⁾	10.2	10.6	11.74	11.59
IVc	(C ₂ H ₅ O) ₂ P(O)OC(OC ₂ H ₅) : CClCOOC ₂ H ₅	81.0	140~142	0.2	1.5387 ⁽²⁵⁾	9.4	9.1	10.74	10.86
IVd	(CH ₃ O) ₂ P(O)OC(OC ₂ H ₅) : CHCN	43.7	119~122	11.1	1.4332 ^(26,8)	14.0	14.3	—	—
IVe	(C ₂ H ₅ O) ₂ P(O)OC(OC ₂ H ₅) : CHCN	51.3	124~126	1.0	1.4320 ⁽²¹⁾	12.4	12.5	—	—

15) Y. Nishizawa, This Journal, **25**, 61 (1961).

TABLE II. THE ACTIVITY AND THE TOXICITY OF PHOSPHORUS COMPOUNDS

No.	<i>Ephestia cautella</i> (WLKR) Topical Method		Hibernating larvae of <i>Chilo suppressalis</i> WALKER Topical Method		<i>Callosobruchus chinensis</i> LINNE Dipping Method		Oral Toxicity towards Mice LD ₅₀ mg/kg.
	r /larva	Kill (%)	r /larva	Kill (%)	Dilution	Kill (%)	
IIIa	2	90	2	90	$\times 800$	53.5	40
IIIb	2	40	—	—	—	—	10.6
IIIc	2	60	—	—	—	—	200
IIId	2	90	—	—	$\times 3,200$	99.0	28.5
IIIe	—	—	2	100	—	—	25.0
IIIf	2	10	6.7	100	—	—	170.0
IIIg	2	90	—	—	$\times 1,800$	84.3	22.5
IVa	—	—	2	84.3	—	—	7.3
IVb	—	—	2	80.0	—	—	12.8
IVc	—	—	2	100	—	—	7.2
IVd	—	—	2	30	—	—	11.0
IVe	—	—	2	40	—	—	3.2

raised to 40°C and stirring was continued for 4 hours at that temperature. After ether was distilled off, the product (IVc) was distilled under a reduced pressure, b.p. 140~142°C/0.2 mm, yielded 58.4 g n_D^{25} 1.5387.

Anal. Found: P, 9.1; Cl, 10.86%.

Calcd. for $C_{11}H_{20}O_3ClP$: P, 9.4; Cl, 10.74%.

4) Chlorination of O,O-dimethyl O-(1-*p*-chlorophenoxy-2,2-dichloro-vinyl) Phosphorate (IIIf).

To a solution of 7.5 g (0.2 M) of chlorine in 100 ml of carbontetrachloride cooled with ice and salt there was gradually added a solution of 34.7 g (0.1 M) of (IIIf) in 50 ml of carbontetrachloride. The reaction mixture was stirred at 0~10°C for one hour and then at room temperature for two hours. After the solvent was evaporated off, distillation of the residual oil gave 39.1 g of O,O-dimethyl-O-(1-*p*-chlorophenoxy-1,2,2,2-tetrachloro-ethyl) phosphorate, b.p. 122~124°C/0.1 mm: n_D^{27} 1.5144.

Anal. Found: P, 7.9; Cl, 42.68%

Calcd. for $C_{10}H_{10}O_3Cl_5P$: P, 7.4; Cl, 42.41%.

5) Reaction of O,O-diethyl O-(1-ethoxy-2-chloro-2-carboethoxy-vinyl) Phosphorate (IVc) with Acetic Acid.

A solution of 33.1 g (0.1 M) of (IVc) and 9.0 g (0.15 M) of acetic acid in 100 ml of absolute toluene was heated at 110°C for 5 hours. After toluene was distilled off under a reduced pressure, fractionated distillation gave 10.3 g of chloromalonate, b.p. 66~68°C/1 mm and 9.6 g of O,O-diethyl-O-acetyl phosphorate, b.p. 119~121°C/1 mm.

The infrared absorption spectrum of chloromalonate

and O,O-diethyl-O-acetyl phosphorate were coincided with that of authentic specimens^{16,17}.

Anal. Found: C, 43.51; H, 5.38; Cl, 16.11%. Calcd. for $C_7H_{11}O_4Cl$: C, 43.19; H, 5.66; Cl, 16.20%.

Anal. Found: P, 15.6%. Calcd. for $C_{10}H_{13}O_5P$: P, 15.8%.

6) Alcoholysis of O,O-diethyl O-(1-ethoxy-2-carboethoxy-vinyl) Phosphorate (IVa).

To a solution of sodium alcoholate prepared from 2.3 g of sodium in 200 ml of absolute alcohol was added 29.6 g (0.1 M) of (IVa) at room temperature. After refluxing for one hour, the reaction mixture was neutralized with 6.0 g of acetic acid and the alcohol was evaporated off. Ether and water were added to the residue, the ether layer was washed with water and dried with anhydrous sodium sulfate. After ether was distilled off, fractionated distillation gave 9.6 g of triethyl phosphorate, b.p. 100~101°C/21 mm and 10.8 g of diethyl malonate, b.p. 138~141°C/21 mm.

The infrared absorption spectra of these products were coincided with commercial diethyl malonate and authentic specimen¹⁷.

Anal. Found: P, 16.3; C, 39.25; H, 8.51%. Calcd. for $C_6H_{10}O_3P$: P, 17.0; C, 39.56; H, 8.24%.

Anal. Found: C, 52.38; H, 7.61%. Calcd. for $C_{17}H_{12}O_4$: C, 52.50; H, 7.50%.

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16) A. K. Macbeth, *J. Chem. Soc.*, **121**, 1120 (1922).

17) Dutton et al., *J. Am. Chem. Soc.*, **55**, 424 (1933).

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α -Aminoisobutyric Acid as the Constituent Amino Acid of Protein

Part XIV. Studies on Muscle Proteins

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α -Aminoisobutyric acid is the only tertiary amino acid which is reported to occur in the proteins. Nevertheless, this amino acid has not been yet isolated from the proteins. Recently we succeeded in isolating this amino acid as white prisms crystalline substance from both acid and pepsin hydrolysate of horse hind leg muscle proteins, and this crystal was identified to be α -amino-isobutyric acid by elementary analysis, properties of this derivatives, etc.

The first report concerning the possible presence of α -aminoisobutyric acid in the protein was made by Foreman¹⁾ (1913). But his report was a mere assumption, because the α -amino-*n*-butyric acid isolated from proline fraction in the hydrochloric acid hydrolysate of casein showed the same values as theoretical from the data of elementary analysis, but the melting point was lower than synthetic one, and he thought that this fact was due to contamination of α -aminoisobutyric acid, which is isomer of *n*-compound. In 1938, T. Yabuta and his coworkers²⁾ isolated 5,5-dimethylhydantoin from the dry distillate of

pupae of silkworm and confirmed that this was formed by combining α -aminoisobutyric acid with ammonium carbonate and postulated the possible presence of α -aminoisobutyric acid in the protein of pupae of silkworm. In 1948, M. Kandatsu³⁾, one of authors, in part VIII of this series, also confirmed the production of acetone from the acid hydrolysate of rabbit muscle proteins on oxidation by hypochlorite and suggested the presence of α -aminoisobutyric acid in this proteins, because it is the only one which causes the acetone on the oxidation of hypochlorite among the amino acid known as constituents of proteins. Later there were few reports concerning this fact, Y.

1) F.W. Foreman, *Biochem. z.*, **56**, 1 (1913).

2) T. Yabuta et al., *Bulletin of the Institute of Physical and Chemical Research*, **17**, 1241 (1938).

3) M. Kandatsu, *J. Agr. Chem. Soc. Japan*, **22**, 7 (1948),