Studies on Organophosphorus Insecticides III

On the Properties of Some α-Hydroxy Phosphonates and Related Compounds

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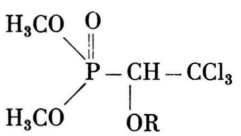
In search for effective organophosphorus esters against the larva of the cotton leaf worm (*Prodenia litura* F), some α-hydroxy phosphonic acid dimethyl esters and related compounds have been synthesized. The effect of substitution on the antiesterase activity and toxicity towards *Prodenia* larvae has been investigated.

The infra-red spectra of the prepared phosphorus esters are discussed.

Recently, it has been reported that *0,0*-dimethyl-2,2,2-trichloro-1-methoxy ethylphosphonate ($\mathbf{1}_b$) possesses almost the same toxicity as Dipterex ($\mathbf{1}_a$) towards the larvae of the cotton leaf worm *Prodenia litura* F. Moreover, $\mathbf{1}_b$ proved to be more stable than Dipterex (*0,0*-dimethyl-2,2,2-trichloro-1-hydroxy ethylphosphonate) and possesses lower anticholinesterase activity [1]. In search for organophosphorus esters with low anticholinesterase activity but

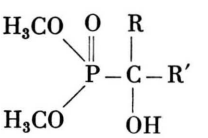


1 a, R = H
b, R = CH_3
c, R = $CONHC_2H_5$

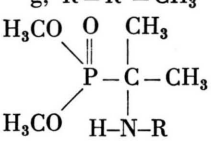
are at the same time effective against *Prodenia* larvae, studies were continued in the series of α-hydroxy phosphonates and related compounds.

For this purpose the α-hydroxyphosphonic acid dimethyl esters $\mathbf{2}_{a-g}$, the *N*-substituted α-aminophosphonates $\mathbf{3}_{a-b}$ and the carbamate $\mathbf{1}_c$ have been prepared. Their infra-red spectra as well as their antiesterase activity were studied.

Of the α-hydroxy phosphonates $\mathbf{2}_a$ [2], $\mathbf{2}_e$ [3], and $\mathbf{2}_g$ [2] have been previously described and are listed here for comparison. $\mathbf{2}_{a-g}$ are readily obtained by condensation of dimethylhydrogen phosphite with the appropriate aldehyde (or ketone) in the presence of sodium methoxide as a catalyst.



2 a, R = H; R′ = CH_3
b, R = H; R′ = C_6H_4Cl-o
c, R = H; R′ = C_6H_4Cl-p
d, R = H; R′ = C_6H_4Br-p
e, R = H; R′ = $C_6H_4NO_2$-p
f, R = H; R′ = C_6H_3Cl-2, NO_2-5
g, R = R′ = CH_3



3 a, R = C_4H_9-n.
b, R = $CH_2C_6H_5$.

Some aromatic α-hydroxy phosphonates, e. g., $\mathbf{2}_{b-d}$ could be prepared directly from the aldehyde, phosphorus trichloride and methanol by a single vessel reaction in a manner similar to that used for the preparation of Dipterex [1].

The α-hydroxy phosphonates are quite stable towards dilute mineral acids. They suffer only a slight decomposition when heated with N hydrochloric acid. Using paperchromatographic analysis, monomethyl- and dimethyl phosphate could be identified as decomposition products. This is in line

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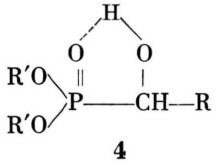
[3] V. S. Abramov, Zhur Obschtschei Khim. **27**, 169 [1957]; C. A. **51**, 12878 [1957].

with previous findings, that most organophosphorus esters are fairly stable in acid medium [4].

In alkaline medium, $\mathbf{2}_{a-g}$ are rapidly decomposed. When treated with cold N sodium hydroxide, the α-hydroxy phosphonates readily liberate the original aldehyde or ketone, which could be identified as its 2,4-dinitrophenylhydrazone.

The α-amino phosphonates $\mathbf{3}_{a-b}$, were obtained by the addition of dimethyl hydrogen phosphite to the Schiff's bases acetone-n-butylimine and acetone-benzylimine, respectively [5, 6, 7]. These compounds are fairly stable towards mineral acids or alkalies and undergo only slight degradation when heated with these reagents. *O,O*-Dimethyl-2,2,2-trichloro-1(*N*-ethyl)carbamoyl ethylphosphonate ($\mathbf{1}_c$) was readily obtained in good yield by condensation of Dipterex with ethylisocyanate in presence of triethyl amine. $\mathbf{1}_c$ has been previously prepared by using dibutyltin dilaurate as a catalyst [8]. It is easily hydrolyzed by acids and alkalies. Cautious hydrolysis with cold N hydrochloric acid leads to the formation of $\mathbf{1}_a$.

In chloroform solution, the infra-red spectra of the α-hydroxy phosphonates $\mathbf{2}_{b-d}$ and $\mathbf{2}_{f-g}$ show a strong absorption in the form of a broadend band in the range of $3265 \pm 15\ cm^{-1}$. Such an associated OH group has been observed in the case of Dipterex ($\mathbf{1}_a$) [9, 10]. In very dilute solution the spectra show, in addition to the bonded OH-absorption, another band of lower intensity around $3530 \pm 10\ cm^{-1}$ due to a free OH-valency vibration. The results strongly suggest the presence of an intramolecular type of hydrogen bonding. Such internal bonding was reported to occur in certain α-hydroxy phosphonates (**4**) [11], where R can be aromatic or aliphatic.



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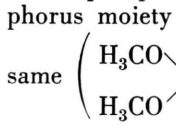
A similar internal bonding may occur also in α-amino phosphonates. In dilute chloroform solution, $\mathbf{3}_a$ does not show a free N-H absorption. A broad absorption band at $2740\ cm^{-1}$ may be attributed to a strongly chelated NH group. $\mathbf{2}_{f-g}$ and $\mathbf{3}_a$ show absorption bands in the regions $1265 \pm 5\ cm^{-1}$ and at $1212 \pm 8\ cm^{-1}$ presumably due to free and associated P = 0 group, respectively; the former band being near the usual phosphonate absorption region [12]. In nujol the band for the associated (P→0) is the dominent one.

In chloroform, $\mathbf{1}_c$ shows an NH-absorption around $3400\ cm^{-1}$. The streching frequency of the free P = 0 group appears as a sharp band at $1250\ cm^{-1}$. In the region of carbonyl absorption $\mathbf{1}_c$ shows a strong band at $1750\ cm^{-1}$. This is in accordance with the usual carbonyl absorption of the carbamate ester group [13]. The strong absorption at $1290\ cm^{-1}$ may be attributed, as in esters [14], to the C – 0 stretching vibration.

Anti-esterase activity and Toxicity

The prepared organophosphates were tested for their anticholinesterase activity in an effort to correlate the findings with the toxicological effects. Fig. 1 and table 1 clearly demonstrate that non of the prepared compounds is active as the parent substance Dipterex. Aromatic or aliphatic substitution of the trichloro radical results in a decreased activity. Also, the replacement of the hydroxy group by a substituted amine does not seem to provide any increase in activity. However, the introduction of a carbamoyl group to Dipterex seems to reduce its anticholinesterase property only slightly. It is known that the reaction of organophosphates with cholinesterase involves a binding (or affinity) constant and a phosphorylation constant [15]. Since the phosphorus moiety of all the compounds tested is the same  , it is believed that it is the

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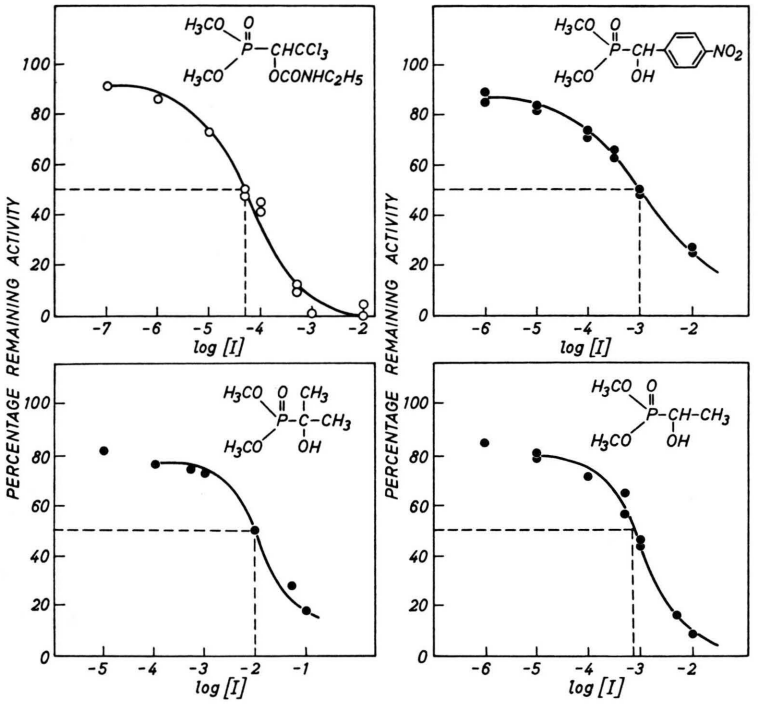

Fig. 1. Inhibition of acetylcholinesterase. Inhibitor concentration [1] expressed as Moles/liter.

Compound	Percentage Inhibition* Rat-brain Acetyl-Cholinesterase	Rat-liver Aliesterase
1_a	100	80.0
1_b	40.0	26.0
1_c	94.5	50.0
2_a	55.1	12.5
2_b	27.3	22.5
2_c	23.4	10.6
2_d	18.0	11.0
2_e	49.4	13.0
2_f	39.3	0.0
2_g	27.3	22.0
3_a	27.6	19.8
3_b	23.5	23.0

Table 1. Anti-esterase activity of the prepared α-hydroxy- and α-amino phosphonate derivatives. * Inhibitor concentration 10^{-3} M.

affinity constant (K_a) rather than the phosphorylation constant (K_p) which determines the degree of inhibition (as influenced by the "leaving" group).

The inhibition of rat liver aliesterase by these compounds shows again the same general trend (Table 1). An important feature of this inhibition is that it is not progressive, i. e., the inhibitor combines reversibly with the enzyme. In this case, it may be assumed that the dephosphorylation rate is equal to or exceeds the rate of phosphorylation of the enzyme.

The toxicological results show no definite pattern, so far as substitution is concerned (Table 2), and again Dipterex seems to be the best organophosphonate. Of the parameters investigated to asses the potency of the prepared organophosphonates, the anticholinesterase activity is the most important. Recently, cholinesterase has been reported to be present in the nerve cord of the *Prodenia* larva, and evidence has been introduced that the organophos-

Compound	Dose [μg]	Mortality*
1_a	20	75
1_b	20	75
1_c	20	12.5
2_a	20	10
2_b	36	5
2_c	36	10
2_d	20	10
2_e	36	15
2_f	72	5
2_g	36	12
3_a	36	8
3_b	36	10

Table 2. Toxicity of the investigated α-hydroxy- and α-amino phosphonate derivatives. * Results are mean of three different experiments.

phonate Dipterex is incapable of penetrating to the target enzyme, through the nerve sheath [16]. It seems,, therefore, feasible to assume that the toxic effect of the organophosphonates is primarily due to inhibition of the hemolymph esterases (and probably also to non-specific inhibition of other enzyme systems) and that cholinesterase is not involved. It also explains the low toxicity of Dipterex itself [16] as well as its derivatives. This assumption is strongly supported by the fact that *O,O*-dimethyl-2,2,2-trichloro-1-methoxyethyl phosphonate ($\mathbf{1}_b$) -though possessing much lower anticholinesterase activity than Dipterexshowed the same toxicity as the parent compound [1]. In fact, it was the latter observation that stimulated the search for compounds of the type described in this paper.

Experimental

Melting points are uncorrected. The infra-red spectra have been carried out on UR 10, Zeiss, Jena, Infrared spectrophotometer.

Preparation of the α-hydroxy phosphonates **2**

General procedure

Freshly distilled dimethyl hydrogen phosphite (0.1 mole) was mixed with an equivalent amount of the appropriate aldehyde or ketone. 1 N sodium methoxide solution (0.5 ml.) was added and the mixture was shaken for 10 minutes. After the originaly evolved heat had ceased, the reaction mixture was cooled and the product collected. It was then purified by crystallization from the appropriate solvent. The analytical data of the new α-hydroxy phosphonates are listed in table 3.

Action of hydrochloric acid on $\mathbf{2}_{a-g}$

50 mg. of the compound were heated with 5 ml. 1 N hydrochloric acid for 30 minutes at 100°. After removal of the unchanged material with chloroform, the aqueous solution was paper chromatographed on Schleicher and Schüll 2043 b using isopropanol-water-ammonia (75:24:1)[17] and isopropanol-ammonia (75:25)[17]. The spots were made visible by spraying with Hanes and Isherwood reagent [18] using U.V. light as a reducing agent. Monomethyl- and dimethyl phosphates could be identified as decomposition products (R_f of monomethyl phosphate 0.11 and 0.05; R_f of dimethyl phosphate 0.6 and 0.50 in the first and second systems, respectively). The recovered unchanged substance ranged from 60 – 80 per cent.

Action of dilute sodium hydroxide on $\mathbf{2}_{a-g}$

50 mg. of the compound were treated with 10 ml. 1 N sodium hydroxide solution. The parent carbonyl compound was readily liberated. After 2 minutes the solution was acidified with dilute hydrochloric acid and the separated carbonyl compound was identified as its 2,4-dinitrophenyl hydrazone.

Preparation of the aminophosphonates $\mathbf{3}_{a-b}$

A mixture of 0.1 mole acetone, 0.12 mole of the appropriate amine (n-butylamine or benzylamine) and 2 g. anhydrous potassium carbonate was heated on a water bath for 30 minutes. Benzene was then added and the organic layer rapidly separated, dried over potassium hydroxide pellets and filtered. The imine obtained after removal of benzene under reduced pressure, was used directly for the preparation of the amino phosphonate derivatives.

Acetone-butylimine (0.05 mole) was mixed with the equivalent amount of dimethyl hydrogen phosphite while shaking. The reaction mixture was allowed to stand till no more heat was evolved. Excess dimethyl

Compd.	m. p.° *	Formula [Mol.Wt.]		Analysis C	H	Halogen	N	P
2_b	88–9	$C_9H_{12}Cl\text{-}O_4P$	Calcd.	43.13	4.82	14.14	—	12.36
	a, c	(250.635)	Found	43.21	4.69	14.18	—	12.21
2_c	80–1	$C_9H_{12}ClO_4P$	Calcd.	43.13	4.82	14.14	—	12.36
	b	(250.635)	Found	42.98	4.71	13.98	—	12.29
2_d	87–8	$C_9H_{12}BrO_4P$	Calcd.	36.63	4.09	27.08	—	10.49
	a	(295.095)	Found	36.70	3.97	26.98	—	10.36
2_f	154–5	$C_9H_{11}ClNO_6P$	Calcd.	36.56	3.75	11.99	4.73	10.47
	a, b	(295.635)	Found	—	—	11.78	4.86	10.38

Table 3. Analytical data of the α-hydroxy phosphonates **2**. * Solvent of crystalization. a) Ether, b) Benzene, c) Benzene-Pet. ether (b.p. 40–60°).

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hydrogen phosphite was then removed under vacuum (in vacuum desiccator for 4 days); whereupon the oily product solidifies.

3a gave colourless crystals from methyl alcohol–ether mixture, m.p. 205–206°; yield 70 per cent.

Analysis
Calcd. for $C_9H_{22}NO_3P$ (223.26)
C 48.41 H 9.93 N 6.27 P 13.87,
Found: C 48.45 H 9.89 N 6.25 P 13.81.

3b gave colourless crystals from a mixture of ethanol and ether, m.p. 212–213°; yield 80 per cent.

Analysis
Calcd. for $C_{12}H_{20}NO_3P$ (257.28)
C 56.02 H 7.83 N 5.44 P 12.04,
Found: C 56.10 H 7.81 N 5.39 P 12.11.

50 mg. of **3a** were heated with 1 N hydrochloric acid as previously described. It proved to be fairly stable.

3a (50 mg.) was heated in 1 N sodium hydroxide solution for 20 minutes at 100°. The cooled mixture was acidified with dilute hydrochloric acid and analyzed by paper chromatography in isopropanol-water-ammonia (75:24:1)[17]. In addition to the unchanged material (R_f = 0.75), smaller spots appeared with R_f-values 0.1 and 0.58 due to monomethyl- and dimethyl phosphate, respectively. The percentage of decomposition was less than 30 per cent.

O,O-Dimethyl-2,2,2-trichloro-1-(N-ethyl)carbamoyl ethyl phosphonate **1c**

To a solution of Dipterex (0.01 mole) in dry benzene (20 ml.), ethyl isocyanate (0.015 mole) was added followed by addition of 0.5 ml. of triethyl amine. The reaction mixture was refluxed for five hours and then cooled. After acidification with 1 N hydrochloric acid, the product was extracted with benzene. The solvent was removed in a vacuum and the residue crystallized from benzene-petroleum ether (b.p. 40–50°). **1c** formed colourless needles, m.p. 96–97°, Lit. m.p. 97–98°)[8].
Analysis
Calcd. for $C_7H_{13}Cl_3NO_5P$ (328.55)
C 25.59 H 3.98 Cl 32.37 N 4.26 P 9.43,
Found: C 25.61 H 4.00 Cl 32.21 N 4.16 P 9.28.

Action of hydrochloric acid on **1c**

50 mg. of the carbamate was treated with 10 ml. 1 N hydrochlorid acid and the mixture was left for one hour at room temperature. The mixture was then extracted with chloroform and the chloroform layer was analyzed by paper chromatography in isopropanol-water-ammonia (75:24:1)[17], and n-butanol-pyridine-water (12:8:6)[19]. The chromatograms showed the presence of Dipterex alone (R_f = 0.79 and 0.95 in the first and second systems, respectively).

Determination of Acetylcholinesterase activity

The anticholinesterase activity has been determined according to the method of HESTRIN [20]. A 10% rat-brain homogenate was used as a source of acetylcholinesterase. The reaction mixture had the following composition: 0.9 ml. 0.2 M. phsphate buffer pH 7.2; 0.3 ml. 1.0 M. magnesium chloride solution; 0.3 ml. 1.0 M. sodium chloride solution; 0.5 ml. 10% rat-brain homogenate in isotonic potassium chloride; 0.5 ml. distilled water or variable concentrations of the tested compound (inhibitor) in distilled water (final concentration 10^{-2} M. – 10^{-7} M.). The reaction mixture was incubated for 30 minutes at 37°. 0.5 ml. 48 millimolar acetylcholine chloride in 0.001 M. sodium acetate (final concentration 8 millimolar) was then added to stop the E-I reaction and the residual acetylcholine was assayed after 30 minutes.

Determination of Aliesterase activity

Rat-liver homogenate in isotonic KCl (10%) served as the enzyme source. The enzyme was assayed titrimetrically, according to the procedure of HARRER and KING [21]; using a freshly prepared saturated solution of ethyl butyrate as a substrate. The organophosphate was preincubated with the enzyme for 30 minutes, followed by the addition of the substrate. In some experiments the inhibitor and the substrate were incubated simulatneously with the liver homogenate.

Toxicological studies

For the evaluation of the prepared organophosphonates as to their effectiveness against *Prodenia* larvae, the third larval instar was treated topically with the organophosphonate in acetone. Each larva received 1 µl. acetone containing the desired amount of the toxicant. Control larvae received acetone alone. Fifty larvae were used for each determination and mortality counts were taken 24 hours after treatment.

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