604. *Studies on Phosphorylation. Part XVII.* The Hydrolysis of Methyl* 3-(OO-*Dimethylphosphoryloxy*)*but*-2-*enoate.*

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Hydrolysis of the esters (Ia and b) has been examined; the results indicate that partial hydrolysis products are unlikely to account for their biological activity. Preliminary data on hydrolysis under oxidising conditions suggest that such processes may be involved in their activation *in vivo*.

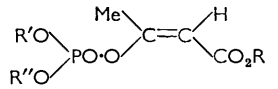
Recent investigation [1] in these laboratories has had as its ultimate aim the development of reagents for phosphorylation in aqueous media. In a broad sense many organophosphorus insecticides may be considered as reagents of this type since the mode of inhibition of certain hydrolases, *e.g.*, chymotrypsin,[2] has been demonstrated to involve phosphorylation of the enzyme. It was considered that a further insight into phosphorylation processes might be obtained by a study of certain of these organophosphorus compounds *in vivo* and *in vitro* and, in particular, we wished to investigate those compounds which show low reactivity both in hydrolysis and towards enzymes *in vitro* but are rapidly metabolised and are highly toxic *in vivo*. This behaviour, typical of certain insecticides, is usually attributed to the conversion of the compounds, in the plant, insect, or mammal into unstable and active phosphorylating agents,[3] a typical example being the activation of octamethylpyrophosphoramide ("Schradan") by oxidation.[4] The present paper deals with the hydrolysis and derivatives of a recently reported [5] systemic insecticide of this type methyl 3-(*OO*-dimethylphosphoryloxy)but-2-enoate (I) ("Phosdrin") and attempts to elucidate the nature of its activation. Two possible modes of activation appeared possible, one involving oxidation to a ketal derivative (II) and the other a partial hydrolysis, it being expected that the *cis*-derivative (Ia; R, R′, R″ = Me) might display the high reactivity of the similarly constituted salicylyl and *o*-carboxynaphthyl phosphate.[6] The instability of "Phosdrin" and especially of the *cis*-isomer in the plant has been demonstrated by Casida *et al.*[7]

"Phosdrin" was readily separated into two geometrical isomers (Ia and b) by countercurrent distribution in ether–water, the separation being followed by ultraviolet

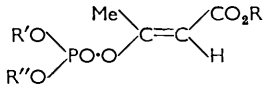
* Part XVI, *J.*, 1958, 528.

[1] Clark, Kirby, and Todd, *J.*, 1957, 1497; Khorana and Todd, *J.*, 1953, 2257.
[2] Schaffer, May, and Summerson, *J. Biol. Chem.*, 1953, **202**, 69; 1954, **206**, 201; Aldridge, *Ann. Reports*, 1956, **53**, 294.
[3] O'Brien and Spencer, *Ann. Rev. Entomology*, 1957, **2**, 261.
[4] Spencer, *Chem. Soc. Special Publ.*, No. 8, 1957, p. 171.
[5] Stiles, U.S.P., 2,685,552/1954.
[6] Chandley, Gindler, and Sobotka, *J. Amer. Chem. Soc.*, 1952, **74**, 4347; Chandley and Gindler, *ibid.*, 1953, **75**, 4035.
[7] Casida, Gatterdam, Getzin, and Chapman, *J. Agric. Food Chem.*, 1956, **4**, 722.

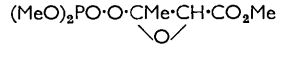
absorption measurements at 218 mμ; the separated components were distinguished by refractive index and by characteristic infrared absorption, the *cis*-isomer showing a strong absorption at 897 and the *trans*- at 913 cm.$^{-1}$. The isomers were separately hydrolysed



(Ia)



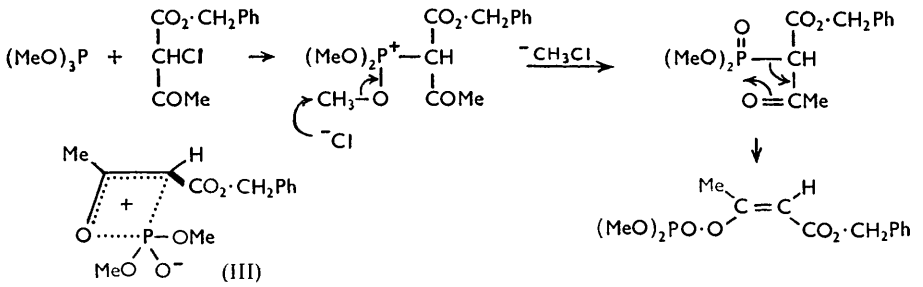
(Ib)



(II)

at pH 11 and 30°; after 24 hours, when hydrolysis was virtually complete, the *trans*-isomer had consumed 2·4 mols. of alkali and the *cis* 1·9 mols., although initially the rate of alkali uptake was approximately the same. Paper chromatography of the hydrolysis products indicated that the *trans*-isomer gave dimethyl hydrogen phosphate together with a trace of a component subsequently identified as methyl *trans*- 3-(*O*-methylphosphoryloxy)but-2-enoate (Ib; R = R′ = Me, R″ = H). The *cis*-isomer gave a more complex mixture containing dimethyl hydrogen phosphate and appreciable amounts of two other components identified as (Ia; R = H, R′ = R″ = Me; and R = R′ = Me, R″ = H); in both cases acetone and a trace of methyl dihydrogen phosphate were also produced. Thus the *trans*-isomer reacts almost exclusively by attack of hydroxide ion on phosphorus, the absence of product (Ib; R = H, R′ = R″ = Me) in spite of its expected stability indicating that this probably occurs more rapidly than hydrolysis at the carboxylic ester. The *cis*-isomer (Ia; R = R′ = R″ = Me) however is hydrolysed at the phosphoric and (more rapidly) at the carboxylic ester groups and further, whilst both isomers are hydrolysed at the phosphate ester group by predominant fission of the enol-phosphate linkage, the *cis*-form gives a higher proportion of P–O–CH_3 cleavage. The results reflect the ability of the *trans*-olefinic system to transmit the inductive effect of the methoxycarbonyl group and the steric effect in the *cis*-isomer, and both effects may be compared with those operating in *cis*- and *trans*-αβ-unsaturated acids where they determine the differing acid strengths.[8]

The greater reactivity of the *cis*-form of " Phosdrin " on hydrolysis at the methoxycarbonyl group was also demonstrated by a partial hydrolysis of the mixed isomers (*cis* 60%, *trans* 40%) in M-sodium carbonate at 30°, the recovered " Phosdrin " being shown to be predominantly the *trans*-form, whilst fractional acidification of the hydrolysate afforded *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoic acid (Ia; R = H, R′ = R″ = Me), identified by paper chromatography and conversion into the ester (Ia; R = R′ = R″ = Me) by diazomethane. The pure *cis*-carboxylic acid (Ia; R = H, R′ = R″ = Me) was obtained by the hydrogenolysis of benzyl *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoate (Ia; R = CH_2Ph, R′ = R″ = Me) prepared by treatment of benzyl α-chloroacetoacetate with trimethyl phosphite. It is of interest that similar treatment of methyl α-chloroacetoacetate with trimethyl phosphite gives a mixture of *cis*- and *trans*-products.[5] The steric control of the reaction with the benzyl ester could be explained by considering the formation of the " Phosdrin " derivative to involve an Arbusov reaction [9] between the chloro-ester and the trimethyl phosphite with a subsequent phosphonate rearrangement:



(III)

[8] Ingold, " Structure and Mechanism in Organic Chemistry," Bell and Co., 1953, Chapter 13, p. 745; Dippy, *Chem. Rev.*, 1939, **25**, 187.

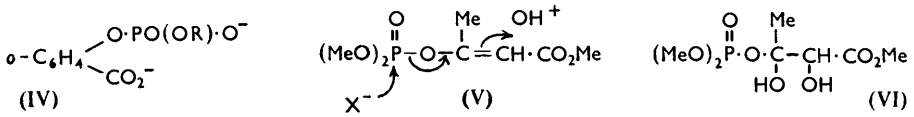
[9] Arbusov, *J. Soc. Phys. Chim. Russ.*, 1906, **38**, 687; Kosolapoff, *J. Amer. Chem. Soc.*, 1944, **66**, 109.

eclipsing of the methyl and the benzyloxycarbonyl group in the formation of the transition state (III) of the phosphonate–phosphate rearrangement is at a minimum for production of the *cis*-isomer. An attempt to determine the configuration of the double bond in the allyl analogue (I; R = CH_2·CH:CH_2, R′ = R″ = Me), prepared from allyl α-chloroacetoacetate and trimethyl phosphite, by hydrogenolysis and esterification with diazomethane could not be made since the allyl derivative underwent reduction to the *n*-propyl derivative and not hydrogenolysis to the carboxylic acid; however, counter-current distribution of the allyl ester indicated that a mixture had been formed.

The *cis*-acid (Ia; R = H, R′ = R″ = Me) was only slowly hydrolysed under mild conditions, having a half-life of 50 hours at pH 11 and 30°, and did not show any maximum in the rate of hydrolysis at intermediate pH values as do salicylyl and α-carboxynaphthylphosphate at pH 5—6. Our results parallel those of Montgomery, Turnbull, and Wilson [10] who were unable to demonstrate easy hydrolysis of steroid esters of salicylyl phosphate (IV; R = steroid residue) under conditions where the dianion (IV; R = H) undergoes rapid hydrolysis to salicylic acid and inorganic phosphate.

A similar slow hydrolysis was shown by methyl *cis*- and *trans*-3-(*O*-methylphosphoryloxy)but-2-enoate (I; R = R′ = Me, R″ = H) which were prepared by anionic demethylation of the corresponding " Phosdrin " derivatives by sodium iodide in acetone.[11] From the resistance to further hydrolysis shown by the partially hydrolysed derivatives of " Phosdrin " it appears that none of these intermediates can be considered as the " active component " in biological reactions of the compound and, although it has been pointed out [12] that the rate of attack by a nucleophilic site in an enzyme does not necessarily parallel that of hydroxide ion, we conclude that some other activation process or processes must be operating *in vivo*.

We therefore turned to an oxidative step as a possible activation process, it being considered that an activation *in vivo* might arise by the process (V), leading to the production of a phosphorylated enzyme and hydroxyacetone. In aqueous pertungstic acid [13] (pH 5·5, 70°) " Phosdrin " was rapidly hydrolysed to dimethyl hydrogen phosphate.



A solution of sodium hypobromite (pH 9·5, 20°) had a similar effect; in the absence of bromine at pH 9·5, 20° " Phosdrin " was only very slowly hydrolysed. These reactions may be interpreted on the basis of attack on the phosphate ester initiated by attack of OH^+ or Br^+ on the olefinic bonds and provide a possible mechanism of oxidative phosphorylation for esterase inhibition by the insecticide. An alternative explanation of the above hydrolysis involving formation and decomposition of a semi-ketal derivative (VI), whilst possible, is considered less likely since it should lead also to products other than dimethyl hydrogen phosphate formed by intermolecular phosphoryl migration involving the vicinal hydroxyl group.[14] Further work is in hand with isolated enzyme systems to determine whether " Phosdrin " or its hydrolysis products are metabolised by an oxidative process analogous to that discussed above.

Experimental

cis- *and* trans-" *Phosdrin.*"—A redistilled commercial sample (12·56 g.) of the mixed isomers was placed in the first 5 tubes of an automatic counter-current battery of 100 tubes with 20·5 c.c. phases, and the system ether–water. After 95 transfers the contents of appropriate

[10] Montgomery, Turnbull, and Wilson, *J.*, 1956, 4603.
[11] Zervas and Dilaris, *J. Amer. Chem. Soc.*, 1955, **77**, 5354.
[12] Aldridge, *Ann. Reports*, 1956, **53**, 297.
[13] Mugdan and Young, *J.*, 1949, 2988.
[14] Brown, Magrath, and Todd, *J.*, 1954, 1442.

tubes were examined for ultraviolet absorption at 218 mμ. The contents of tubes 61—100 were combined, the aqueous phase was extracted with chloroform (3 × 30 c.c.), and the combined extracts were dried (Na_2SO_4) and evaporated. The residue on distillation afforded the *cis*-isomer of " Phosdrin " (7·3 g.), b. p. 114°/2 mm., n_D^{20} 1·4460, $\nu_{max.}$ 897 cm.$^{-1}$. The contents of tubes 18—48 similarly gave the *trans*-isomer (2·88 g.), b. p. 112°/1 mm., n_D^{20} 1·4505, $\nu_{max.}$ 913 cm.$^{-1}$.

Benzyl cis-3-(OO-*Dimethylphosphoryloxy*)*but*-2-*enoate*.—Benzyl α-chloroacetoacetate was prepared from benzyl acetoacetate as described by Day [15] for the ethyl ester and had b. p. 107°/0·3 mm., n_D^{22} 1·5183 (Found: C, 56·3; H, 5·0. $C_{11}H_{11}O_3Cl$ requires C, 56·2; H, 5·1%). Trimethyl phosphite (24·8 g.) was added with stirring during 10 min. to benzyl α-chloroacetoacetate (39·9 g.). After a further 20 min. the temperature was slowly raised to 105° and the unchanged trimethyl phosphite then removed at 0·1 mm. The residue was distilled at 70° (bath-temp.)/0·0005 mm., giving *benzyl cis*-3-(OO-*dimethylphosphoryloxy*)*but*-2-*enoate* (41 g.), n_D^{16} 1·5083 (Found: C, 51·7; H, 5·6. $C_{13}H_{17}O_6P$ requires C, 52·0; H, 5·7%). The ester (6·0 g.), dioxan (65 c.c.), acetic acid (0·1 c.c.), and 10% palladised charcoal (0·3 g.) were stirred under hydrogen; after 6 hr. a further quantity (0·3 g.) of 10% palladised charcoal was added and the mixture stirred under hydrogen for a further 16 hr., then filtered, and evaporated. The product was shown to be essentially pure *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoic acid by its conversion, in almost quantitative yield, into methyl *cis*-3-(*OO*-dimethylphosphoryloxy)-but-2-enoate by ethereal diazomethane, by an infrared max. (CO_2R) at 2200—3200 cm.$^{-1}$, and absence of aromatic absorption (3030, 1590, and 1500 cm.$^{-1}$).

Preparation and Reduction of Allyl 3-(OO-*Dimethylphosphoryloxy*)*but*-2-*enoate*.—This *ester*, b. p. 122°/0·9 mm., n_D^{16} 1·4580 (Found: C, 43·8; H, 6·3. $C_9H_{15}O_6P$ requires C, 43·3; H, 6·1%), was prepared from allyl α-chloroacetoacetate as described for the benzyl ester. Attempted hydrogenolysis gave only n-*propyl* 3-(OO-*dimethylphosphoryloxy*)*but*-2-*enoate*, b. p. 114° (bath-temp./14 mm.), n_D^{25} 1·4435 (Found: C, 42·5; H, 7·0. $C_9H_{17}O_6P$ requires C, 42·9; H, 6·8%), $\nu_{max.}$ 1660 (C:C) and 1722 cm.$^{-1}$ (conjugated CO) [no absorption at 3080 cm.$^{-1}$ (CH_2·CH:CH_2)].

Sodium Methyl 3-(O-*Methylphosphoryloxy*)*but*-2-*enoate*.—(*a*) A solution of methyl *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoate (0·22 g.) and sodium iodide (0·158 g.) in acetone (2 c.c.) was refluxed for 5 min., giving an almost immediate precipitate (0·21 g.) which was washed with acetone and converted into the *cyclo*hexylammonium salt by use of Dowex-50 resin (*cyclo*-hexylammonium form), affording after crystallisation from acetone cyclo*hexylammonium methyl* cis-3-(O-*methylphosphoryloxy*)*but*-2-*enoate* (0·09 g.), m. p. 137—138° (Found: C, 46·0; H, 7·5. $C_{12}H_{24}O_6NP$ requires C, 46·4; H, 8·1%).

(*b*) The *trans*-form (Ib; R = R′ = R″ = Me) (0·22 g.), when similarly treated with sodium iodide (0·158 g.), gave *sodium methyl* trans-3-(O-*methylphosphoryloxy*)*but*-2-*enoate* (0·171 g.) (Found: C, 30·6; H, 4·3. $C_6H_{10}O_6PNa$ requires C, 31·1; H, 4·3%).

Hydrolysis of " Phosdrin " and its Derivatives.—Titrimetric experiments. In a typical experiment a weighed sample (0·572 g.) of methyl *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoate, dissolved in water, was stirred at 30° by means of nitrogen in a covered cell containing the electrodes of a pH meter. The pH was adjusted to 11 and kept thereat by an automatic titrating machine (Raiometer Model TT 2) and the alkali (0·025N) consumption measured at intervals. When hydrolysis was complete the contents of the cell were concentrated *in vacuo* below room temperature and examined by paper chromatography (see Tables) after detection of the phosphorus-containing products by the molybdate spray.[16] The annexed assessments (p. 2972) were made.

Partial hydrolysis. The mixed " Phosdrin " esters [60% of (Ia; R = R′ = R″ = Me) and 40% of (Ib; R = R′ = R″ = Me)] (2·5 g.) were kept at 30° for 24 hr. in M-sodium carbonate (100 c.c.); the pH of the solution fell from 11 to 8·5. The yellow solution was extracted with chloroform, and the extract dried (Na_2SO_4), evaporated, and distilled, giving a product (1·93 g.), $n_D^{22.5}$ 1·4466, shown by paper chromatography and its infrared spectrum to be mainly the *trans*-form (Ib; R = R′ = R″ = Me). The aqueous residue was brought to pH 2·4 by hydrochloric acid and extracted with chloroform (3 × 25 c.c.), dried (Na_2SO_4), and evaporated. The residue (0·48 g.) showed strong infrared absorption at 2960 cm.$^{-1}$ (CO_2H) but no P—OH band was detected at 2300 cm.$^{-1}$ (Found: equiv., 226. Calc. for $C_6H_{11}O_6P$: equiv., 210). The acid on treatment with ethereal diazomethane gave methyl *cis*-3-(*OO*-dimethylphosphoryloxy)but-2-enoate as shown by the refractive index and infrared spectrum of the distilled product.

[15] Day, *J.*, 1915, 1646.
[16] Hanes and Isherwood, *Nature*, 1949, **164**, 1107.

Ester hydrolysed	Conditions	Products	Proportion
Ia; R = R′ = R″ = Me	pH 11, 3 hr.	Ia; R = R′ = R″ = Me	5
		Ia; R = H, R′ = R″ = Me	1
		Ia; R = R′ = Me, R″ = H	1
		Me_2HPO_4	3
	pH 11, 24 hr.	Ia; R = H, R′ = R″ = Me	3
		Me_2HPO_4	6
		MeH_2PO_4	1
Ib; R = R′ = R″ = Me	pH 11, 3 hr.	Ib; R = R′ = R″ = Me	7
		Me_2HPO_4	2
		MeH_2PO_4	1
	pH 11, 24 hr.	Me_2HPO_4	8
		MeH_2PO_4	1
Ia; R = H, R′ = R″ = Me	pH 11, 24 hr.	Ia; R = H, R′ = R″ = Me	3
		Me_2HPO_4	5
		MeH_2PO_4	1
	pH 6, 72 hr.	Ia; R = H, R′ = R″ = Me	5
		Ia; R = R′ = H, R″ = Me *	1
		Me_2HPO_4	3
		MeH_2PO_4	1
Ia; R = R′ = Me, R″ = H	pH 11, 24 hr.	Ia; R = R′ = Me, R″ = H	5
		Ia, R = R″ = H, R′ = Me	2
		MeH_2PO_4	2
Ib; R = R′ = Me, R″ = H	pH 11, 24 hr.	Ib; R = R′ = Me, R″ = H	5
		MeH_2PO_4	5

* Prepared by anionic demethylation of (Ia; R = CH_2Ph, R′ = R″ = Me) and hydrogenolysis as described above.

Peracid-catalysed Hydrolysis of "Phosdrin."—The mixed "Phosdrin" derivatives (0·05 g.) were added to a solution of pertungstic acid [13] (30 c.c.; 2M-hydrogen peroxide + 10 mg. of WO_3) previously adjusted to pH 5·5 and the solution kept at 70° and pH 5·5. The uptake of alkali was rapid. There were obtained dimethyl hydrogen phosphate, as main product, and unchanged material. Omission of the tungstic oxide from the above reaction gave much slower uptake of alkali, and the product was shown to be (Ia; R = H, R′ = R″ = Me), with unchanged material.

Sodium Hypobromite Treatment of "Phosdrin."—A saturated solution of bromine water (40 c.c.) was adjusted to pH 9·5 with alkali, and the mixed "Phosdrin" isomers were then added (0·05 g.); the solution was kept at 20° and pH at 9·5 (titration with 0·025N-alkali). One mol. of alkali was consumed in 1 hr. and at this point the product was shown to be dimethyl hydrogen phosphate with a trace of methyl dihydrogen phosphate; alkali alone under these conditions effected negligible breakdown of "Phosdrin."

Paper Chromatography of Phosdrin and Derivatives.—Ascending chromatograms on Whatman No. 1 paper gave the annexed values for (*A*) propan-2-ol–ammonia (*d* 0·88) (2·5 : 1) and (*B*) butan-1-ol–acetic acid–water (5 : 2 : 3).

	R_F in solvent			R_F in solvent	
	A	*B*		*A*	*B*
Ia; R = R′ = R″ = Me	0·92	0·96	Ia; R = R′ = H, R = Me	0·25	0·44
Ia; R = R′ = Me, R″ = H	0·73	0·61	Ib; R = R′ = R″ = Me	0·86	0·93
Ia; R = H, R′ = R″ = Me	0·62	0·47	Ib; R = R′ = Me, R″ = H ...	0·67	0·61
Ia; R = CH_2Ph, R′ = R″ = Me	0·86	—	Me_2HPO_4	0·50	0·60
Ia; R = CH_2Ph, R′ = Me, R″ = H	0·73	—	MeH_2PO_4	0·08	0·48

We are indebted to Dr. N. Sheppard for assistance with the interpretation of infrared absorption spectra, and to the Shell Chemical Company for a gift of "Phosdrin."

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