from the zones with acetone and the extracts were evaporated under reduced pressure to sirups. Zone 1 material crystallized from ethanol-methanol; yield 82 mg. of anhydrotrisaccharide nonaacetate I. Further crystallization from ethanol produced pure material; m.p. 256–256.5°, $[\alpha]_D^{25}$ +20° (*c* 0.3, chloroform), x-ray powder diffraction pattern[15]: 13.81s(3), 10.98m, 10.02m, 9.21w, 6.78vw, 6.08w, 5.44s, 4.86vs(1), 4.63w, 4.56w, 4.33s(2), 4.12vw, 3.74m, 3.59vw, 3.40w, 3.26vw, 3.14vw, 3.02vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7. Found: C, 49.93; H, 5.86; mol. wt. (Rast), 820.

Zone 2 material failed to crystallize. Zone 3 material crystallized from ethanol; yield 110 mg. of anhydrotrisaccharide nonaacetate II. Pure material was obtained upon recrystallization from ethanol; m.p. 209–209.5°, mixed m.p. with anhydrotrisaccharide nonaacetate I, 206–240°, $[\alpha]_D^{24}$ +26° (*c* 3, chloroform), x-ray powder diffraction pattern[15]: 11.67 vw, 10.62vs(1), 9.85m, 7.90m, 6.69s, 6.47vw, 6.15m, 5.99s, 5.68m, 5.32s(3), 4.93w, 4.64vs(2), 4.18s, 4.03s, 3.87m, 3.72w, 3.54w, 3.48m, 3.32m, 3.22vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7 Found: C, 49.94; H, 5.57; mol. wt. (Rast), 721.

Zones 4 and 5 produced material which crystallized from methanol-ethanol; yield 162 mg. of anhydrotrisaccharide nonaacetate III. Further crystallization from ethanol produced pure material, m.p. 230–230.5°, mixed m.p. with anhydrotrisaccharide nonaacetate I, 224–228°, mixed m.p. with anhydrotrisaccharide nonaacetate II, 204–215°, $[\alpha]_D^{24}$ −46.9° (*c* 0.8, chloroform), x-ray powder diffraction pattern[15]: 13.70vw, 10.68w, 10.08m. 9.36s, 8.54w, 6.47vw, 5.48-vs(2), 5.33vw, 5.04m, 4.75w, 4.44vs(3), 4.12vs(1), 3.80vw, 3.57m, 2.94w, 2.51vw, 2.40vw, 1.99vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7. Found: C, 50.38; H, 5.56; mol. wt. (Rast), 874.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION]

Configuration of the α-and β-Isomers of Methyl 3-(Dimethoxyphosphinyloxy)-crotonate (Phosdrin®)[1,2]

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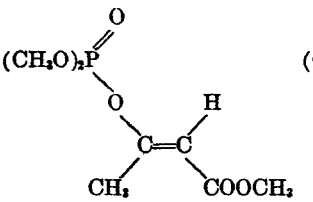
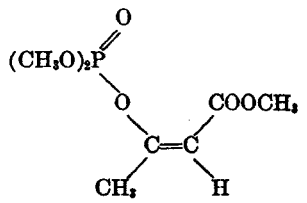
From proton NMR spectra and enzyme inhibition data the α- and β-isomers of methyl 3-(dimethoxyphosphinyloxy) crotonate (Phosdrin®) have been assigned the *cis*-crotonate and *trans*-crotonate configuration, respectively. The higher rate of inhibition of fly-brain cholinesterase by the α-isomer has been attributed to steric factors.

The assignment of configuration of the α- and β-isomers of methyl 3-(dimethoxyphosphinyloxy)-crotonate (hereafter referred to as Phosdrin®) is of interest because of the widely differing biological properties exhibited by the two forms. The technical isomeric mixture, prepared through the condensation of trimethyl phosphite and methyl 2-chloroacetoacetate, is being used extensively as a wide spectrum insecticide of short residual action. That technical Phosdrin consists primarily of *cis-trans* isomers was first demonstrated by Casida[3] who was able to separate an α- and β-form by column chromatography. He also found when either the α- or β-fractions were irradiated with ultraviolet light a mixture of approximately 30% α- and 70% β-isomers was obtained. On the assumption that ultraviolet irradiation should result in a predominance of the more stable isomer, the α-fraction was assigned the *trans*-crotonate (II) configuration and the β-fraction the *cis*-crotonate (I) configuration since II with two bulky groups on one side of the olefinic bond would be expected to be the thermodynamically less stable form.



I. *cis*-crotonate



II. *trans*-crotonate

It was also found that the α-form was considerably more active as a cholinesterase inhibitor, less stable to hydrolytic splitting of the P—O—C bond, and more toxic to mammals and insects.

To assign configurations upon results obtained from ultraviolet irradiation may lead to erroneous conclusions. In fact, ultraviolet irradiation of the stable isomer often results in the formation of the labile form and is often used as preparative method for the unstable isomer. For example, fumaric acid is transformed into maleic acid upon exposure to ultraviolet light.[4] Although, for thermodynamic reasons, I may be considered the more stable form in reactions involving the olefinic bond, the difference in reactivity of the P—O—C ester bond, particularly in the case of enzyme inactivation, may more likely be attributed to steric factors. For these reasons it was decided that further investigation was needed and this paper reports the application of NMR spectrometry and enzyme

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(1) Paper No. 1304, University of California Citrus Experiment Station.

(2) Supported in part by a research grant from the U. S. Public Health Service, No. RG-5433(C3).

(3) J. E. Casida, *Science*, 122, 597 (1955).

(4) R. Stoermer, *Ber.*, 42, 4865 (1909).

inhibition studies for the assignment of configuration of the Phosdrin isomers.

EXPERIMENTAL

Pure samples of α-, n_D^{25} 1.4426, and β-, n_D^{25} 1.4482, Phosdrin were obtained through the courtesy of the Shell Development Co., Modesto, Calif.

NMR spectra of the pure liquids were obtained with a Varian V-4302 high resolution NMR spectrometer with associated V-4102-SM magnet system equipped with a VK-3506 flux stabilizer. Hydrogen spectra of the pure liquid samples were obtained at 56.4 mc. and 24.3 mc. and phosphorus spectra at 24.3 mc. Proton spectra of degassed solutions of the isomers, 20% in carbon tetrachloride, were obtained using 5% tetramethylsilane (TMS) as an internal reference. The chemical shifts were determined by the audiofrequency side band technique. Phosphoric acid (85%) was used as an external reference for the phosphorus spectra and was contained in the annulus of a precision coaxial cell. All spectra were recorded with spinning samples and the chemical shift values reported are the average values of at least six determinations, reproducible to ±.5 c.p.s.

The rate of reaction of the Phosdrin isomers with fly-brain cholinesterase was determined manometrically by the method described by Aldridge,[5,6] using double-armed Warburg flasks. The reagents used in the manometric procedure have already been described.[7] The values for the bimolecular inhibition constants given in Table I represent two or more independent determinations.

RESULTS AND DISCUSSION

Both the H^1 and P^{31} NMR spectra of α- and β-Phosdrin showed distinct differences. The hydrogen spectra of the isomers at 56.4 mc. are given in Fig. 1, the five peaks labeled A–E. On the basis of the chemical shift values the following assignments can be made. The low intensity peak A must be attributed to the single olefinic proton. The resonance at high field, peak E, is due to the highly shielded protons in the $CH_3C{=}C$ moiety.

To determine which of the remaining peaks B, C, and D were due to the $(CH_3O)_2PO$ doublet and the singlet from the CH_3OCO moiety, the hydrogen spectra of the two isomers were measured also at 24.3 mc. Since the spin coupling constant, J(POCH) is independent of the applied field the separation of the doublet should be the same at the two frequencies. At 24.3 mc. peaks C and D of β-Phosdrin are superimposed and the separation between B and C (and D) was found to be 11.7 ± 0.5 c.p.s., the value of the spin coupling constant. This value is in agreement with the splitting in the phosphorus spectra of the two isomers in which the J(POCH) value of 11.3 ± 0.5 c.p.s. for α- and 11.1 ± 0.5 c.p.s. for β-Phosdrin was found. The H^1–P^{31} spin coupling constants in trimethyl phosphate has been reported as 11.19 ± 0.2 c.p.s.[8]

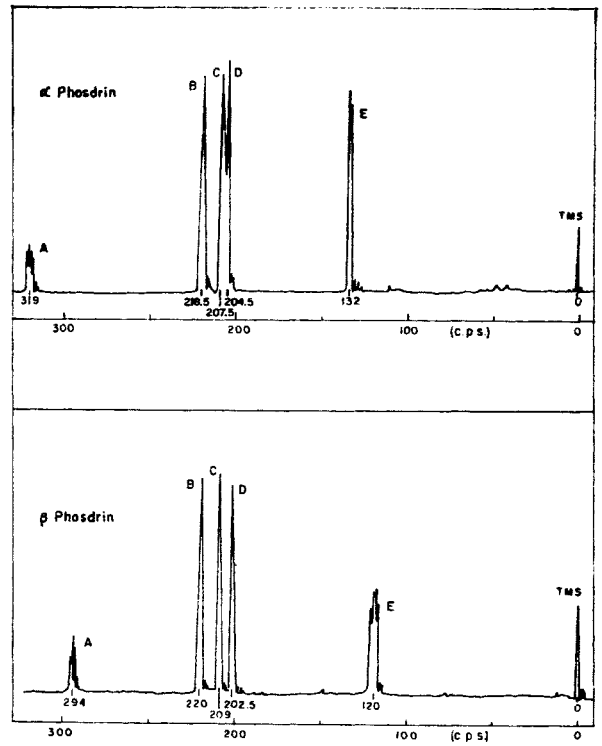

Fig. 1. Proton NMR spectra of α- and β-phosdrin at 56.4 mc., 20% solutions in carbon tetrachloride, internal reference tetramethylsilane

Peaks B and C with a separation of 11.0 ± 0.5 c.p.s. (56.4 mc.), therefore, are assigned as the $(CH_3O)_2PO$ doublet and peak D the singlet from the CH_3OCO resonance.

The assignment of the configuration of ethylenic geometrical isomers by NMR spectroscopy has been discussed by Jackman.[9,10] It has been shown that various β-substituents cause differential shielding of *cis-trans* olefinic protons and also *cis-trans* methyl groups, one of the most effective substituents being the carbomethoxy group. The chemical shifts of β-olefinic hydrogens in methyl esters of α,β-unsaturated monocarboxylic acids, are consistently lower in the isomer in which the olefinic hydrogen is *cis* to the carbomethoxy moiety, being deshielded by 0.5 to 0.9 p.p.m. Similarly, β-methyl groups *cis* to carbomethoxy give chemical shift values 0.20 to 0.30 p.p.m. less than when they are *trans*.[9,11] Others[12] have also reported similar differential shielding of olefinic and C-methyl protons in *cis* and *trans* isomers.

The spectra of α- and β-Phosdrin, Fig. 1, show that both C-methyl proton and olefinic proton resonances occur at lower field in α- than in β-

(5) W. N. Aldridge and A. N. Davison, *Biochem. J.*, 51, 62 (1952).
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(7) T. R. Fukuto and R. L. Metcalf, *J. Am. Chem. Soc.*, 81, 372 (1959).
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(11) R. R. Frazer, *Can. J. Chem.*, 38, 549 (1960).
(12) S. Fujiwara, H. Shimizu, Y. Arata, and S. Akahori, *Bull. Chem. Soc. Japan*, 33, 428 (1960).

Phosdrin. In structure I (*cis*-crotonate configuration) the C-methyl is *cis* to the carbomethoxy group. The difference in chemical shift of the C-methyl protons of the two isomers is 12 c.p.s. or 0.21 p.p.m. The magnitude of this difference is in agreement with the values reported by Jackman[9] and by Frazer.[11] On this basis α-Phosdrin is assigned structure I and β-Phosdrin structure II.

It is noteworthy that the olefinic proton resonance also occurs at lower field in α- than in β-Phosdrin. The long range shielding by the dialkylphosphoryloxy moiety has not been studied previously. Jackman[9,10] has reported, however, that the structurally analogous acetoxy moiety does not produce differential shielding of either olefinic or β-methyl protons and, therefore, direct correlation cannot be made.

The phosphorus spectra of α- and β-Phosdrin at 24.3 mc. are very similar. In each a seven-fold peak was observed with the intensities expected for the phosphorus resonance split by six equivalent neighboring hydrogens. The chemical shift, with an external reference from 85% phosphoric acid, for the P^{31} resonance in the pure liquid samples was slightly lower in α-Phosdrin (5.98 p.p.m.) than in β-Phosdrin (6.40 p.p.m.). It is possible that the different values may be due to a difference in the degree of molecular association in the isomers. The isomers were not examined in dilute solutions because of the weak phosphorus signal, however.

The striking difference in the phosphorus spectra of the isomers is that each of the seven peaks in α-Phosdrin is split reproducibly and uniformly into a doublet with a separation of the order of 1.5 c.p.s. while the peaks in β-Phosdrin could not be resolved further. The additional splitting of the phosphorus spectrum in the α-isomer is presumably due to spin coupling between P^{31} and the vinyl hydrogen. In molecules of fixed configurations the values of coupling constants between two nuclei can differ considerably depending on their spatial arrangement. The close proximity of the vinyl hydrogen to the phosphoryl oxygen in the α-isomer (structure I) leads one to speculate the possibility of weak intramolecular hydrogen bonding occurring here. However, infrared spectra gave no evidence of hydrogen bonding by the vinyl hydrogen in either isomer.

The rate of inhibition of housefly-head cholinesterase by α- and β-Phosdrin was determined at three temperatures. The rate constants (K_e) for the reaction with the enzyme (En-H) shown

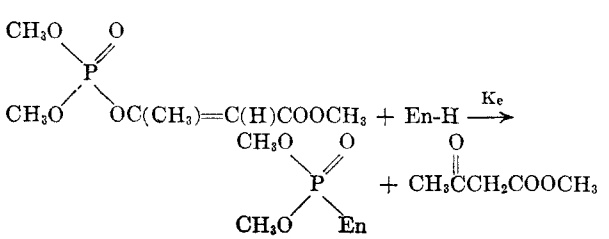

TABLE I

BIMOLECULAR RATE CONSTANTS (K_e) FOR THE INHIBITION OF FLY-BRAIN CHOLINESTERASE BY α- AND β-PHOSDRIN

T	K_e (l. moles^{-1} min.$^{-1}$) α-Phosdrin	β-Phosdrin
29.0	3.9×10^4	8.7×10^3
33.0	1.0×10^5	1.4×10^4
37.5	2.8×10^5	2.2×10^4

below are given in Table I. Activation energies, ΔE* for the inhibition reaction were obtained from plots of log K_e *vs.* 1/T at the three given temperatures. The activation energy of the reaction of α-Phosdrin with fly-head cholinesterase (41.6 kcal.) is much higher than that of β-Phosdrin (19.9 kcal.), indicating that the ester linkage in the β-isomer is more reactive. The higher rate of inhibition by the α- over the β-isomer must then be attributed to its greater PZ factor or entropy of activation, ΔS*. The values for log PZ and ΔS* for α-Phosdrin are 32.9 and 90.3 e.u., respectively, compared to 14.7 and 6.6 e.u. for β-Phosdrin. These values indicate that steric factors strongly influence the reactivity of the isomers with the cholinesterase enzyme.

The importance of steric factors in reactions involving a nucleophile and an organophosphorus ester has been demonstrated by others. Hudson and Keay[13] have shown that in the reaction between hydroxide ion and diisopropyl methylphosphonate and methylphosphonodithiolate, the activation energy was lower and the PZ factor was higher for the thiol ester. The increase in the PZ factor was attributed to the greater covalent radius of sulfur than of oxygen, thus removing the isopropyl group farther from the phosphorus atom in the thiol ester and causing less strain in the formation of the transition state. Thain[14] also has shown that similar considerations apply in the alkaline hydrolysis of triethyl phosphorotrithiolate and triethyl phosphate. If one considers the cholinesterase enzyme as a large bulky molecule containing at least one nucleophilic center and the reaction between Phosdrin and the enzyme as a single bimolecular reaction involving the nucleophilic center and the phosphorus atom in Phosdrin[5,15], the isomer in which the carbomethoxy moiety is *trans* to the phosphoryloxy moiety would be expected to show the higher degree of inhibition. In the isomer in which these groups are *cis* and, therefore, are in closer proximity to each other, the carbomethoxy moiety might be expected to interfere sterically with the nucleophilic attack of the bulky cholinesterase

(13) R. F. Hudson and L. Keay, *J. Chem. Soc.*, 3269 (1956).

(14) E. M. Thain, *J. Chem. Soc.*, 4694 (1957).

(15) T. R. Fukuto, in *Advances in Pest Control Research*, Vol. I, Interscience Publishers, Inc., New York, N. Y., 1957, 147–192.

enzyme on the phosphorus atom. In view of these findings, the α-isomer which shows the higher degree of inhibition and the higher entropy of activation is assigned the structure I and the β-isomer structure II. The conclusions expressed here are in agreement with results obtained from NMR spectrometric data.

RIVERSIDE, CALIF.

[A CONTRIBUTION FROM THE CHEMICAL RESEARCH DEPARTMENT, CENTRAL RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Phosphorus-Containing Monomers. I. The Synthesis of Vinyl Phosphines, Oxides, Sulfides, and Phosphonium Compounds

ROBERT RABINOWITZ AND JOSEPH PELLON

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Synthetic routes to a series of new vinyl phosphorus, nonester compounds are presented. Specifically the preparations of diphenylvinylphosphine, diphenylvinylphosphine oxide, diphenylvinylphosphine sulfide, diphenylmethylvinylphosphonium iodide, and diisobutylvinylphosphine oxide are described. The paths are general and suggest many analogous compounds. Preliminary polymerization work indicates that free radical polymerization of these monomers is difficult. They enter into copolymers; however, in a much smaller ratio than in the feed.

Very little information exists in the literature concerning vinyl phosphines and their derivatives.[1-4a] Two recent publications by Kabachnik and co-workers[5,6] describe convenient routes to a series of vinyl phosphines and vinyl phosphine oxides. In none of the above articles was any reference to polymerization or copolymerization uncovered. Furthermore, a search of the literature revealed no mention of vinyl phosphine sulfides.

It was the object of this work to prepare representative vinyl phosphines, oxides, sulfides, and phosphonium compounds in order to compare them as to polymerization reactivity with existing vinyl monomers.

Diisobutylvinylphosphine oxide and diphenylvinylphosphine oxide were synthesized by a combination of the following reactions:

$$R_2PCl + HOC_2H_5 \xrightarrow{(C_2H_5)_3N} R_2POC_2H_5 \quad (1)$$

$$R_2POC_2H_5 + BrCH_2CH_2Br \longrightarrow R_2P(O)CH_2CH_2Br \quad (2)$$

$$R_2P(O)CH_2CH_2Br + (C_2H_5)_3N \longrightarrow (C_2H_5)_3N\cdot HBr + R_2P(O)CH{=}CH_2 \quad (3)$$

Yields, without any attempts at optimizing, were 62% in the diphenyl case and 21% for the dibutyl, both based on R_2PCl. In the diphenyl synthesis, some 1,2-ethanebis(diphenylphosphine oxide) was isolated. This is the product of the reaction of ethyl diphenylphosphinite with 2-bromoethyldiphenylphosphine oxide and can be kept at a minimum by use of a large excess of 1,2-dibromoethane.

The reaction of vinylmagnesium chloride with diphenylchlorophosphine was used to prepare diphenylvinylphosphine:

$$CH_2{=}CHMgCl + (C_6H_5)_2PCl \longrightarrow (C_6H_5)_2PCH{=}CH_2$$

In several experiments the yields varied from 15–40%. A variable amount of 1,2-ethanebis(diphenylphosphine oxide) was obtained from the pot residues after the distillation.

The diphenylvinylphosphine was used to prepare the corresponding sulfide and methylphosphonium iodide. It was also oxidized to the phosphine oxide, and this product was identical to the material prepared by dehydrobrominating 2-bromoethyldiphenylphosphine oxide.

$$(C_6H_5)_2PCH{=}CH_2 \xrightarrow{t\text{-}C_4H_9OOH} (C_6H_5)_2P(O)CH{=}CH_2 \quad (72\%)$$

$$(C_6H_5)_2PCH{=}CH_2 \xrightarrow{S} (C_6H_5)_2P(S)CH{=}CH_2 \quad (86\%)$$

$$(C_6H_5)_2PCH{=}CH_2 \xrightarrow{CH_3I} [(C_6H_5)_2\overset{+}{P}(CH_3)CH{=}CH_2]I^- \quad (80\%)$$

The literature reveals that polymerization studies on vinylphosphorus compounds have been limited to esters of phosphorus acids. These compounds have a rather low tendency to homo- and copolymerize under free-radical conditions.[7-12]

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(8) R. M. Pike and R. A. Cohen, *J. Org. Chem.*, **44**, 533 (1960).
(9) C. S. Marvel and J. C. Wright, *J. Polymer Sci.*, **8**, 255 (1952).
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