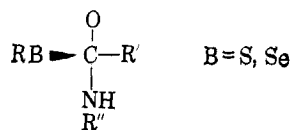tion of sulfur or selenium, respectively, one should expect both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values to differ in comparing thio and seleno esters. Since, however, $\Delta H^{\ddagger}$ values for the aminolysis of I and II are quite similar, while the $\Delta S^{\ddagger}$ value of the selenoacyl compound is considerably less negative than that of its thioacyl analog, it seems more reasonable to postulate that the differences in reactivity and entropies of activation are related to leaving tendencies of the selenomercaptide as compared to that of the mercaptide group from the tetrahedral transition state

$$RB \longrightarrow \underset{\underset{R''}{\overset{\displaystyle \overset{O}{\|}}{\underset{|}{\overset{|}{C}}}}{C} - R' \qquad B = S, Se$$

This postulate is reinforced by the observation that the addition of lithium chloride accelerates the aminolysis of the sulfur compound II more than that of its seleno analog I. In the latter case, the carbon–selenium bond is presumably polarized highly even in the absence of salt, while the carbon–sulfur bond, which in the transition state of the analogous thiolester is polarized to a lesser degree, is more susceptible to an increase in polarization induced by lithium chloride addition, which in turn alters the entropy of activation.

Phosphorylations and Phosphonations of Glycerol by Recoil Atoms[1,2]

By TATSUHIKO YAGI, S. A. EL-KINAWY,[3] AND A. A. BENSON[4]

The labeled products of neutron activation of phosphorus oxyacids in glycerol were studied by paper chromatographic techniques. Irradiation of mixtures of phosphoric acid and glycerol produced P^{32}-labeled H_3PO_3, H_3PO_2, glycerophosphorous acid, 3-phosphinicopropanediol-1,2, and 1-phosphonoglycerol. Other C_2- and C_3-organic phosphorus compounds were suggested. Neither $H_3P^{32}O_4$ nor its esters was produced.

Introduction

Neutron irradiation of phosphorus oxyacids has, so far, suggested that most of the P^{32} *in statu nascendi* reverts to the oxidation level of the parent compounds. Thus, neutron activation of crystalline or aqueous ortho- and condensed phosphoric acids or their salts yielded mainly phosphoric acids-P^{32} or their salts,[5,6] the only change, if any, being that of the degree of polymerization.[6] Formation of more than trace amounts of reduced compounds such as phosphorous, hypophosphoric, or hypophosphorous acids was also repeatedly reported.[7] Lindner and Harbottle[8a] reported the neu-

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(2) Neutron irradiations were provided by the staffs of the Nuclear Reactor Facility of the Pennsylvania State University and of the Engineering Nuclear Reactor, University of California, Los Angeles. We are indebted to Professor E. V. Jensen for a sample of 2,3-dihydroxypropylphosphonic acid (phosphonopropanediol).

(3) An International Atomic Energy Agency Fellowship to S. A. El-K. is gratefully acknowledged.

(4) Department of Marine Biology, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, Calif.

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tron activation products of various phosphate salts as a mixture of oxyanions of phosphorus containing one, two, or three phosphorus atoms, in which distribution of P^{32} was critically dependent upon the irradiation conditions. Recent work by Campbell, *et al.*,[8b] defined the products of neutron activation of tributyl phosphate as di- and monobutyl phosphates, unchanged tributyl phosphate, and phosphoric and phosphorous acids. Studies of the radioactive products from PCl_3 reported formation of $P^{32}Cl_3$ in yields as high as 88% of the total radioactivity induced.[9]

A P^{32} nucleus, recoiling from the (n,γ) capture process, has more than enough energy to break its covalent chemical bonds. Except in the case of symmetrical distribution of γ-recoil momenta, a "hot atom" will be produced which can return to its original state only by proper recombination with ions and radicals formed in its path. When inorganic phosphorus oxyacids are activated in the presence of organic substances, combination of the recoiling nucleus and organic radicals may occur. Although labeling of organic molecules by recoiling atoms such as C^{14} or halogens has been extensively investigated,[10,11] syntheses of organic phosphorus compounds by such methods have been relatively unsuccessful.[12]

The present paper reports phosphorylations and phosphonations of the biologically important compound, glycerol, as a result of thermal neutron capture in mixtures of glycerol and phosphoric acid. Radiochromatographic techniques were applied in the elucidation of the structures of the products. Recognition

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of these products provides information on the mechanism of hot-atom reactions involved in their formation. The radioactive products include glycerophosphorous acid-P^{32}, which can be converted to glycerophosphoric acid-P^{32}, a metabolic intermediate. In view of the usefulness of labeled organic phosphorus compounds in biochemical studies, this method could provide important materials by simple procedures.

Experimental

Neutron Irradiations.—The mixtures of glycerol and phosphoric acid or other phosphorus oxyacids to be irradiated were sealed in polyethylene tubes (inside diameter, 1 mm.). These tubes, contained in a larger polyethylene tube, were irradiated by thermal neutrons (10^{12} neutrons/cm.²/sec., 25°, about equal fast neutron flux, 15×10^6 r./hr. γ-radiation, in the 200 kw. swimming pool-type reactor at the Pennsylvania State University; 2×10^{11} neutrons/cm.²/sec., 40°, in the Argonaut 10 kw. reactor at the University of California, Los Angeles; or in the thermal column of the CP-5 reactor, Argonne National Laboratory, at 6×10^{12} thermal neutrons/cm.²/sec. and 2×10^9 epithermal neutrons/cm²./sec.).

γ-Irradiations.—Solutions of radiophosphate with and without H_3PO_4 carrier in dry glycerol were sealed in Pyrex ampoules and irradiated for increasing time periods with 50 kv. X-rays and Co^{60} γ-radiation. The maximum Co^{60} γ-dose, 7×10^8 rads, which was several fold greater than those obtained during the neutron activations, 6×10^7 rads, produced appreciable decomposition of the glycerol. γ-Doses comparable to 6×10^7 rads produced no detectable P^{32} compounds from the glycerol.

Separation of the Products.—The irradiated mixture after being stored several days for decay of radioactive impurities contained only P^{32} as a radioactive nuclide. The products were separated by two-dimensional paper chromatography on Whatman No. 4 filter paper using phenol–water for the x-direction and butanol–propionic acid–water for the y-direction[13] for small scale separations. R_f values are reported on the basis of solvent movement equal to 100. Larger scale separations involved unidimensional (phenol–water) chromatography on Whatman No. 3 MM paper.

Detection of Radioactive Compounds on the Paper Chromatograms.—Radiograms were prepared using 14×17 in. Eastman Kodak single coated blue-sensitive X-ray film. Radioactivities in chromatographically separated compounds were measured using 1.75 in. diameter G.-M. tubes (Radiation Detector, 1001 T, Lionel Electronic Laboratories, Inc., Brooklyn, N. Y.).

Detection of Nonradioactive Compounds on the Paper Chromatograms.—The spray reagent for phosphates by Hanes and Isherwood[14] was used for detection of phosphoric acid, phosphorous acid, their esters, and hypophosphorous acid, all of which produced the characteristic blue color by the phosphate spray reagent. 3-Phosphonopropanediol-1,2,[15] which did not give a color by the phosphate spray reagent, was detected by the periodate–Schiff spray reagent for vicinal diols.[16]

Materials.—Crystalline phosphorous acid was prepared by mixing 100 g. of 85% phosphoric acid and 40 g. of P_2O_5 followed by crystallization and filtration in the cold. Glycerophosphorous acid and its barium salt were prepared by the method of Carré.[17] Phosphoric esters were obtained commercially (California Biochemicals, Inc.; Los Angeles Calif.).

Identification of Neutron Activation Products. Phosphorous Acid.—The product of R_f 27, 36 was resistant to the treatment with 3 N HCl at 100° for 3 hr., but was readily oxidized to produce H_3PO_4 by aqueous Br_2 solution or silver ion. It cochromatographed precisely with authentic H_3PO_3. It had electrophoretic mobilities identical with those of authentic H_3PO_3 at pH 2 and at pH 6. The radioactive yield was about 19% of the total radioactivity induced.

Hypophosphorous Acid.—The product of R_f 49, 41 was partially converted to H_3PO_3 after treatment with 3 N HCl at 100° for 3 hr., or treatment with SO_2. It was readily oxidized to H_3PO_4 by treatment with aqueous Br_2. It was finally identified by cochromatography and by identical electrophoretic mobilities with those of authentic H_3PO_3. Its radioactive yield was 11%.

Glycerophosphorous Acid.—The product of R_f 45, 29 yielded H_3PO_3 upon treatment with 0.1 N HCl at 100° for 30 min. It cochromatographed precisely with synthetic glycerophosphorous acid. The radioactive yield was 8%.

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(17) P. Carré, *Compt. rend.*, **133**, 882 (1901).

The radioactivity of this component was eluted from the paper with water and oxidized with Br_2 water at pH 8 in a sealed tube at 5°. After 24 hr. the Br_2 was removed by extraction with CCl_4 and the aqueous solution of the product was treated with Dowex 50 (H^+) and cochromatographed with authentic α-glycerophosphoric acid (sodium salt obtained from the Sigma Chemical Co. was treated with Dowex 50 (H^+) and applied to paper for chromatography). The major radioactive component, containing 78% of the total radioactivity, cochromatographed precisely with the authentic α-glycerophosphoric acid, while another lesser component, containing 14% of the total activity, was identified as the β-isomer; the third component, containing 8% of the total, was the unchanged glycerophosphorous acid.

Glycerophosphorous acid, an ester of H_3PO_3, is, like other phosphite esters,[18] very resistant to oxidative reagents. To produce glycerophosphoric acid, controlled Br_2 oxidation was found to be the most effective. Treatment with 5% H_2O_2 and ultraviolet resulted in 100% yield of H_3PO_4 in 30 min. at room temperature. Without ultraviolet irradiation, the oxidation was incomplete at 100°. Oxygen in the presence of palladium black was ineffective; Silver ion, at pH 8, was reduced at room temperature by glycerophosphorous acid[17]; the organic part of the product was shown to be glycerophosphoric acid by cochromatography.

The oxidized product, *i.e.*, glycerophosphoric acid, was attacked by "Polidase S" (Schwarz BioResearch, Inc.), a phosphatase preparation, orthophosphate being the product. The original glycerophosphorus acid was resistant under the same conditions.

Phosphorous acid-P^{32} was mixed with a glycerol–phosphoric acid mixture, or glycerol–K_2HPO_4 mixture, and kept for 2 weeks at room temperature. Only a small amount of glycerophosphorous acid was produced from the former, none from the latter. These facts suggest that esterification was caused by the recoil phosphorus atom and not by the usual chemical equilibrium established between glycerol and $H_3P^{32}O_3$.

3-Phosphinicopropanediol-1,2.—The product of R_f 44, 34 which was resistant to the treatment with 2 N HCl at 100° for *30 min.* was buffered at pH 8 with $KHCO_3$ and treated with Br_2 at 5° for 24 hr. in a sealed tube. The remaining Br_2 was extracted and the aqueous solution was chromatographed. The product was further identified as 3-phosphonopropanediol-1,2 by cochromatography. The original compound, before Br_2 oxidation, had almost the same electrophoretic mobility as that of glycerophosphorous acid at pH 6. Since 3-phosphinopropanediol-1,2, which will give 3-phosphonopropanediol-1,2 by Br_2 treatment, must be an almost neutral compound, 3-phosphinicopropanediol-1,2 is the only possibility for this product. The radioactive yield was 3%.

1-Phosphonoglycerol.—The compound of R_f 30, 26, which was resistant to acid or Br_2 treatment, was considered to be 1-phosphonoglycerol or a mixture of the two diastereoisomers, as a result of the following observations. The striking similarity of the paper chromatographic behavior of this unknown to those of α-glycerophosphoric acid and 3-phosphonopropanediol-1,2 suggested the structural similarity of these three compounds. The unknown and 3-phosphonopropanediol-1,2 were treated with $NaIO_4$ solution overnight in the cold. The former gave H_3PO_4 and H_3PO_3 while the latter did not yield either of them but a compound which behaved like phosphonoacetaldehyde. This fact suggests that the phosphonic acid group of the unknown is on one of the carbons bearing vicinal hydroxyls. The unknown and the 3-phosphonopropanediol-1,2 were acetylated with acetic anhydride in pyridine (1:2 by volume) at 100° for 1 hr. The products were chromatographed in the butanol–propionic acid–water solvent. The shift of R_f value caused by acetylation (from 26 to 71) was almost 150% as much as that observed for the identified 3-phosphonopropanediol-1,2 (from 29 to 52). This fact indicates that the unknown had three hydroxyls instead of two. Since 1-phosphonoglycerol has not yet been studied or prepared, cochromatography was not possible. The radioactive yield of this compound was 4%.

"C_2"-Components.—The compounds of R_f 40, 35 and R_f 58, 52 were studied by comparing their electrophoretic mobilities with that of 2-hydroxyethylphosphorous acid-P^{32} which had been produced by the neutron activation of a mixture of ethylene glycol and H_3PO_4 and was proved by cochromatography with the authentic 2-hydroxyethylphosphorous acid synthesized by the method of Carré.[17] The electrophoretic mobilities of these three were nearly the same, suggesting the unknowns to be some C_2-organophosphorus acids.

Activation of Organic Phosphoric Esters.—Aqueous and crystalline sodium α-glycerophosphate, crystalline salts of adenylic, guanylic, cytidylic, and uridylic acids, and adenosine triphosphate sealed in polyethylene capillaries were activated with 3×10^{16} neutrons/cm.². Radiograms of the separated products revealed phosphorous and hypophosphorous acids as the major products. No significant differences were observed when the activation was done in the purely thermal neutron flux or when
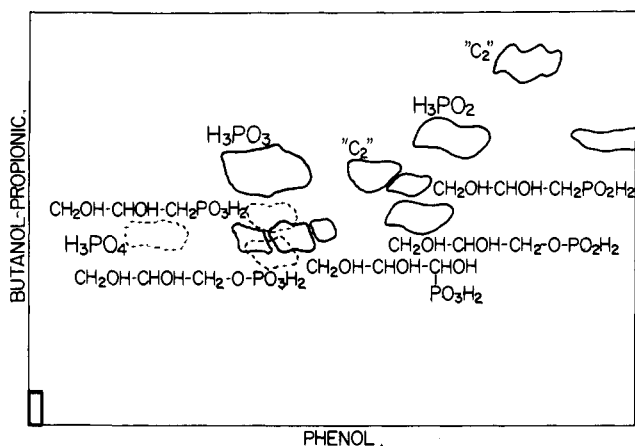
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Fig. 1.—Relative chromatographic positions of activation products and their derivatives. Chromatograms were developed first in phenol–water and second in butanol–propionic acid–water solvents.

the samples were sealed in quartz under nitrogen. Less than 1% of the induced activity was observed in the original substances.

Results

A schematic radioautogram of the products after 40 hr. irradiation in a flux of 2×10^{11} thermal neutrons/cm.²/sec. of a mixture of glycerol and phosphoric acid (molar ratio 2:1, water-free) is shown in Fig. 1. Chromatographic coordinates and radiochemical yields are given in Table I.

TABLE I

PRODUCTS OF NEUTRON ACTIVATION OF GLYCEROL–PHOSPHORIC ACID MIXTURES AND THEIR DERIVATIVES

Compound	R_f[a,b]	Electrophoretic mobility at pH 6	Radioactive yield, %
H₃PO₄	14, 27	100 (std.)	0
H₃PO₃	27, 36	122	19 ± 5
H₃PO₂	49, 41	129	11 ± 3
α-Glycerophosphoric acid	28, 24	94	0
1-Phosphonoglycerol (erythro- or	30, 26	81	4 ± 1
threo-)	26, 26	81	2 ± 1
3-Phosphonopropanediole-1,2	28, 29	91	0
Glycerophosphorous acid	45, 29	81	9 ± 2
3-Phosphinicopropanediol-1,2	44, 34	81	3 ± 1
"C₂"	40, 35	101	3 ± 1
"C₂"	58, 52	111	5 ± 1
Remainder[c]	60–90[a]		Over 40

[a] R_f in the phenol–water solvent, in %. [b] R_f in the butanol–propionic acid–water solvent. [c] The remainder, not sharply separated by paper chromatography, was water soluble. It included both neutral and anionic components. It is suspected that the latter are C_1 and C_2 derivatives and that the former are substituted phosphines.

The chromatogram patterns were quite reproducible regardless of the neutron dose (15 hr. and 40 hr. at 2×10^{11} n./cm.²/sec., or 3 hr. and 8 hr. at 10^{12} n./cm.²/sec.). Similar chromatograms were obtained from the products of irradiation of mixtures of glycerol–phosphoric acid–water (3:1:2),[19] glycerol–phosphorous acid (3:1), glycerol–hypophosphorous acid–water (3:1:4),[18] or glycerol–K₂HPO₄(9:1). Of the P³²-labeled products, phosphorous acid, hypophosphorous acid, glycerophosphorous acid, and 3-phosphinicopropanediol-1,2 were identified using the reference compounds described in the preceding section; formation of 1-phosphonoglycerol and some C₂ compounds bearing P³² was suggested. Neither radioactive phosphoric

(19) Calculated from the water contents of the materials employed.

acid nor glycerophosphoric acid was detected in any case (cf. Table I).

Activation of 85% phosphoric acid gave a 100% yield of $H_3P^{32}O_4$; of crystalline phosphorous acid gave $H_3P^{32}O_3$ and $H_3P^{32}O_2$ in a ratio of 20:1; and of 50% aqueous hypophosphorous acid gave $H_3P^{32}O_2$ (60% yield), $H_2P^{32}O_3$ (40% yield), and $H_3P^{32}O_4$ (trace). These yields, obtained in polyethylene capillary tubing, are in accord with those of other workers.[5]

The products of γ-irradiation of glycerol–$H_3P^{32}O_4$ mixtures were examined in order to ascertain that the above reactions could be attributed to recoil-induced reactions. The amounts examined were large enough to allow detection of 0.05% conversion to new products. With the highest γ-dose there was appreciable discoloration of the glycerol with the production of detectable amounts of a wide spectrum of organic phosphate compounds. Only one product could be recognized as a discrete chromatographic spot. It was shown to be glycerophosphate by electrophoresis and by cochromatography with α-glycerophosphate. It was hydrolyzed by the action of phosphatase to yield orthophosphate. Formation of this compound would be anticipated as a result of direct esterification. Neutron activation, on the other hand, produced a simple group of readily separable products.

Neutron activation of crystalline and aqueous glycerophosphate salts and other phosphoric monoester salts, purine and pyrimidine nucleotides, adenosine-5'-triphosphate, etc., produced good yields of inorganic phosphorous and hypophosphorous acids. In no case were the starting materials labeled with P^{32}. The ratios of phosphorous to hypophosphorous acids produced varied with the nature of the organic starting material irradiated.

Discussion

It is a general opinion that the nuclide in statu nascendi produced by neutron activation is a positive ion deprived of some of its orbital electrons and bears, in some cases, other atoms combined in the parent compound. The "hot atom," thus produced, will revert to a stable oxidation state, having exhausted its recoil energy by reacting with the medium, its parent compound, or other species available.

In case of the neutron irradiation of H_3PO_4 (or its salts), alone or in aqueous medium, the most stable oxidation state of the phosphorus atom must be that of phosphoric acid. The results of many workers[5–8] who observed retention of P^{32} in phosphoric acids can be considered as examples of this case. Sato and Strain[5e] concluded that no bond cleavage occurred upon activation of crystalline phosphate salts.

The neutron irradiation of phosphoric acid or its salts in a reduced medium like glycerol induces different reactions. The "hot" P^{32} atom, after dissipating its recoil energy by reacting with the organic medium, will find itself in a state less oxidized than it would in water. The P^{32} compounds identified in the present paper appear to be reaction products of glycerol and "hot" phosphite and hypophosphite radicals. Thus, formation of 3-phosphinicopropanediol-1,2 can be explained as a result of the reaction: $CH_2OHCHOH$-$CH_2OH + "H_3P^{32}O_2" = CH_2OHCHOHCH_2P^{32}O_2H_2 + H_2O$. 1-Phosphonoglycerol may be formed by the reaction: $CH_2OHCHOHCHO + H_3P^{32}O_3 = CH_2OH$-$CHOHCHOHP^{32}O_3H_2$, glyceraldehyde being a product of oxidation of glycerol by oxidizing fragments formed in the activation process.

Recognition of C_2 compounds among the products suggests that the P^{32} atoms, secondary products, or neutron or γ-radiations caused some rupture of C–C

bonds. The resulting C_2 radicals proved to be effective in capturing the "hot" P^{32} atoms for formation of chemically stable C_2–P^{32} derivatives.

The possibility that the organic P^{32} compounds described in this paper are the chemical reaction products of P^{32}-oxyacids and glycerol was eliminated by the experimental observation that a mixture of glycerol and $H_3P^{32}O_3$ at room temperature yielded very little glycerophosphorous acid; no other compounds were observed. γ-Irradiation products of a $H_3P^{32}O_4$–glycerol mixture contained unchanged $H_3P^{32}O_4$, some glycerophosphoric acid-P^{32} (probably in an equilibrium concentration), and other unrecognizable substances which were not identical with the neutron activation products. None of the neutron activation products was detectable by paper chromatographic spray reagents, whereas the starting materials, $H_3P^{31}O_4$ and glycerol, were easily detected by these reagents. Thus the organic products are considered to be products of "hot atom" reactions of P^{32} with the medium.

Nascent P^{31} atoms from related nuclear processes

have recoil energies comparable to those of P^{32} atoms. The products of their reactions would be organophosphorus compounds of P^{31}. One might consider the sources of the phosphate esters which could accumulate in extra-terrestrial[20] environments during chemical evolution[21] and prior to origin of life. Some of these might result from nuclear processes on silicates to yield organophosphorus derivatives which could be oxidized by suitable minerals. The surprisingly rapid metabolism of phosphonic acids by primitive microorganisms[22] and the occurrence of a phosphonic acid (2-aminoethanephosphonic acid)[23] in nature suggest synthetic applications and possible relationships of reactions revealed in this study.

(20) It is recognized that terrestial conditions did not include an appreciable neutron flux during the era of chemical evolution.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE STATE UNIVERSITY OF NEW YORK AT STONY BROOK, N. Y.]

Cyclic Saturated Oxyphosphoranes and their Hydrolysis to Cyclic Phosphate Esters. The Diastereomeric 2:1 Biacetyl–Trimethyl Phosphite Adducts[1]

BY FAUSTO RAMIREZ,[2] N. RAMANATHAN, AND N. B. DESAI

Trimethyl phosphite reacts rapidly with the α-diketone biacetyl to form exclusively a *1:1 adduct* having a cyclic unsaturated oxyphosphorane structure with the new 1,3-dioxaphospholene ring system. The 1:1 adduct reacts slowly with more biacetyl to form two diastereomeric forms of a *2:1 adduct* having a cyclic saturated oxyphosphorane structure with the new 1,3-dioxaphospholane ring system. The pentavalent-phosphorus structures are based on: solubility in pentane, P^{31} n.m.r., H^1 n.m.r., and infrared spectra, and on the results of hydrolysis in aprotic solvents. The diastereomeric 2:1 adducts undergo a very rapid and exothermic reaction with one mole equivalent of water and yield diastereomeric cyclic phosphotriesters. A stereomutation *at phosphorus* was observed in the *meso*-cyclic phosphotriester.

We have described new organic compounds of phosphorus in which the phosphorus atom appears to be covalently bound to five oxygen atoms.[1c] Typical of these substances is the biacetyl–trimethyl phosphite *1:1 adduct* I. This is formed rapidly and exothermally when the diketone biacetyl is added to one mole equivalent of the phosphite ester at 10°. The properties of this pentane-soluble adduct are in better agreement with a *cyclic unsaturated oxyphosphorane* structure, I, derived from the 1,3-dioxaphospholene ring system, than with ionic structures.[1c]
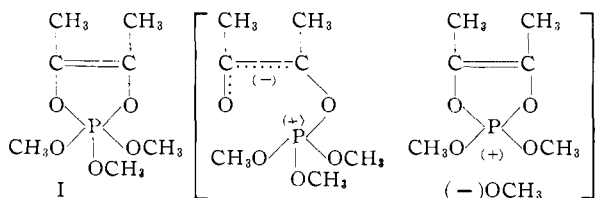
Experiments in which the biacetyl/phosphite mole ratio was greater than one, and in which the reaction was allowed to proceed for several hours, yielded a new substance. This observation was investigated in greater detail and the results are described in this paper.[3]

Results

The biacetyl–trimethyl phosphite *1:1 adduct* I reacted slowly with a second mole of biacetyl yielding a colorless, distillable oil. Analysis of this material agreed with the formula $C_{11}H_{21}O_7P$, which corresponds to a biacetyl–trimethyl phosphite *2:1 adduct*. The data discussed below show that the oil is a mixture of the two possible diastereomeric forms of a *cyclic saturated oxyphosphorane*, IIa and IIb, a new type of organic compound with pentavalent phosphorus, derived from the 1,3-dioxaphospholane ring system. In one of the diastereomers, IIa, the two acetyl groups are *cis* to each other; in the other, IIb, they are *trans* to each other. The isomers are formed in the approximate proportion of 4:1, respectively.
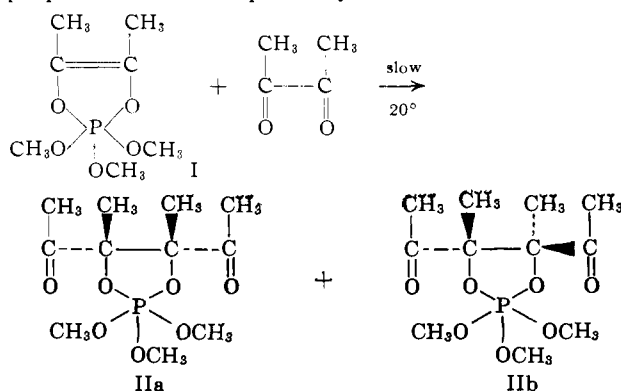

Table I (expt. 3 and 1) shows that an excess of biacetyl leads to higher yields of 2:1 adducts, IIa +