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Nuclear Spin Coupling and Conformation of the P–O–C–H Group

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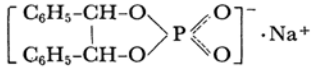
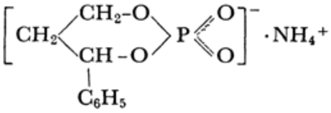
Proton magnetic resonance spectra have been observed of eight phosphoric acid esters in heavy water solutions. Among these esters, 1-phenyltrimethylene phosphate (1-phenyl-1, 3-propanediol cyclic phosphate) is a new compound. It was shown that the six-membered ring in this compound is fixed in a conformation at room temperature. On assuming that it is a chair form, 1-proton should be on the axial bond and this should be in a gauche relation with respect to the O-P bond around the C_1-O_1 bond. The proton-phosphorus coupling constant, J_{HP}, in this H-C_1-O_1-P system was found to be 1.5 cps. The J_{HP} in the CH_3OP system was found to be 10.3—10.5 cps, and, therefore, J_{HP} in the trans form is considered to be much greater ($\sim 28._2$ cps) than that in the gauche form. The J_{HP} in the RCH_2OP systems were found to be 6.3—7.0 cps, and, therefore, C–R bond in these systems are considered to have a greater chance of taking trans position with respect to the O–P bond around the C–O bond than the chance of taking gauche position.

The purpose of this work is to obtain a basis for determining molecular conformations of mono- and polynucleotides *in aqueous solutions* by means of the nuclear magnetic resonance. Nucleic acids involve the H-C-O-P group, and the P···H spin-coupling constant J_{HP} of this group is expected to be as sensitive to its conformation as, in the H-C-C-H group, the H···H spin-coupling constant is sensitive to the conformation of the H-C-C-H group.[1-6] This has been found to be actually the case as shown below.

J_{HP} in the CH_3OP System

Proton magnetic resonance spectra have been observed of the eight phosphoric acid esters listed in the first column of Table 1 in heavy water solutions. Dimethyl phosphate anion, $(CH_3O)_2PO_2^-$, shows a signal of methyl protons at a position shifted about 1.15 ppm from the H_2O signal towards higher field (see Fig. 1). The signal consists of two equal peaks

TABLE 1. PROTON-PHOSPHORUS COUPLING CONSTANTS IN THE H-C-O-P SYSTEM

	Compound (in D_2O)	J_{HP} (cycles per second)
(I)	$(CH_3OPO_3)^{2-}\cdot 2Na^+$	10.3
(II)	$[(CH_3O)_2PO_2]^-\cdot \frac{1}{2}Ba^{2+}$	10.5
(III)	$(CH_3CH_2OPO_3)^{2-}\cdot 2Na^+$	6.3
(IV)	$[(CH_3CH_2O)_2PO_2]^-\cdot \frac{1}{2}Ba^{2+}$	7.0
(V)	$[(CH_3CH_2CH_2O)_2PO_2]^-\cdot \frac{1}{2}Ba^{2+}$	6.5
(VI)	$[(CH_3CH_2CH_2CH_2O)_2PO_2]^-\cdot$ $\frac{1}{2}Ba^{2+}$	6.4
(VII)		6.9
(VIII)		1.5

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[1] M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
[2] M. Karplus, *J. Phys. Chem.*, **64**, 1793 (1960).
[3] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.*, **79**, 1005 (1957).
[4] N. Sheppard and J. J. Turner, *Proc. Roy. Soc. (London)*, **A252**, 506 (1959).
[5] C. N. Banwell, N. Sheppard and J. J. Turner, *Spectrochim. Acta*, **16**, 794 (1960).
[6] H. S. Gutowsky, G. G. Belford and P. E. McMahon, *J. Chem. Phys.*, **36**, 3353 (1962).

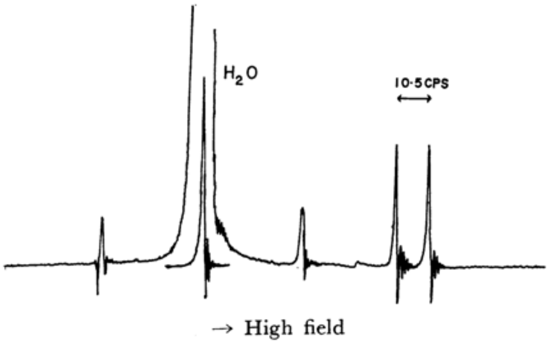

Fig. 1. Proton magnetic resonance spectrum (60 Mc) of dimethyl phosphate anion in D_2O solution.

separated 10.5 cps from each other. All the six methyl protons should be equivalent and no reason can be found that there are two kinds of protons with different chemical shifts. Thus, the two peaks are attributed to a spin-spin coupling between the methyl protons and phosphorus nucleus. The coupling constant J_{HP} is 10.5 cps. In a similar way, the J_{HP} in monomethyl phosphate anion is found to be 10.3 cps.

We attempt to relate these J_{HP} values with the problem of internal rotation of the CH_3OP group around the C–O bond as axis. On the basis of a generalization led from what are known on the internal rotation around the C–C or C–O single bond,[7,8] the potential curve of the internal rotation of the CH_3OP group is considered to have three minima. These minima correspond to the staggered conformations, and should have equal depth as shown in Fig. 2. The interconversion of these conformations is sufficiently rapid, and the observed proton-phosphorus spin-spin coupling constant $10._4$ cps may be expressed as

$$10._4 = \frac{1}{3}J_t + \frac{2}{3}J_g \tag{1}$$

Here, J_t is the coupling constant between the proton and phosphorus in the trans relation and J_g that in the gauche relation.

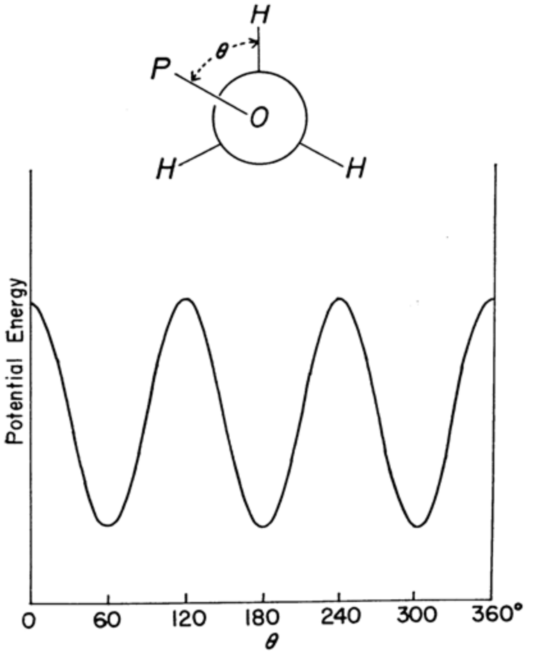

Fig. 2. A schematic drawing of the potential curve of the internal rotation around the C–O single bond in the CH_3OP group.

J_{HP} in the Gauche Relation (J_g)

In an attempt to freeze the internal rotation now in question, we prepared a ring compound given in the last line of Table 1, namely 1-phenyltrimethylene phosphate (1-phenyl-1, 3-propanediol cyclic phosphate). This is a new compound*[1] and was obtained as is described in the section of Experimental. The proton magnetic resonance spectrum of this anion (in D_2O) is shown in Fig. 3. The signal of the proton attached to the 1-C (H_A, see Fig. 3) can easily be found, because it should be situated at the lowest field next to the phenyl protons. It consists of eight peaks as shown in Fig. 4, (a). This is considered to be caused by the spin couplings of H_A with H_K, with H_L, and with P. A triple resonance experiment in which both of the signals of H_K and H_L are saturated indicates that the $H_A \cdots P$ spin-coupling constant is 1.5 cps (see Fig. 4 (b)). Therefore, one of the $H_A \cdots H_K$ and $H_A \cdots H_L$ spin-coupling constants should be about 3.3 cps and the other 10.7 cps.

The fact that H_K and H_L are not equivalent indicates that the six-membered ring C–C–C–O–P–O has a greater chance to take one conformation than other conformations. It is probable that that conformation is a chair form, on the basis of what are known of other six-membered ring with single bonds, and we assume here that the molecule in question is *fixed* in a chair form. On this assumption, the 1-phenyl group should be on the equatorial bond and the 1-H_A should be on the axial bond, because the spin coupling constants observed indicates that H_A is in nearly trans position with H_K and in nearly gauche position with H_L. (If the 1-H_A were on the equatorial bond, it should be in nearly gauche relation with both of H_K and H_L.) Thus, H_A should be nearly

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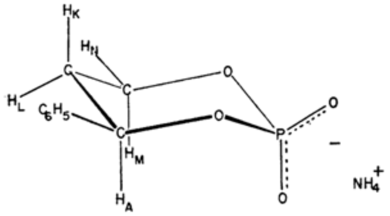
[7] S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, New York (1964).

[8] E. Ivash and D. M. Dennison, *J. Chem. Phys.*, **21**, 1804 (1953).

*[1] After the completion of this manuscript, we have been informed that the chloride of this cyclic phosphate was prepared before (W. M. Lanham, U. S. Pat. 2894016, July 7 (1959)).



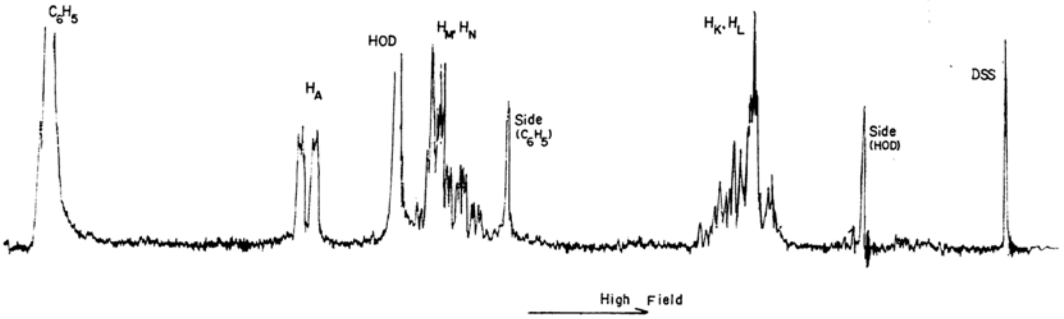


Fig. 3. Proton magnetic resonance spectrum (100 Mc) of 1-phenyl-1, 3-propanediol cyclic phosphate in D_2O solution. DSS: Sodium 2, 2-dimethyl 2-silapentane 5-sulfonate.

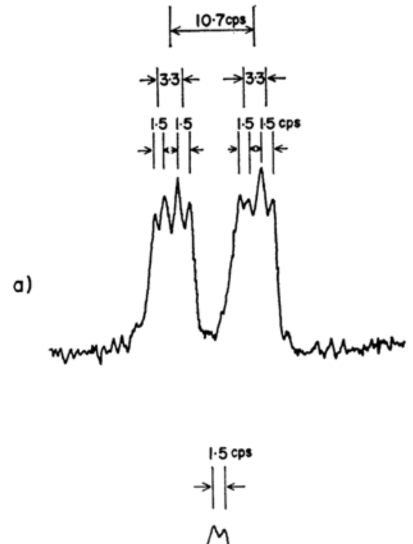


Fig. 4. H_A signal in the proton magnetic resonance spectrum of 1-phenyl-1, 3-propanediol cyclic phosphate in D_2O solution. (a) Observed in an ordinary state. No decoupling device is applied. (b) Observed when the H_K and H_L signals are saturated (triple resonance experiment).

gauche relation with P in the H_A–C–O–P system now in question, and it may now be concluded that J_{HP} in the gauche relation (J_g) is about 1.5 cps. If so, $J_t=28._2$ cps from Eq. (1).

J_{HP} in the RCH_2OP Systems

Diethyl phosphate anion, $(CH_3CH_2O)_2PO_2^-$, shows two signals due to the methyl protons and methylene protons respectively at 3.45 and 0.75 ppm from the H_2O signal (see Fig. 5). The former consists primarily of three components separated about 7.0 cps as usual. In detail, however, each component consists of two peaks separated about 0.8 cps. This may be due to the proton-phosphorus spin-spin coupling in the H–C–C–O–P system. The coupling constant is about 0.8 cps. One might consider a possibility that this separation is due to the second order perturbation of the methyl-methylene spin coupling Hamiltonian. This may be eliminated, however, on the basis of the fact that no splitting is observed for the components of the methylene signal separated 7.0 cps. The methylene signal consists primarily of five components instead of four. This fact may well be interpreted by considering that the spin-spin coupling constant between the methylene proton and the phosphorus nucleus has accidentally an equal value (7.0 cps) to that between the methylene proton and methyl proton.

The fact that the H(–C–O–)P spin-spin coupling constant is smaller (7.0 cps) in diethyl phosphate anion than that (10.5 cps) in dimethyl phosphate anion is now connected with the problem of internal rotation around the C–O single bond as axis. The potential curve of the internal rotation in diethyl phosphate anion may have also three

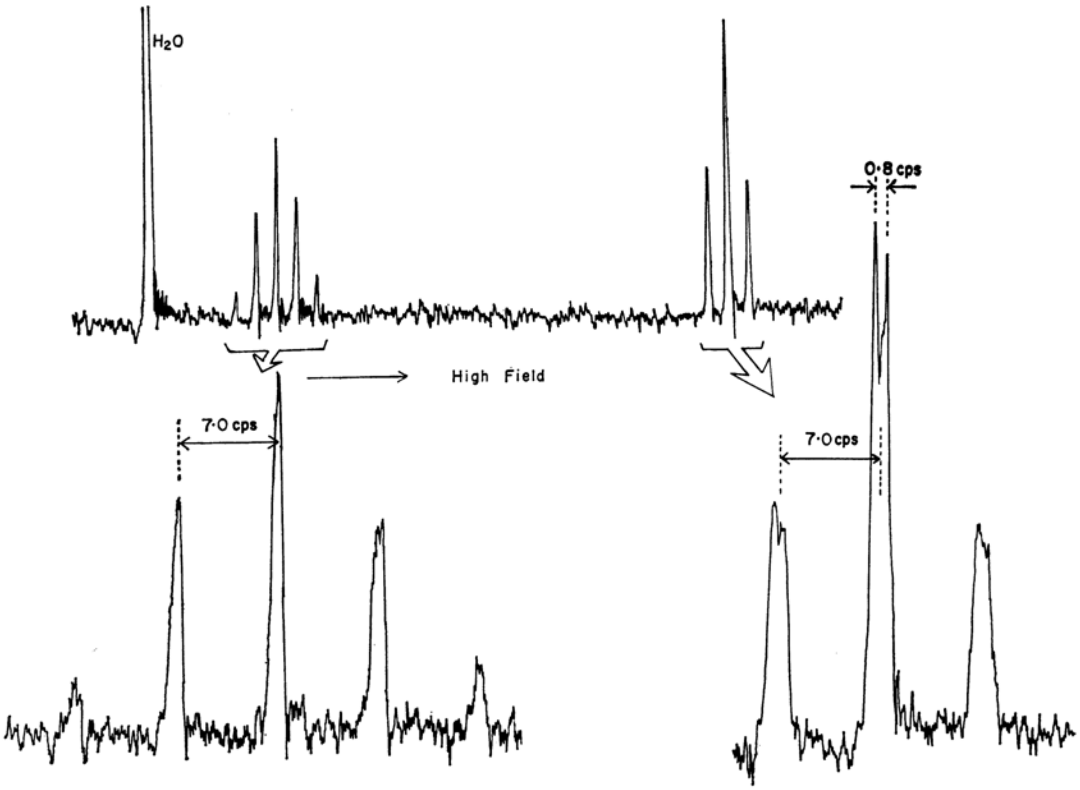


Fig. 5. Proton magnetic resonance spectrum (60 Mc) of diethyl phosphate anion in D_2O solution.

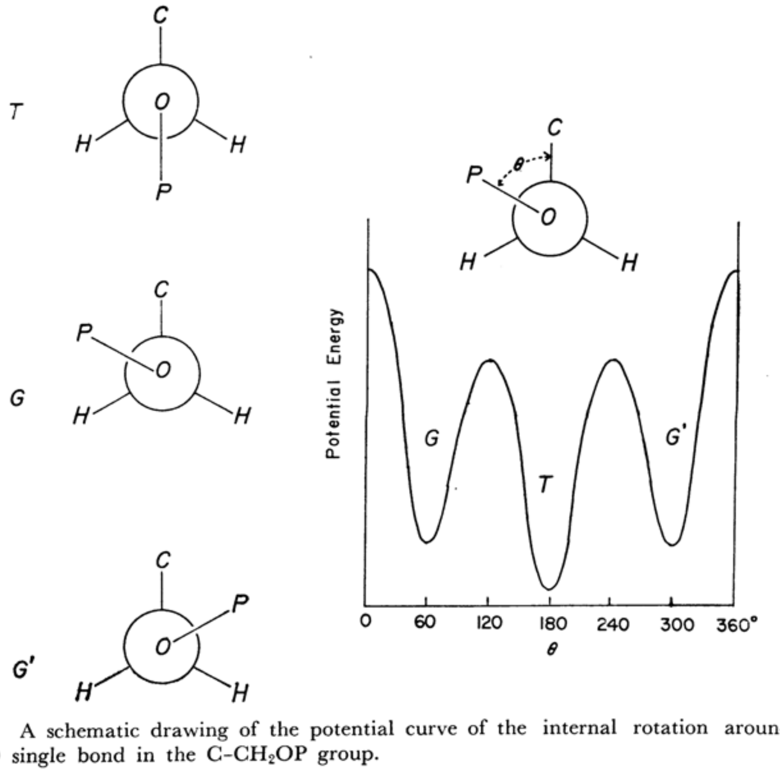


Fig. 6. A schematic drawing of the potential curve of the internal rotation around the C–O single bond in the C–CH_2OP group.

minima, but their depths would not be equal any more (Fig. 6). One of the three minima corresponds to the form (Fig. 6, T) where C is trans to P, and the other two correspond to the forms (Fig. 6, G and G′) where C is gauche to P. Representing the populations of the T form by α_T, and of G or G′ forms by $\frac{1}{2}(1-\alpha_T)$, the relation,

$$7.0=\frac{1-\alpha_T}{2}J_t+\frac{1+\alpha_T}{2}J_g, \qquad (2)$$

is obtained.*2 On the basis of what is found above, it is reasonable to assume that $J_t > J_g$ here. This leads to a conclusion that $\alpha_T < \frac{1}{3}$. If it is further assumed that $J_t = 28._2$ and $J_g = 1.5$ as above, α_T should be 0.59. This means that the trans form (Fig. 6, T) is 0.6 kcal/mol more stable than the gauche form (Fig. 6, G or G′) in the aqueous solution.

In a similar way, the J_{HP}'s in the H–C–O–P systems of monoethyl phosphate, di-*n*-propyl phosphate, and di-*n*-butyl phosphate anions were examined. In each of these three cases, the J_{HP} value was found to be almost equal to a J_{HH} value, and, therefore, the J_{HP} value determined (given in Table 1) may be less accurate than otherwise. It is certain, however, that J_{HP} is in the range of 6.0—7.0 in every case. Therefore, it may be concluded that the trans form is 0.6—0.8_4 kcal/mol more stable than the gauche form in the C–C–O–P groups of these anions in the aqueous solutions.

The two protons on the ring carbons of hydrobenzoin cyclic phosphate anion (meso, VII of Table 1) were found to be equivalent as are expected. The spin-spin coupling constant of this proton and the phosphorus nucleus in the five-membered ring was found to be 6.9 cps (Table 1). This J_{HP} value should be connected with the problem⁹⁾ of the puckering motion or pseudo-rotation of the C–C–O–P–O ring. It is probable that one of the potential minima in such an intramolecular motion corresponds to a form where one of the H's is nearly trans to P (*e. g.* Fig. 7, a—This form corresponds to the C_s form cyclopentane¹⁰⁾) and another corresponds to a form where the both H's are nearly gauche to P (*e. g.* Fig. 7, b—This form corresponds to the C_2 form of cyclopentane¹⁰⁾). If so, it should be concluded that the ring stays in the former form for almost an equal time to that for which the ring stays in the latter form.

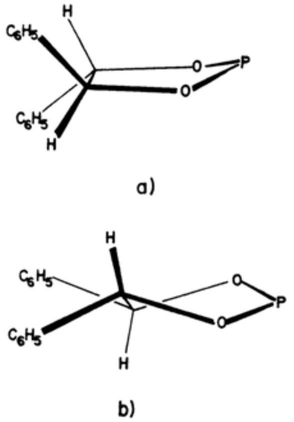


Fig. 7. Possible conformations of the five-membered ring in the hydrobenzoin cyclic phosphate anion.

Experimental

Preparation of Ammonium 1-Phenyltrimethylene Phosphate (1-Phenyl-1, 3-propanediol Cyclic Phosphate). First, 1-phenyl-1, 3-propanediol was prepared by a reaction of styrene, trioxane, and acetic acid.¹¹⁾ A mixture of 16.5 g of trioxane, 152 m*l* of glacial acetic acid, and 9.2 m*l* of concentrated sulfuric acid was heated to effect solution. This was cooled to 25°C, and 57.4 m*l* of styrene was added drop by drop, the temperature being maintained below 40°C. After standing overnight, the mixture was poured into 500 m*l* of water, and taken up in ethylether. The ether solution was washed with sodium carbonate, and the ethyl ether was removed. The remaining oil was saponified with slightly more than the theoretical amount of alcoholic KOH and then was subjected to a fractional distillation in vacuum. The fraction of 155°C/4 mmHg was found to be the aimed dialcohol by means of the NMR and infrared spectra as well as the elementary analysis (Found: C, 71.04; H, 7.87%. Calcd for $C_9H_{12}O_2$: C, 71.09; H, 7.89%).

The cyclic phosphate of this diol was prepared by a method developed by Ukita *et al.*¹²⁾ To an ice-cooled solution of 2.5 g of phosphorus oxychloride in 10 m*l* of dry pyridine was added 1.534 g of 1-phenyl-1, 3-propanediol dissolved in 60 m*l* of dry pyridine with vigorous stirring over a period of 1 hr. Stirring was continued for an additional 30 min at room temperature and then pyridine was removed *in vacuo*. The residue, dissolved in 30 m*l* of cooled distilled water, was shaken three times with 15 m*l* portions of ether. The pH of the aqueous layer was adjusted to 8.0 with solid sodium carbonate. A precipitate which occurs in this aqueous solution was removed by filtration, and to the filtrate dowex 50 [H^+] was added so that Na^+ was removed and the pH of the solution was lowered to 2.8—3.0. By adding NH_4OH, the pH of the solution was brought up to 9. This solution was placed in a column of 300 mesh cellulose powder, and a chromatographic separation was made by using isopropyl alcohol+water+NH_4OH (7 : 2 : 1) as the effluent. Fractions in which phosphorus was detected by the Hanes and Isherwood

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*2 Suffix *T* means that C is trans to P, while suffix *t* means that H is trans to P.

9) See, for example, A. Y. Hirakawa and M. Tsuboi, *Kagaku-to-Kogyo (Chem. & Chem. Ind.)*, **19**, 344 (1966).

10) K. Pitzer, *Science*, **101**, 672 (1945).

11) W. S. Emerson, *Chem. Revs.*, **45**, 319 (1949).

12) T. Ukita, K. Nagasawa and M. Irie, *J. Am. Chem. Soc.*, **80**, 1373 (1958).

method[13] were collected, and the solute was concentrated by removing the solvent in vacuum. The product was dissolved into water, and this was passed through two sheets of filter paper. From the filtrate the water was removed, and the residue was washed with acetone for five times. The product was dried over P_2O_5, purified with ethanol, and dried over P_2O_5 again.

Found: C, 46.71; H, 6.45; N, 6.14; P, 13.29%. Calcd for $C_9H_{14}NPO_4$: C, 46.80; H, 6.10; N, 6.10; P, 13.30%.

Preparations of Other Esters. Dimethyl, diethyl, di-*n*-propyl, and di-*n*-butyl esters (II, IV, V, and VI) were obtained by the partial hydrolysis of the corresponding triesters in 10% alcoholic potassium hydroxide.[14] After the removal of alcohol by distillation, the solution was acidified with HCl and then neutralized with $Ba(OH)_2$. When the hydrolysate was found to be a mixture of mono- and diesters, they were separated from each other by the use of solubility difference of the barium salts in water. The purification was effected by recrystallization from alcohol-water solution.[15]

Trimethyl and triethyl phosphates were purchased from Tokyo Kasei Co., and tri-*n*-propyl and tri-*n*-butyl phosphates were kindly supplied by Dr. R. Chō, Tama Chemical Co.

Sodium monoethyl phosphate (III) was prepared by the reaction of sodium pyrophosphate and ethyl alcohol saturated with hydrochloric acid.[16] Barium monomethyl phosphate (I) was obtained from commercial samples supplied by Tokyo Kasei Co., which was found to be a mixture of the corresponding mono- and dimethyl phosphate. The separation was made by the fractional crystallization from hot water.

Every purification of the esters was followed by the observation of infrared spectrum and then an elementary analysis of P, C, and H.

Sodium hydrobenzoin cyclic phosphate (meso) (VII) was a gift from Professor T. Ukita,[12] here in the University of Tokyo.

NMR Spectra. The proton magnetic resonance of ammonium 1-phenyltrimethylene phosphate was observed in D_2O solutions by the use of a Varian Model HA-100 NMR Spectrometer. The triple-resonance experiment was done according to the direction given in the manual of the instrument. The proton magnetic resonance of other esters were examined by the use of a Varian A-60 NMR Spectrometer or a Japan Electron Optics Lab. Model JNM Spectrometer (60 Mc). All the spectra were observed at room temperature.

We wish to express our sincere thanks to Professor T. Ukita and Dr. N. Imura for their valuable advices in our preparation of 1-phenyltrimethylene phosphate, to Professor S. Fujiwara and Dr. Y. Fujiwara for their kindness in the NMR measurements by the use of the 100 Mc Varian NMR Spectrometer and for their instructive discussions, and to Dr. A. Nakamura for his kindness in the NMR measurements by the use of a Varian A60 NMR Spectrometer and for his valuable suggestions in the early stage of this work. This work was supported partly by a grant from the Ministry of Education of Japan and partly by a grant form United States Public Health Service GM 10024-03.

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13) C. S. Hanes and F. A. Isherwood, *Nature,* **164**, 1107 (1949).

14) R. Bentley, *J. Am. Chem. Soc.,* **70**, 2183 (1948).

15) R. H. A. Plimmer and W. J. N. Burch, *J. Chem. Soc.,* **1929**, 292.

16) E. Cherbulitz and H. Weniger, *Helv. Chim. Acta,* **29**, 2006 (1946).