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# The Reaction of *cyclo*-Triphosphate with Ethanolamines

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The reaction of *cyclo*-triphosphate($P_{3m}$) with mono-(MEA), di-(DEA), or triethanolamine(TEA) in an aqueous solution was investigated under various conditions (mixing ratio of MEA, DEA, or TEA to $P_{3m}$, pH, temperature, and reaction time). 1) In the pH region of 7—12, $P_{3m}$ reacted with MEA or DEA to yield tri-, di-, and monophosphate derivatives of MEA or DEA. The phosphoric acid ester of MEA or DEA was not formed at all. 2) The reactivities of MEA and DEA to $P_{3m}$ were in the order of MEA > DEA, and TEA did not react with $P_{3m}$. 3) The reactivity of $P_{3m}$ with MEA or DEA decreased with the decrease in the pH, and no reaction took place under acidic conditions. 4) The maximum yields of triphosphate derivatives of MEA, *N*-(2-hydroxyethyl)-triphosphoramidate($P_3$-(N)MEA) and of DEA, *N*-bis(2-hydroxyethyl)triphosphoramidate ($P_3$-(N)DEA), were about 75 and 60% respectively at a molar ratio of 1 : 1, at pH 12, and at room temperature. 5) $P_3$-(N)MEA and $P_3$-(N)DEA easily recyclized to the raw material, $P_{3m}$, under acidic conditions. 6) The mechanism of the reaction between $P_{3m}$ and MEA or DEA was investigated.

Inorganic condensed phosphates can be roughly classified into the following three types: 1) linear polyphosphates, 2) cyclic phosphates, and 3) ultraphosphates (network-like phosphates).[1] Among them, linear poly- and ultraphosphates are widely used for drugs, food additives, fertilizers, detergents, water treating agents, and industrial chemicals.[2–4] However, as to cyclic phosphates, few practical applications[5–7] have been known, and few reports[8,9] have been presented on fundamental investigations of them. Since Feldmann reported, in 1965—1969, that $P_{3m}$ reacts with alkylamines,[10] phenols,[11] and amino acids,[12] $P_{3m}$ has been attracting much attention as a phosphorylating agent for organic compounds. Rabinowitz[13,14] reported the reaction of $P_{3m}$ with amino acids (glycine and alanine); Saffhill,[15] the phosphorylation of nucleosides with $P_{3m}$, and Dombrovskii and Dorosh,[16] the reaction of $P_{3m}$ with 1,6-hexanediamine. However, in these studies paper chromatography has been principally used to analyze the reaction products; it provides only a semiquantitative discussion. In the present study, the reaction of $P_{3m}$ with mono- (MEA), di- (DEA), or triethanolamine (TEA) has been examined in order to develop studies of the application of $P_{3m}$ as a phosphorylating agent. Both $^{31}$P-NMR and anion-exchange chromatography have been used to determine the structure and the amounts of the reaction products. The reaction mechanism is also discussed.

## Experimental

**Chemicals.** Sodium *cyclo*-triphosphate hexahydrate, $Na_3P_3O_9 \cdot 6H_2O$, was obtained by recrystallizing it three times from an aqueous solution of industrial-grade anhydrous sodium *cyclo*-triphosphate (98%) from Rasa Kogyo, Ltd. Unless otherwise stated, guaranteed grade mono-, di-, and triethanolamine were used without further purification.

**Reaction between *cyclo*-Triphosphate and Ethanolamine.** In the reaction of $P_{3m}$ with MEA, DEA, or TEA, 50 cm$^3$ of a 0.5 mol dm$^{-3}$ aqueous sodium *cyclo*-triphosphate ($P_{3m}$) solution and 50 cm$^3$ of a 0.5—5 mol dm$^{-3}$ aqueous mono-, di-, or triethanolamine solution were mixed. The pH values of the mixed solution were about 12.0—12.5 in MEA, 11.6—11.9 in DEA, and 10.5—10.7 in TEA. These mixed solutions were then adjusted to the prescribed pH value (pH 12, 10, 7, or 4) by the use of a 6 mol dm$^{-3}$ sodium hydroxide aqueous solution and hydrochloric acid, and the reaction was allowed to proceed at room temperature or 50 °C. Since the pH of the mixed solution gradually fell with the progress of the reaction, a sodium hydroxide solution was added to it to maintain the prescribed pH value.

Anion-exchange chromatography and the measurement of $^{31}$P-NMR were carried out by the methods described in a previous paper.[17]

## Results and Discussion

**Reaction of $P_{3m}$ with MEA.** Aqueous solutions of $P_{3m}$ and MEA were mixed in a molar ratio of 1 : 1. The pH of the mixed solution was adjusted to 12 with a sodium hydroxide solution, and the reaction was allowed to proceed at room temperature. With the progress of the reaction, the pH of the mixed solution gradually decreased to about 9.3 after 1 d. This fact indicates that $P_{3m}$ reacts with water or MEA to produce linear phosphates or their derivatives. In order to examine this, the present authors investigated the reaction products and their amounts by the use of anion-exchange chromatography and $^{31}$P-NMR.

As an example, two representative anion-exchange chromatograms of the reaction products are shown in Fig. 1. Immediately after the reaction, a large peak due to an unknown compound appeared at about 880 cm$^3$ of the effluent (designated as Compound C). The peak of the pyrophosphate ($P_2$) was also observed, though its amount was slight. The amount of Compound C increased with the passage of the reaction time, but in a reaction over a long period its amount gradually decreased due to hydrolysis. As can be seen from Fig. 1, when the reaction was allowed to proceed for 44 d, in addition to Compound C four peaks due to un-

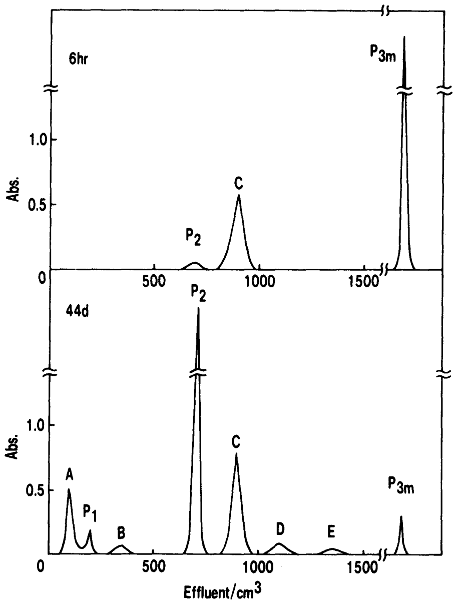
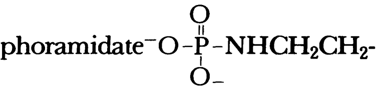


Fig. 1. Elution patterns of the reaction products of $P_{3m}$ with MEA at pH 12.
$P_{3m}$ : MEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$.

known compounds (Compounds A, B, D, and E) were found at about 100, 350, 1150, and 1360 cm$^3$ of the effluent. Moreover, almost no production of triphosphate ($P_3$) was found in this reaction.

Figure 2 shows the $^{31}$P-NMR spectra of the reaction products of $P_{3m}$ and MEA at a molar ratio of 1 : 10 and pH 12. It can be seen that the main product, Compound C, is a triphosphate derivative of MEA, *N*-(2-hydroxyethyl)triphosphoramidate($P_3$-(N)MEA). In the $^1$H-decoupling spectrum of Fig. 2, the doublets at $\delta$ − 0.5 and −4.3 ppm were assigned to the end phosphorus atoms of $P_3$-(N)MEA, $P_\alpha$ and $P_\gamma$, respectively, while the triplet at −20.3 ppm corresponded to the middle phosphorus atom, $P_\beta$. In the $^1$H-coupling spectrum, each of the doublets of $P_\alpha$ ought to split into triplets because of the two hydrogen atoms of the methylene (-NHCH$_2$-) group, but actually a quintet was observed as a result of overlapping.

The $^{31}$P-NMR spectra do not always indicate that Compound C is $P_3$-(N)MEA, with a P-N bond in the molecule. There is another possibility that Compound C is a triphosphoric acid ester of MEA, 2-aminoethyl triphosphate, with a P-O-C bond in the molecule. In order to check this possibility, an aqueous solution of Compound C was made acidic (pH 3). Since Compound C easily recyclized to $P_{3m}$, it was concluded that Compound C is a triphosphate derivative of MEA, $P_3$-(N)MEA, with a P-N bond in the molecule.[18,19] The singlet at $\delta$ 4.7 ppm in Fig. 2 is attributed to the phosphorus atom of a monophosphate derivative of MEA, *N*-(2-hydroxyethyl)phosphoramidate  $^-O-\underset{O^-}{\overset{O}{\overset{\|}{P}}}-NHCH_2CH_2-OH$ ($P_1$-(N)MEA). This phosphorus atom splits into a triplet in the $^1$H-coupling due to the influence of two hydrogen atoms of the methylene group. Compound A, whose amount is next to $P_3$-(N)MEA, was estimated to be a monophosphate derivative of MEA, $P_1$-(N)MEA, with a P-N bond in the molecule. Singlets at $\delta$ 4.0 and −5.1 ppm were attributed to $PO_4^{3-}$ and $P_2O_7^{4-}$ ions.[20]

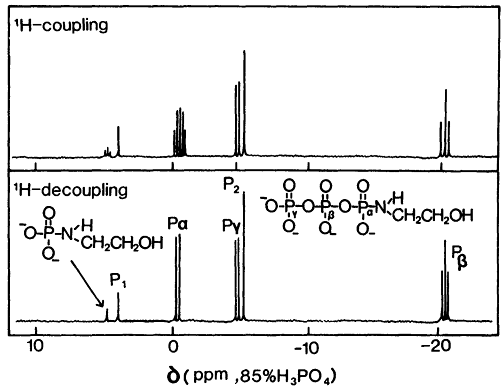


Fig. 2. $^{31}$P-NMR spectra of the reaction products in the $P_{3m}$-MEA series.

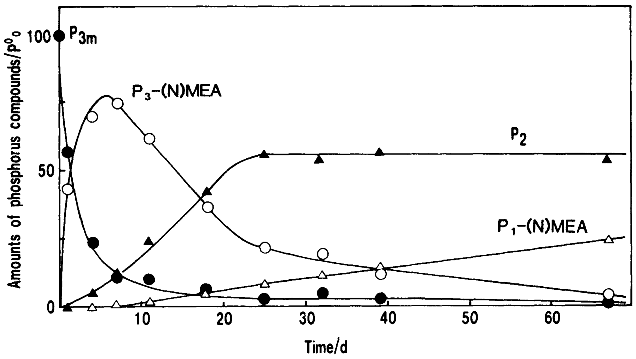


Fig. 3. Changes of the amounts of phosphorus compounds in the reaction of $P_{3m}$ with MEA at pH 12.
$P_{3m}$ : MEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$.

The structures of Compounds B, D, and E have not yet been characterized because they were formed in only minute amounts and could not be isolated in a pure form. However, based upon the fact that, when a solution containing much of Compound B was made acidic (pH=3), it was hydrolyzed to produce $P_2$, Compound B was estimated to be *N*-(2-hydroxyethyl)diphosphoramidate($P_2$-(N)MEA). On the other hand, the orthophosphoric acid ester of MEA, 2-aminoethyl dihydrogenphosphate, was not produced in the reaction of $P_{3m}$ with MEA. Thus, it was found that the phosphorylation of MEA with $P_{3m}$ takes place at the position of the NH$_2$ group in MEA.

**Amounts of $P_3$-(N)MEA, $P_2$-(N)MEA, and $P_1$-(N)MEA.** Figure 3 shows the changes in the amounts of the various phosphates formed by the reaction of $P_{3m}$ with MEA at a molar ratio of 1 : 1, pH 12, and room temperature. $P_3$-(N)MEA began to be produced immediately after the start of the reaction; the yield reached

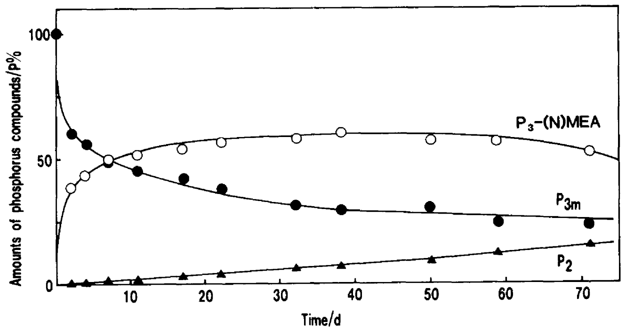


Fig. 4. Changes of the amounts of phosphorus compounds in the reaction of $P_{3m}$ with MEA at pH 10.

$P_{3m}$ : MEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$.

about 75% after 7 d. After that, however, it gradually decreased as the reaction progressed. On the other hand, $P_1$-(N)MEA increased with the reaction time, attaining about 24% after 67 d. Also, the amount of $P_2$ increased with the progress of the reaction, and the crystals of tetrasodium pyrophosphate decahydrate, $Na_4P_2O_7 \cdot 10H_2O$, began to precipitate after 25 d. However, the amount of $Na_4P_2O_7 \cdot 10H_2O$ precipitated was not taken into consideration in Fig. 3. The amounts of $P_1$, $P_2$-(N)MEA, and Compounds D and E were so small ($P_1$, 4.4; $P_2$-(N)MEA, 4.7; Compound D, 6.6, and E, 11.5%) that they were omitted in Fig. 3.

Figure 4 shows the changes in the amounts of phosphorus compounds formed by the reaction between $P_{3m}$ and MEA ($P_{3m}$ : MEA = 1 : 1) at pH 10 and room temperature. The raw material, $P_{3m}$, gradually decreased with the progress of the reaction, showing that the reaction of $P_{3m}$ with MEA at pH 10 was slower than that at pH 12. The main product, $P_3$-(N)MEA, attained to about 50% after 7 d. After that, the amount was constant (about 55—60%) until 60 d after the reaction. $P_1$, $P_1$-(N)MEA, $P_2$-(N)MEA, and Compounds D and E were not formed until 20 d after the reaction. No precipitate of $Na_4P_2O_7 \cdot 10H_2O$ was observed under these conditions.

In the reaction of $P_{3m}$ with MEA at a molar ratio of 1 : 1, pH 7, and room temperature, the amount of $P_3$-(N)MEA was very small amounting only to about 2.3% even after 60 d. At pH 4, the phosphorylation of MEA with $P_{3m}$ was not observed at all. Instead, the hydrolysis products of $P_{3m}$, i.e., $P_3$, $P_2$, and $P_1$ were obtained. From the above experimental results (pH 12, 10, 7, and 4), it can be concluded that $P_{3m}$ and MEA react well in highly alkaline regions, that the reactivity decreases with the decrease in the pH, and that no reaction takes place under acidic conditions. This is ascribable to the formation of inactive $H_3{}^+NCH_2CH_2OH$ under acidic conditions.

Figure 5 shows the changes in the amounts of phosphorus compounds when the reaction was allowed to proceed at a molar ratio of 1 : 10, pH 12, and room temperature. $P_{3m}$ rapidly decreased after the start of the reaction, and it became almost zero after several hours. On the contrary, $P_3$-(N)MEA began to be produced rapidly, and it was produced about 92% within 3.5 h. The amount of $P_3$-(N)MEA gradually decreased after that with the lapse of the reaction time. The production rate of $P_3$-(N)MEA was faster, and its amount was larger at a molar ratio of 1 : 10 than that at 1 : 1 (Fig. 3). Since the amounts of $P_1$, $P_1$-(N)MEA, and $P_2$-(N)MEA were small (about 7.1, 8.0, and 2.7% respectively), they are not shown in Fig. 5. No Compound D or E was produced under these experimental conditions.

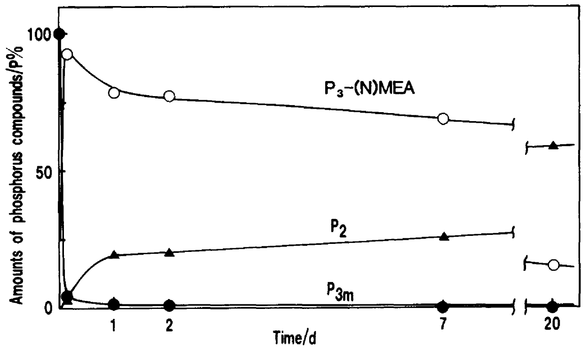


Fig. 5. Changes of the amounts of phosphorus compounds in the reaction of $P_{3m}$ with MEA at pH 12.

$P_{3m}$ : MEA = 0.5 mol dm$^{-3}$ : 5 mol dm$^{-3}$.

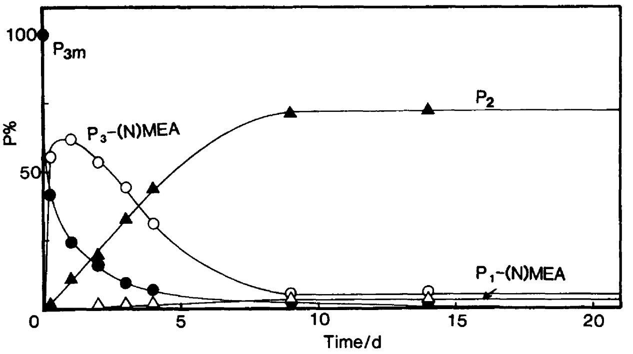


Fig. 6. Changes of the amounts of phosphorus compounds in the reaction of $P_{3m}$ with MEA at 50 °C.
$P_{3m}$ : MEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$, pH 12.

In Fig. 6, the amounts of $P_3$-(N)MEA, $P_1$-(N)MEA, and $P_2$ formed by the reaction of $P_{3m}$ with MEA (molar ratio = 1 : 1) at pH 12 and 50 °C are plotted against the time. The reaction proceeded faster than that at room temperature (Fig. 3). The pattern of the formation of $P_3$-(N)MEA, $P_1$-(N)MEA, and $P_2$ was, however, similar to that at room temperature. The amount of $P_3$-(N)MEA increased to the maximum value of about 61% after 1 d, thereafter, it gradually decreased.

**Reaction of $P_{3m}$ with DEA.** A representative anion-exchange chromatogram for the products resulting from the reaction of $P_{3m}$ and DEA at a molar ratio of 1 : 1, pH 12, and room temperature is shown in Fig. 7. Other than the peaks of $P_1$, $P_2$, and $P_3$, three peaks due to unknown compounds were found at about 100, 440, and 815 cm$^3$ of the effluent (designated as Compounds I, II, and III). In the reaction of $P_{3m}$ with DEA, the production of $P_3$ was remarkable. This may be due to the lower reactivity of

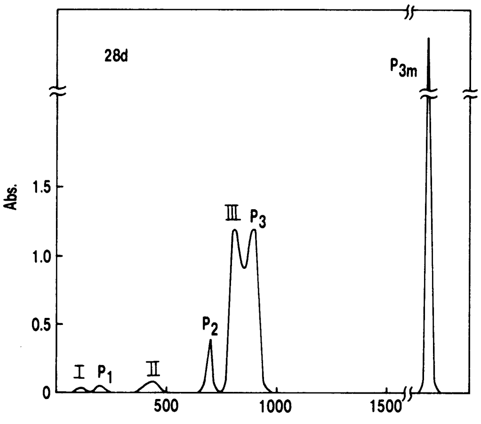


Fig. 7. Elution pattern of the reaction products of $P_{3m}$ with DEA at pH 12.
$P_{3m}$ : DEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$.

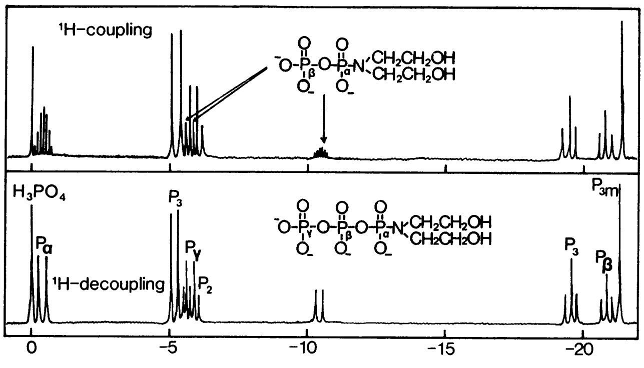
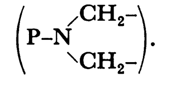


Fig. 8. $^{31}$P-NMR spectra of the reaction products in the $P_{3m}$–DEA series.

$P_{3m}$ with DEA compared with that with MEA. The hydrolysis of $P_{3m}$ in such an alkaline solution can not be neglected in this case. In order to clarify the structures of Compounds I, II, and III, $^{31}$P-NMR of the reaction products of $P_{3m}$ and DEA was measured (Fig. 8). At $\delta$ −0.3, −5.4, and −20.8 ppm in the $^{1}$H-decoupling spectrum, peaks due to $P_\alpha$ (doublet), $P_\gamma$ (doublet), and $P_\beta$ (triplet) of a triphosphate derivative of DEA, *N*-bis(2-hydroxyethyl)-triphosphoramidate ($P_3$-(N)DEA) are found. In the $^{1}$H-coupling, the $P_\alpha$ of $P_3$-(N)DEA was split into seven peaks due to the four hydrogen atoms of two methylene groups,


$$\left(P\text{-}N\begin{matrix}CH_2\text{-}\\CH_2\text{-}\end{matrix}\right).$$

Since $P_\beta$ and $P_\gamma$ are not influenced by these hydrogen atoms, no coupling due to a proton is recognized. It is interesting that, at $\delta$ −5.3 and −10.3 ppm, two doublets due to the $P_\beta$ and $P_\gamma$ of a diphosphate derivative of DEA, *N*-bis(2-hydroxyethyl)diphosphoramidate ($P_2$-(N)DEA), were found. One doublet ($\delta$ −5.1 ppm) and one triplet ($\delta$ −19.7 ppm) are due to phosphorus atoms of the end $PO_4$ and to the middle $PO_3$ of $P_3$[20] respectively. From the peak heights (amounts) of Compounds II and III on an anion-exchange chromatogram (Fig. 7), it was deduced that Compound II is a diphosphate derivative of DEA, $P_2$-(N)DEA, while III is a triphosphate derivative of DEA, $P_3$-(N)DEA. Another proof that Compound III is a triphosphate derivative of DEA with a P–N bond in the molecule is the fact that Compound III was easily recyclized to $P_{3m}$ when the solution of Compound III was made acidic (pH 3).

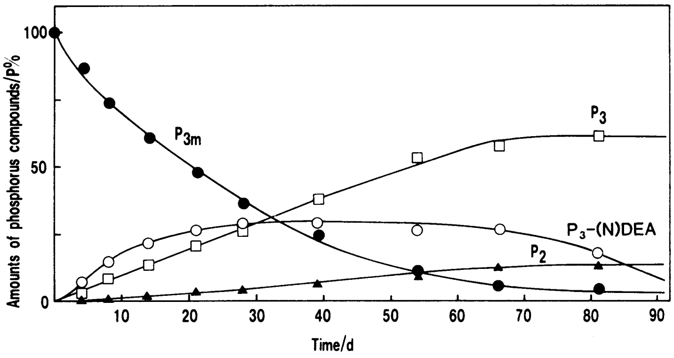


Fig. 9. Changes of the amounts of phosphorus compounds in the reaction of $P_{3m}$ with DEA at pH 12.
$P_{3m}$ : DEA = 0.5 mol dm$^{-3}$ : 0.5 mol dm$^{-3}$.

From the fact that $P_2$-(N)DEA was not produced at the initial stage of the reaction, but was produced with the progress of the reaction, it can be considered that $P_2$-(N)DEA is produced by the hydrolysis of $P_3$-(N)DEA. That is, the $P_3$-(N)DEA formed at the initial stage of the reaction is gradually hydrolyzed to yield $P_2$-(N)DEA and $P_1$.

It was impossible to determine the structure of Compound I because of its small yield. However, it can be anticipated that Compound I is a monophosphate derivative of DEA, *N*-bis(2-hydroxyethyl)phosphoramidate ($P_1$-(N)DEA), because $P_1$ was produced when an aqueous solution of Compound I was made acidic (pH 3).

**Amounts of $P_3$-(N)DEA, $P_2$-(N)DEA, and $P_1$-(N)-DEA.** Figure 9 shows the changes in the amounts of phosphates formed by the reaction of $P_{3m}$ and DEA at a molar ratio of 1 : 1, pH 12, and room temperature. Compared with the reaction of MEA, the production of $P_3$-(N)DEA was very slow; after 28 d it attained a maximum level of about 30%, smaller than that observed in the reaction of MEA. After that, it was gradually hydrolyzed. On the other hand, the amount of $P_3$ increased linearly up to 70 d, and it showed a constant value of about 60%. Since the amounts of $P_1$-(N)DEA, $P_2$-(N)DEA, and $P_1$ were very small ($P_1$-(N)DEA, 0.8; $P_2$-(N)DEA, 2.0; $P_1$, 0.5%), they are omitted in Fig. 9. A characteristic phenomenon is that the production of $P_3$ was dominant in the $P_{3m}$–DEA series, although the production of $P_2$ was remarkable in the $P_{3m}$–MEA series. Therefore, it is considered that, in the $P_{3m}$–MEA series, $P_3$-(N)MEA is produced at the first stage of the reaction and that this is hydrolyzed principally to $P_1$-(N)MEA and $P_2$. On the contrary, in the $P_{3m}$–DEA series, the

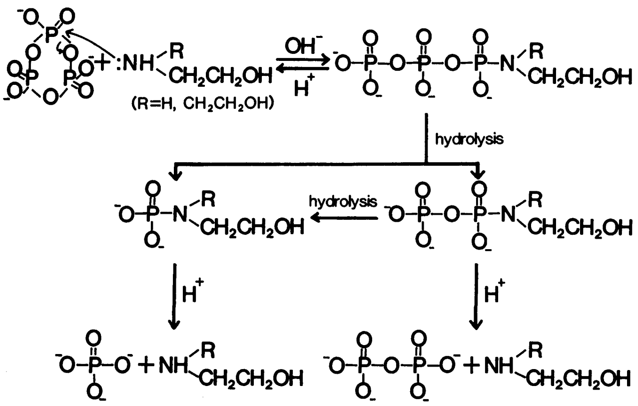


Fig. 10. The reaction mechanism of $P_{3m}$ with MEA or DEA.

hydrolysis of $P_{3m}$ to $P_3$ proceeded at the same time as the phosphorylation of DEA. With the lapse of the reaction time, $P_3$-(N)DEA was gradually hydrolyzed to $P_2$-(N)DEA and $P_1$.

The reaction of $P_{3m}$ with TEA was allowed to proceed under the same conditions as those in the $P_{3m}$-MEA and $P_{3m}$-DEA series. However, no reaction took place in the $P_{3m}$-TEA series. This is due to the absence of a hydrogen atom directly bonded to the nitrogen atom in the TEA molecule. That is, no ring cleavage of $P_{3m}$ takes place because of the absence of a dissociable hydrogen atom in TEA when the lone-pair of the nitrogen atom of TEA nucleophilically attacks the phosphorus atom of $P_{3m}$. On the other hand, Feldmann[10] reported that $P_{3m}$ easily reacts with methylamine and dimethylamine to yield triphosphate derivatives of them, but not with trimethylamine. That is, $P_{3m}$ easily reacts with primary and secondary amines in alkaline regions, but does not react with tertiary amines. This fact agrees well with our experimental results.

**Mechanism of the Reaction of $P_{3m}$ with MEA or DEA.** The mechanism of the reaction of $P_{3m}$ with MEA or DEA in alkaline solutions is shown in Fig. 10. The lone-pair of the nitrogen atom of MEA or DEA nucleophilically attacks the phosphorus atom of $P_{3m}$, causing a ring cleavage of $P_{3m}$ to produce a triphosphate derivative of MEA or DEA, $P_3$-(N)MEA or $P_3$-(N)DEA, with a P-N bond in the molecule. These compounds are gradually hydrolyzed in the reaction solution to form di- and monophosphate derivatives of MEA or DEA. When $P_3$-(N)MEA and $P_3$-(N)DEA were made acidic (pH 3), they were easily recyclized to $P_{3m}$. From this fact, it is clear that the reaction of $P_{3m}$ with MEA or DEA proceeds reversibly, depending upon the pH. When di- and monophosphate derivatives of MEA or DEA are made acidic, they are easily hydrolyzed to yield $P_2$ and $P_1$ respectively. Based upon the fact that the phosphoric acid ester of MEA or DEA was not formed in this reaction, it can be estimated that the reactivity of the $NH_2$ group to $P_{3m}$ is higher than that of the OH group in ethanolamines. When the reaction of $P_{3m}$ with MEA, DEA, or TEA was compared, the reactivity was in the order of MEA>DEA, and TEA did not react with $P_{3m}$.

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