January, 1981] © *1981 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, *54*, *289—290 (1981)* 289

The Reaction of *cyclo*-Tetraphosphate with L-Valine

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(Received March 22, 1980)

Synopsis. *cyclo*-Tetraphosphate (P_{4m}) reacted with L-valine at pH 12 to form a certain phosphorus-containing compound (Compound X). Compound X was obtained in amounts so small as about 3.2% at room temperature after 91 d and about 0.3% at 70 °C after 1 d. Based on the results of paper chromatography, anion-exchange chromatography, and IR spectroscopy, Compound X was proved to be the same orthophosphate derivative of valine, an anion of *N*-(1-carboxy-2-methylpropyl)phosphoramidic acid, as that formed by the reaction of *cyclo*-triphosphate (P_{3m}) with L-valine. The mechanism of the reaction of P_{4m} with L-valine was discussed.

Quimby and Flautt¹⁾ first reported that cyclic phosphates, such as P_{3m}, could be subjected to ring opening with ammonia to form amidophosphates. Since then, the reactions of P_{3m} with alkylamines,²,³⁾ sodium fluoride,⁴⁾ alcohols,⁵⁾ and glycine⁶⁾ have been actively studied by Feldmann and Rabinowitz. The present authors have already reported that, by the reaction of P_{3m} with L-valine one of the α-amino acids, a P_1-derivative of valine, was produced in the pH range from 10 to 12.⁷⁾ Thus, in the present study, the reaction of P_{4m}, one of cyclic phosphates, with L-valine was investigated.

Experimental

Chemicals. Sodium *cyclo*-tetraphosphate tetrahydrate,⁸⁾ $Na_4P_4O_{12}\cdot 4H_2O$, was prepared in the following manner: 50 g of diphosphorus pentaoxide was gradually hydrolyzed in 300 cm³ of cold water, after which the hydrolyzate was neutralized to pH 7 with a cold, concentrated sodium hydroxide solution. Then, by repeating the recrystallization 5 times from water and drying at 40 °C, $Na_4P_4O_{12}\cdot 4H_2O$ was obtained. Reagent-grade L-valine was used without purification.

Reaction between cyclo-*Tetraphosphate and* L-*Valine.* Aqueous solutions of 0.2 mol dm⁻³ of sodium *cyclo*-tetraphosphate tetrahydrate and 0.2 mol dm⁻³ of L-valine were mixed in a volume ratio of 1:1. The pH of the mixture was about 5.97. The solution was adjusted with 6 mol dm⁻³ of a sodium hydroxide solution to pH 12 and then allowed to stand at room temperature and at 70 °C. With the progress of the reaction the pH of the solution was gradually lowered, after which the sodium hydroxide solution was added to maintain the pH 12.

Anion-exchange chromatography, paper chromatography, and IR spectroscopy were carried out by the methods described in a previous paper.⁷⁾

Results and Discussion

Figure 1 shows the anion-exchange chromatograms obtained for the reaction mixtures of P_{4m} with L-valine in a molar ratio of 1:1 after 14 and 91 d at room temperature and at pH 12. In addition to the peaks of ortho-(P_1), pyro-(P_2), tri-(P_3), and tetraphosphate(P_4), the peak of Compound X appeared between those of P_1 and P_2. Though the formation of Compound X was small, it tended to increase gradually with the reaction time. Figure 2 shows the change in the amounts of Compound X, P_3, and P_4, as well as the amount of remaining P_{4m}, determined by means of anion-exchange chromatography. The amounts of P_1 and P_2 formed are omitted here because their amounts were very small (about 0.4 and 0.1% respectively), even after 91 d. The starting material, P_{4m}, decreased gradually with the time, but still about 73% of it remained after 91 d. On the other hand, the yields of P_4, P_3, and Compound X were about 12.5, 11.0, and 3.2% respectively.

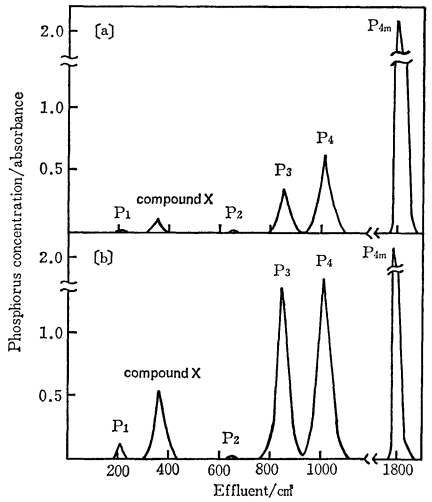


Fig. 1. Elution patterns for the reaction products of P_{4m} with L-valine at room temperature and pH 12. (a) after 14 d, (b) after 91 d.

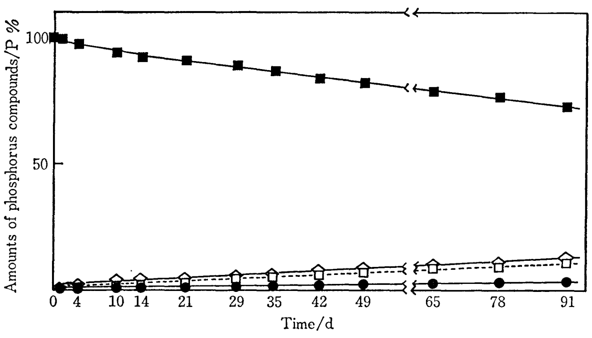
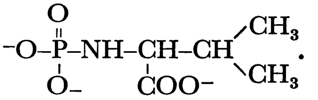


Fig. 2. Change of the amounts of phosphorus compounds in the reaction of P_{4m} with L-valine at room temperature and pH 12.
—●—: P_1-(N)Val, --□--: P_3, —◇—: P_4, —■—: P_{4m}.

Based on the position of the elution peak in the anion-exchange chromatography, and on the results of paper chromatography, ninhydrin reaction, and IR spectroscopy, Compound X was proved to be identical with the orthophosphate derivative of valine formed by the reaction of P_{3m} and L-valine, *i.e.*, an anion of *N*-(1-carboxy-2-methylpropyl)phosphoramidic acid (P_1-(N)Val),  $^{-}O\text{-}\underset{O^{-}}{\overset{\overset{O}{\|}}{P}}\text{-}NH\text{-}\underset{COO^{-}}{CH}\text{-}CH\begin{smallmatrix}\diagup CH_3\\ \diagdown CH_3\end{smallmatrix}$. Comparing the reaction of P_{4m} and L-valine with that of P_{3m} and L-valine, it was found that, though the same type of phosphate was obtained in both reactions, there were great differences in the yields of the phosphates formed and in the reaction rates.

In the reaction of P_{4m} with L-valine at 70 °C, we obtained the same products as those obtained by the reaction at room temperature, namely P_1, P_2, P_3, P_4, and P_1-(N)Val. Their amounts formed at 70 °C were, however, different from those formed at room temperature. P_3 was predominantly formed at the early stage of the reaction (3—14 d), while P_1 and P_2 were predominant at the later stage (more than 20 d). The formation of P_1-(N)Val was observed immediately after the start of the reaction, but its yield was as low as about 0.3% after 1 d and about 0.1% after 3 d. After 6 d and thereafter, it could not be observed at all. On the other hand, the residual amount of P_{4m} decreased very rapidly, becoming almost zero after 20 d. The fact that, at 70 °C, in spite of the rapid decrease of P_{4m}, P_1-(N)Val was formed in only a very small amount, suggests that the hydrolysis of P_{4m} itself may proceed in preference to the reaction of P_{4m} with L-valine.

It was found that, at room temperature, the reaction of P_{4m} with L-valine is slower than the reaction of P_{3m} with L-valine. This can be understood by analogy with the fact that, in an alkaline solution, the hydrolysis of P_{4m} is slower than that of P_{3m}.

The mechanism of the reaction of P_{4m} with L-valine at pH 12 is summarized in Fig. 3. A phosphorus of P_{4m} may be attacked by the lone pair of the nitrogen of L-valine; consequently, the ring-opening reaction of P_{4m} may proceed to form a tetraphosphate derivative of valine (P_4-(N)Val), but this compound is very unstable and is immediately hydrolyzed to produce a P_1-(N)Val and P_3. P_2, which is a minor component in the reaction products, may be produced by the hydrolysis of P_3 and P_4.

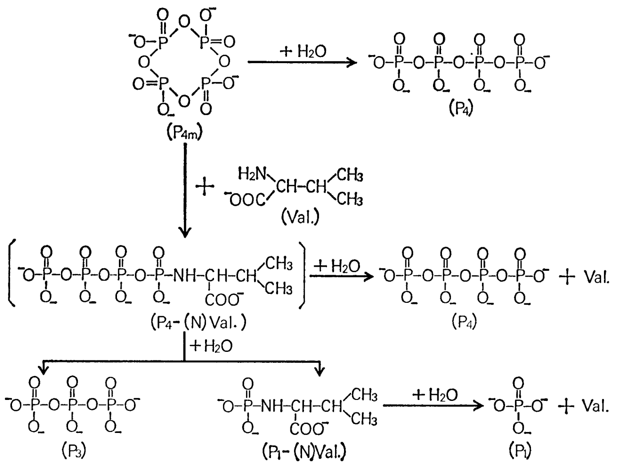


Fig. 3. Mechanism of the reaction of P_{4m} with L-valine.

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