colorless fluffy powder, yield 120 mg. Paper chromatography showed the presence of one major component (R_f 0.40) and two faint impurities with R_f values of 0.32 and 0.47, respectively. For purification, this product (110 mg.) was dissolved in $0.05M$ ammonium acetate buffer (20 ml.) and the solution was applied to a CMC column (1.5 × 20.0 cm.) which was eluted successively with these pH 6.5 ammonium acetate buffers: $0.05M$ (100 ml.), $0.075M$ (10 ml.), $0.10M$ (150 ml.) and $0.12M$ (150 ml.). Individual fractions (10 ml. each) were collected with an automatic fraction collector at a flow rate of 3 to 4 ml. per minute and absorbancy at 275 mμ was determined for each fraction. The desired material was present in the $0.10M$ eluates (tubes 21–29) which were pooled, evaporated to a small volume and lyophilized to constant weight; fluffy colorless powder, yield 91 mg. (74%), $[\alpha]^{28}$D $-90.0°$ (c 0.3 in 10% acetic

acid); sharp single spot ninhydrin, Sakaguchi and Pauly positive; R_f 0.40; amino acid ratios in acid hydrolysate lys$_{3.89}$pro$_{2.04}$val$_{2.98}$gly$_{1.02}$arg$_{1.87}$tyr$_{0.93}$ (98%); amino acid ratios in LAP digest N$^\epsilon$-formyl$_{4.22}$pro$_{2.11}$val$_{2.97}$gly$_{0.97}$arg$_{1.78}$tyr$_{0.86}$ (77%); amino acid ratios in trypsin plus LAP digest N$^\epsilon$-formyl$_{3.83}$pro$_{2.05}$val$_{3.08}$gly$_{1.00}$arg$_{2.02}$tyr$_{1.00}$ (84%).

Anal. Calcd. for $C_{82}H_{140}O_{24}N_{24}\cdot6H_2O$: C, 50.7; H, 7.3; N, 17.3. Found: C, 50.2; H, 7.9; N, 17.3.

[CONTRIBUTION FROM RESEARCH DEPARTMENT, VICTOR CHEMICAL WORKS, DIVISION OF STAUFFER CHEMICAL CO., CHICAGO HEIGHTS, ILL.]

Chemistry of Chloromethylphosphinic Acid. I. Preparation and Alkaline Hydrolysis

BY EUGENE UHING, KENNETH RATTENBURY AND A. D. F. TOY[1]

The rate of hydrolysis of the chlorine atom in chloromethylphosphinate was found to be much more rapid than that in the corresponding chloromethylphosphonate. The product of hydrolysis of chloromethylphosphinate in an excess of 1 to 15 N sodium hydroxide solution ($NaOH:ClCH_2P(O)(H)ONa$ = 2 to 5:1) was shown to be predominantly the methylphosphonate instead of the expected hydroxymethylphosphinate. A mechanism for this reaction is proposed. The chloromethylphosphinic acid, $ClCH_2P(O)(H)OH$, used for this study was obtained by the hydrolysis of chloromethylphosphonous dichloride, $ClCH_2PCl_2$. The latter was prepared through the sulfur exchange reaction between chloromethylphosphonothioic dichloride, $ClCH_2P(S)Cl_2$, and phenylphosphonous dichloride, $C_6H_5PCl_2$. The preparation of the required chloromethylphosphonothioic dichloride was carried out by the action of chloromethylphosphonic dichloride, $ClCH_2P(O)Cl_2$, on P_4S_{10} or $PSCl_3$.

Introduction

Previous studies[2] in this Laboratory have shown that the chlorine atom in sodium chloromethylphosphonate is rather inert toward substitution reactions. It has been reported in the literature[3] that the reaction of sodium chloromethylphosphonate with iminodiacetic acid in the presence of sodium hydroxide was very sluggish. Kabachnik and Medved[4] in their study of the conversion of chloromethylphosphonate to the aminomethylphosphonate stated that the "PO$_3$" grouping may be said to be passivating rather than activating. The low degree of activity of this chlorine atom in chloromethylphosphonate makes the preparation of derivatives through its replacement by organic substituents a difficult task.

It was thought that the derivatives of the chloromethylphosphonate might readily be prepared by using the chloromethylphosphinate as the starting material. Inasmuch as the chloromethylphosphinate ion bears a single negative charge while the chloromethylphosphonate ion carries a double negative charge, it was thought that the chlorine atom in the former would be more readily replaced as chloride ion by negative groups than in the latter. (Once the substituted phosphinate is obtained, it then can be readily converted to the phosphonate by oxidation reactions.) In order

to check this hypothesis, it is necessary to measure, experimentally, the relative reactivity of the α-chlorine atoms in these two compounds. As the first part of this study, the relative rates of hydrolysis of these two compounds in alkaline medium were measured.

Discussion

The rates of hydrolysis of chloromethylphosphinate and chloromethylphosphonate conducted in a refluxing aqueous alkaline system were measured by the rate of formation of the chloride ions. In experiments carried out by heating 2.28 g. (0.02 mole) of chloromethylphosphinic acid with 50 cc. of 1.2 N sodium hydroxide and 2.6 g. (0.02 mole) of chloromethylphosphonic acid, $ClCH_2P(O)(OH)_2$, with 50 cc. of 1.6 N sodium hydroxide, it was found that the chlorine atom in the phosphinate was liberated at a much faster rate than that from the phosphonate (Table I). These results indicated a possible way for the synthesis of various organic substituted methylphosphinates and phosphonates through the use of the chloromethylphosphinate as the starting material.
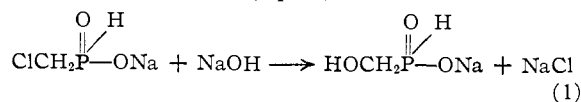
(1) To whom correspondence concerning this paper should be directed.

(2) E. N. Walsh, T. M. Beck and A. D. F. Toy, *J. Am. Chem. Soc.,* **78**, 4455 (1956).

(3) G. Schwarzenbach, H. Ackermann and P. Ruckstuhl, *Helv. Chim. Acta,* **32**, 1175 (1949).

(4) M. I. Kabachnik and T. Ya. Medved, *Izvest. Akad. Nauk S.S.S.R. Otdel. Khim Nauk,* 95 (1951).

TABLE I

	Cl⁻ formed, %	
Hours of reflux	$ClCH_2\overset{O}{\overset{\|}{P}}(ONa)_2$	$ClCH_2\overset{O}{\overset{\|}{P}}HONa$
1	5	86
2	10	93
3	14	96
15	58	..

In the hydrolysis of sodium chloromethylphosphinate, one would expect the product to be the

sodium salt of hydroxymethylphosphinic acid, a monobasic acid (eq. 1). The results of our

$$\underset{\substack{\text{O} \quad \text{H}\\ \backslash\!\!\!\diagup}}{ClCH_2P}-ONa + NaOH \longrightarrow \underset{\substack{\text{O} \quad \text{H}\\ \backslash\!\!\!\diagup}}{HOCH_2P}-ONa + NaCl$$

(1)

experiments, however, showed that when the hydrolysis was carried out in the presence of an excess of sodium hydroxide, the main product isolated after acidification was mainly a dibasic acid contaminated with a little monobasic acid. Subsequent analytical studies by means of acid–base titrations, elemental analysis, paper chromatographic and ion exchange separations, indicated that the product consisted of approximately 80–88% methylphosphonic acid and other minor impurities.

The formation of methylphosphonate in the hydrolysis of chloromethylphosphinate was unexpected. Methylphosphonate is a structural isomer of hydroxymethylphosphinate, the anticipated product from this hydrolysis reaction. It was thought that the methylphosphonate may have been formed by the intramolecular rearrangement of the hydroxymethylphosphinate in the refluxing sodium hydroxide solution used. This, however, was shown not to be the case by heating under reflux for 3 hours 1.08 g. (0.011 mole) of hydroxymethylphosphinic acid with 25 cc. of 1.34 N sodium hydroxide solution. Under these conditions, no methylphosphonate was detected, and no significant change in the original trivalent phosphorus content was noted. The formation of methylphosphonate in the hydrolysis reaction, therefore, must occur through a different mechanism. One mechanism consistent with the results would involve coördination of the hydroxide ion to the phosphorus atom, followed by an internal displacement of the hydride ion from the phosphorus atom to the carbon atom and simultaneous displacement of the chloride ion from the carbon atom to the solvent. The over-all reaction is as shown in eq. 2.

$$\begin{bmatrix} O\bar{H} & & \\ & \searrow & O \\ & & \parallel \\ ClCH_2PO & & \\ \diagup & \nwarrow & H \end{bmatrix}$$

$$\underset{\substack{\text{O} \quad \text{H}\\ \backslash\!\!\!\diagup}}{ClCH_2P}-OH + 3NaOH \longrightarrow \underset{\substack{\text{O}\\ \parallel}}{CH_3P}(ONa)_2 + NaCl$$

(2)
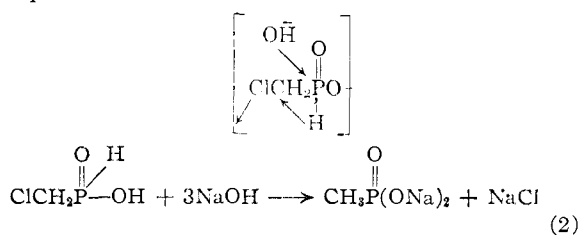
The competitive reaction is the displacement of the chloride ion by the hydroxide ion to form a small percentage of hydroxymethylphosphinate as expected from eq. 1.

The displacement of the hydride ion by the hydroxide ion is analogous to the reaction of sodium hypophosphite in hot, aqueous sodium hydroxide solution which results in the formation of disodium phosphite and hydrogen.[5] It is also analogous to the rearrangement of the hydride ion from chloromethyldimethylsilane to form trimethylsilanol and potassium chloride in aqueous potassium hy-

droxide solution.[6] In the alkaline hydrolysis of chloromethylphosphinate it was found that larger quantities of methylphosphonate were formed in the reaction using an excess of the alkali or by the addition of the chloromethylphosphinate to the refluxing alkaline solution. This result shows the necessity of a large concentration of the hydroxide ion in order to displace the more basic hydride ion. This displacement reaction appears to be the slow and the rate-controlling step while the displacement of the chloride ion by the hydride ion through internal hydride shift apparently constitutes the fast step. However, in the presence of an excess of the hydroxide ion, the reaction involving the direct displacement of the chloride ion, by the hydroxide ion in chloromethylphosphinate, eq. 1, must be slower than the above two reactions and that is why, in the presence of an excess of alkali, the formation of hydroxymethylphosphinate occurs only to the extent of 12–20%.

The reaction involving the displacement of the hydride ion in chloromethylphosphinate by the hydroxide ion is accelerated by the large concentration of the latter and by the subsequent fast displacement of the chloride ion. Apparently, the concentration of hydroxide ion needed to displace a hydride ion depends on the stability of the hydride ion in a particular compound. For example, our experimental evidence showed that even the hydrogen bound to phosphorus in hydroxymethylphosphinate can be displaced by hydroxide ion. However, in this case the concentration of the hydroxide ion needed for such a displacement reaction is much greater than that required for the chloromethylphosphinate. For example, when 15.5 g. (0.161 mole) of hydroxymethylphosphinic acid was heated to reflux at 130–132° with 50 cc. of 18 N sodium hydroxide, the evolution of hydrogen was very rapid. After completion of the reaction, the solution was acidified. It was found that of the original 31.3% trivalent phosphorus, only 3.3% remained. The major reaction product isolated in 80% yield through its lead salt was shown to be hydroxymethylphosphonic acid.[7,8] In another example, the hydrogen bound to phosphorus in disodium phosphite is sufficiently stable that it is not possible to liberate it even when the phosphite is heated to 130° in an 18 N sodium hydroxide solution. Blaser and Worms[9] recently reported that only by heating for 6 hours in 85% NaOH is the phosphite quantitatively oxidized to the orthophosphate with the evolution of hydrogen. In order to check the hypothesis that the hydride shift is an intramolecular and not an intermolecular reaction, a model experiment was carried out, namely, the reaction of sodium chloromethylphosphonate with sodium hypophosphite in the presence

(5) D. M. Yost and H. Russell, Jr., "Systematic Inorganic Chemistry," Prentice Hall, Inc., New York, N. Y., 1944, p. 192.

(6) L. H. Sommer, W. P. Barie, Jr., and D. R. Weyenberg, J. Am. Chem. Soc., 81, 251 (1959).

(7) G. M. Kosolapoff. "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 130 and 156.

(8) Hydroxymethylphosphonic acid was also synthesized by (1) the hydrolysis of chloromethylphosphonic acid with 20 M sodium hydroxide solution at 120°, followed by acidification (yield 97%); (2) the oxidation of hydroxymethylphosphinic acid with mercuric chloride in aqueous solution under reflux.

(9) B. Blaser and K. H. Worms, Z. anorg. allgem. Chem., 300, 229 (1959).

of an excess of aqueous sodium hydroxide. The displaced hydride ions from the hypophosphite did not react with the α-chlorine atom of the chloromethylphosphonate. Instead, it reacted with the solvent and the hydrogen gas liberated and collected was quantitative.

The hypothesis that the displacement of the more basic hydride ion in the chloromethylphosphinate by the less basic hydroxide ion is a slow one which necessitates the use of a large concentration of hydroxide ions to drive it forward, was checked also by the hydrolysis of chloromethylphosphinate in an aqueous suspension of calcium hydroxide (pH of saturated $Ca(OH)_2$ solution at room temperature $= 12.3$). In this reaction, after four hours of heating under reflux, 96% of the chlorine atoms was replaced. The product obtained after acidification and isolation consisted of approximately 39% hydroxymethylphosphinic acid and 53% of its reaction product with chloromethylphosphinate, the α,α'-bis-(phosphinyl)-dimethyl ether. In other words, in the presence of a smaller concentration of hydroxide ions, the hydroxide–chloride displacement reaction as shown in eq. 1 became the predominant one.

The hydrolysis of chloromethylphosphinate by a weaker base was also checked by using magnesium hydroxide as the base (pH of saturated aqueous $Mg(OH)_2$ at room temperature $= 10.3$). After 62 hours of heating at reflux only 68% of the chlorine atoms was liberated as chloride ions. Paper chromatographic analysis showed that the product of hydrolysis was much higher in hydroxymethylphosphinic acid content than in methylphosphonic acid.

The effect of a lower concentration of hydroxide ion on the hydrolysis was also shown by maintaining the reaction at a pH of 11.6 to 11.7 through the addition of a controlled quantity of sodium hydroxide solution to the chloromethylphosphinate. This reaction was stopped after thirty hours, at which time one equivalent of NaOH had been consumed by one equivalent of the chloromethylphosphinate. Paper chromatographic analysis of the reaction mixture showed that 46.7% of the chloromethylphosphinate remained unreacted. Of the reacted portion, the products of reaction showed the ratio of P^{5+} to P^{+3} compounds to be 1:0.52, a figure considerably smaller than when more concentrated sodium hydroxide was used.

Experimental

A. Preparation of Reactants and Intermediates. 1. Chloromethylphosphinic Acid.—Freshly redistilled chloromethylphosphonous chloride (502 g., 3.32 moles) was added dropwise at 20–30° to 400 cc. of concentrated HCl under a nitrogen atmosphere. The reaction mixture was warmed to 50° for 15 minutes and then the water and HCl were removed, first under water aspirator vacuum and finally by heating to 90° at 1–2 mm. A water-white, viscous liquid product weighing 375 g. (99% of theory) was obtained, n^{25}D 1.4905, d^{25}_{25} 1.5369.

$$\text{O}$$
$$\underset{\text{H}}{\overset{\parallel}{\text{Anal. Calcd. for ClCH}_2\text{P—OH}}}: \text{P, 27.0; Cl, 31.0;}$$

neut. equiv., 114.5. Found: P, 27.0; Cl, 31.0; neut. equiv., 113.1.

A titration curve showed the compound to be a monobasic acid. Attempts to obtain it in crystalline form by
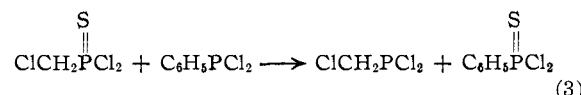
cooling have thus far not been successful. It became a glass at $-40°$. Assaying the product from several preparations by mercuric chloride oxidation indicated that it is at least 96–98% pure.

2. Sodium Chloromethylphosphinate.—A solution of 5.72 g. (0.05 mole) of chloromethylphosphinic acid dissolved in 20 cc. of ethyl alcohol was titrated with 12 N NaOH to a phenolphthalein end-point at 25°. The solution was evaporated to dryness by heating at 30° at 1 mm. The product obtained had a weight which corresponded to that expected for the anhydrous salt. The solid obtained was analyzed. This salt decomposes with the liberation of a gas at a temperature above 124°.

$$\text{O}$$
$$\overset{\parallel}{}$$

Anal. Calcd. for $\text{ClCH}_2\text{PHONa}$: P, 22.70; Cl, 25.95. Found: P, 22.40; Cl, 25.90; free Cl$^-$ ion, 0.8.

3. Chloromethylphosphonous dichloride was prepared by the sulfur exchange reaction as shown in eq. 3.

$$\underset{}{\overset{\text{S}}{\overset{\parallel}{\text{ClCH}_2\text{PCl}_2}}} + C_6H_5PCl_2 \longrightarrow ClCH_2PCl_2 + \underset{}{\overset{\text{S}}{\overset{\parallel}{C_6H_5PCl_2}}}$$
$$(3)$$

The mixture of 1437 g. (7.85 moles) of chloromethylphosphonothioic dichloride and 1612 g. (9.05 moles or 15% excess) of phenylphosphonous dichloride was heated at reflux (175–165°) under an atmosphere of nitrogen for 3 hours. It was then cooled and fractionally distilled; the fraction collected boiling at 67–120° at 100 mm. was then carefully fractionated under atmospheric pressure. The fraction collected boiling at 128–132° weighed 996.4 g. or 84% of the theory. The pure material has a b.p. of 129° at atmospheric pressure; n^{25}D 1.5282, d^{20}_{20} 1.5209, d^{25}_{25} 1.5144.

Anal. Calcd. for $\text{ClCH}_2\text{PCl}_2$: P, 20.4; Cl$^-$, 46.8; total Cl, 70.2, Found: P, 20.4; Cl$^-$, 46.5; total Cl, 69.2.

The compound is readily oxidized and hydrolyzed when exposed to the atmosphere. It is reconverted to chloromethylphosphonothioic dichloride when heated with sulfur in the presence of a small amount of Al_2Cl_6 catalyst to reflux at 140–193° under a CO_2 atmosphere.

The preparation of chloromethylphosphonous dichloride was first reported by Yakubovich and Ginsberg.[10] They prepared it by the action of diazomethane on phosphorus trichloride at $-70°$; along with various by-products, the desired product was obtained in 40% yield. The reported constants are: b.p. 80–81° (140 mm.), d^{20}_{20} 1.5289, n^{20}D 1.5247.

4. Chloromethylphosphonothioic Dichloride. (a) By Reaction of $\text{ClCH}_2\text{POCl}_2$ with P_4S_{10}.—Subsequent to the completion of this phase of the work in our laboratory, Kabachnik and his co-workers[11] reported the independent discovery of this reaction for the preparation of a series of phosphono- and phosphinothioic chlorides from the corresponding phosphonic and phosphinic chlorides by the use of P_4S_{10} as the reagent. Chloromethylphosphonothioic dichloride was included in the series. Since they did not present the experimental details for obtaining this compound, our method of preparation is included.

A mixture of 625 g. (1.4 moles or 17% excess) of commercial, distilled P_4S_{10} and 2000 g. (12 moles) of chloromethylphosphonic dichloride[3] was heated to reflux at 174–179° for 6 hours with stirring, and then distilled at 50–110° under 40 to 10 mm. of pressure. Near the end of the distillation, the residue became quite thick, necessitating the stopping of the stirrer and the distillation. The crude chloromethylphosphonothioic dichloride thus obtained was purified from the contaminating unreacted chloromethylphosphonic dichloride by adding dropwise with vigorous stirring 400 cc. of water at 20–25°. The water-insoluble $\text{ClCH}_2\text{PSCl}_2$ was separated from the aqueous layer and fractionally distilled under reduced pressure, first to take off the adhering water and then the product. The fraction collected had b.p. 91–93° at 30 mm. and weighed 1437 g. (65%). A purified sample had n^{25}D 1.5741, d^{25}_{25} 1.5891, b.p. 89° (30 mm.).

(10) A. Ya. Yakubovich and V H. Ginsberg. *Zhur. Obshchei Khim.* **22**, 1534 (1952).

(11) M. I. Kabachnik and N. N. Godovikov, *Doklady Akad. Nauk, S.S.S.R.*, **110**, 217 (1956).

Anal. Calcd. for $ClCH_2PSCl_2$: P, 16.9; Cl. 58.0; S, 17.4. Found: P, 17.1; Cl, 58.0; S, 17.1.

The thick residue, which consisted mostly of a mixture of P_4O_{10}, P_4S_{10} and phosphorus oxysulfides, obtained in the first distillation, may be eliminated by treatment with phosphorus pentachloride. For example, after the completion of the reaction between $ClCH_2POCl_2$ and P_4S_{10}, the mixture was cooled to room temperature, and for a reaction using 0.15 mole of P_4S_{10}, 0.925 mole of PCl_5 was added and the mixture was heated gradually to reflux at 125° for 1 hour. The product was then fractionated. In a typical run the product obtained on the first fractionation gave a yield of 90% of the 94% pure material. The purity in this case was estimated from the index of refraction and sulfur analysis, on the assumption that the contaminating impurity was $ClCH_2PCl_2$.

$$\overset{O}{\underset{\|}{}}$$

(b) By the Reaction of $ClCH_2POCl_2$ with $PSCl_3$.—A solution consisting of 83.8 g. (0.5 mole) of $ClCH_2PCl_2$ and 169.5 g. (1 mole) of $PSCl_3$ was heated with shaking under 300 lb. of N_2 pressure in a glass liner in a pressure autoclave for 5.5 hours at 200 ± 10°. Phosphorus oxychloride and phosphorus thiochloride were removed by heating to a liquid temperature of 187° and the vapor temperature of 129° at atmospheric pressure. The residue thus obtained was treated with stirring with 18 cc. of water at 15–20°. The mixture was then washed first with 50 cc. and then with 100 cc. of ice-water. The organic layer was dried over $CaCl_2$ and fractionally distilled. A yield of 51.2 g. (53.7%) of the roduct was obtained, $n^{25}D$ 1.5739, d^{25}_{25} 1.589.

Anal. Found: P, 16.8; Cl, 58.0; S, 16.9.

When the above reaction was carried out at a temperature below 175°, the reaction went much slower, whereas at a temperature above 250°, a lowering of the yield was observed.

5. Hydroxymethylphosphinic acid was prepared by the action of aqueous formaldehyde on hypophosphorous acid, a reaction analogous to that used for the preparation of α-hydroxyethylphosphinic acid.[12]

$$\overset{O\ \ H}{\underset{\|/}{HOCH_2P-OH}}$$

Anal. Calcd. for $HOCH_2P$—OH: P, 32.3; OH, 17.7. Found: P, 30.4; OH, 18.

A titration curve showed the product to be monobasic acid. Paper chromatographic separations showed that it contained 97% hydroxymethylphosphinic acid, 0.9% unreacted hypophosphorous acid and 2.1% phosphorus-containing impurities.

B. Hydrolysis of Chloromethylphosphinic Acid and its Salts. 1. Hydrolysis with an Excess of Sodium Hydroxide.

To 80 g. (2 moles) of sodium hydroxide dissolved in 150 cc. of water was added slowly with stirring a solution of 136.5 g. (1 mole) of sodium chloromethylphosphinate in 110 cc. of water and the mixture was heated to 100° under a nitrogen atmosphere. The reaction was exothermic and very little external heat was needed to maintain the temperature at 100°. After the completion of the addition of the phosphinate (2 hours), the mixture was heated for 3 more hours at 100°. It was then cooled, acidified with an excess of concd. HCl (200 cc., 2.4 moles), evaporated to dryness under reduced pressure, and extracted with a total of 1 liter of ethyl alcohol. The alcoholic extract was evaporated under reduced pressure to a final temperature of 100° at 1 mm. The viscous liquid residue thus obtained became a mushy solid on standing. It weighed 94.2 g. and contained 30.8% P and negligible amounts of chlorine. The various components in the product were separated by paper chromatography and the separated components analyzed. The results of this analysis showed that the hydrolyzed material contained 81.4% methylphosphonic acid and 18.6% hydroxymethylphosphinic acid. The determination of hydroxymethylphosphinic acid content by chromic acid oxidation and by ion exchange separation, however, showed that it was present only to the extent of 13.2 to 12.3%. The fact that the product was mainly methylphosphonic acid was confirmed also by the conversion to methylphosphonic dichloride as described below.

To 72.4 g. of the above reaction product was added slowly with stirring, 236 g. (2 moles) of thionyl chloride at 20–30°. A large quantity of HCl was evolved. The reaction mixture was fractionated under reduced pressure. In the first distillation, a fraction was collected, boiling at 63–87° at 50 mm. This fraction was refractionated, the portion coming over at 100–110° at 100 mm. being collected. It weighed 76.1 g. and became a solid on standing, m.p. 30–32°.[13] The theoretical yield of methylphosphonic dichloride based on the methylphosphonic acid content of the starting crude material as determined by paper chromatography is 81.6 g.

Anal. Calcd. for CH_3POCl_2: P, 23.3; Cl, 53.4. Found: P, 23.0; Cl, 53.6.

The methylphosphonic dichloride thus obtained was further identified by hydrolysis in water. Upon evaporation of this hydrolyzate, a product (m.p. 103–104°) was obtained which was analyzed. Paper chromatographic separation indicated only one component.

$$\overset{O}{\underset{\|}{}}$$

Anal. Calcd. for $CH_3P(OH)_2$: P, 32.3; neut. equiv., 48. Found: P, 32; neut. equiv., 48.

In order to be sure that the formation of hydroxymethylphosphinate is due to a competing reaction which occurs even when a large excess of sodium hydroxide is present, and that this competing reaction did not occur only near the end of the reaction when the sodium hydroxide was near depletion, another hydrolysis reaction was carried out by the addition of 0.2 mole of the phosphinate to 0.8 mole of NaOH. This amount of NaOH represents 100% excess beyond that required to convert the chloromethylphosphinate to the methylphosphonate. Analytical studies of this reaction product showed that, in spite of the excess sodium hydroxide used, there was still formed about 12.5% of hydroxymethylphosphinate.

When the hydrolysis of sodium chloromethylphosphinate in an excess of aqueous sodium hydroxide was carried out by adding the reactants together at room temperature and then slowly heating the mixture, the reaction became vigorously exothermic at around 70°, necessitating the use of an ice–water-bath coolant to maintain the temperature at 70°. After the reaction had subsided, the reaction mixture was heated slowly to 100°. After a total heating of about 3 hours, analysis showed that 99.3% of the original chlorine present in chloromethylphosphinic acid had become ionic chlorine.

After isolation, the product obtained from this hydrolysis was shown to consist mostly of methylphosphonic acid contaminated with some hydroxymethylphosphinic acid. Similar results were obtained by the direct hydrolysis of one mole of chloromethylphosphonous dichloride in an aqueous solution of five moles of sodium hydroxide, first at 20°–30° and then at reflux for 2 hours at 100°.

2. Hydrolysis with Dilute Sodium Hydroxide at pH 11.6 to 11.7.—Five and seven-tenths grams (0.05 mole) of chloromethylphosphinic acid, dissolved in 300 cc. of water, was neutralized with 0.05 mole of NaOH. The solution was then heated to 90° and increments of 0.25 N NaOH solution were added at hourly intervals in order to maintain the solution at pH 11.6–11.7. The pH value was measured by the immersion of a calomel electrode from a pH meter into the solution. After 30 hours a total of 200 cc. of 0.25 N (0.05 mole) NaOH had been added. The product after isolation was separated by paper chromatography. The actual chromatogram showed the following compounds, in the order as obtained on the chromatogram: unreacted

$$\overset{O\ \ H}{\underset{\|/}{ClCH_2P-OH}},\ 47\%;\quad \overset{O}{\underset{\downarrow}{HOCH_2PHOH}},\ 7\%;\quad \overset{O}{\underset{\|}{O(CH_2PH}}$$

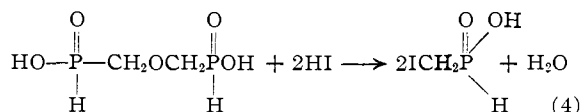$OH)_2$, 11%; $CH_3P(OH)_2$, 29%; unidentified p^{+5} cmpd., 6%. From these data, it is evident that the ratio of methylphosphonic to hydroxymethylphosphinic is considerably less than when concentrated sodium hydroxide was used for the hydrolysis. The α,α'-bis-(phosphinyl)-dimethyl ether was probably formed by the coupling of the hydroxymethylphosphinate with the unreacted chloromethylphosphinate in the presence of an excess of alkali. The identi-

(12) J. Villa, *Ann. phys. chim.*, **28**, 289 (1891).

(13) Reference 7, p. 73.

fication of this compound was made first through separation by paper chromatography and then by a gradient elution ion exchange method.

A paper chromatographic solvent consisting of 80 ml. of acetone, 25 ml. of water and 5 ml. of ammonium hydroxide was found effectively to resolve chloromethyl-, hydroxymethyl- and methylphosphinic acid from each other, as well as from all the phosphonic acids, phosphorous acid, hypophosphorous acid and the ether compound. The ether compound was found to be a trivalent phosphorus compound that titrated as a monobasic acid. By infrared analysis, and by the acetic anhydride method, it was shown that it did not contain any alcoholic OH group. A sample of the ether was isolated by gradient elution through a Dowex anion exchange resin column by means of $1\ M$ KCl solution. The eluted fractions were checked by paper chromatograms. The fraction containing this compound had the same R_f value as that assigned to this fraction in the original paper chromatogram of the reaction mixture. It was evaporated, weighed, and the KCl content determined by flame photometry for potassium and silver nitrate titration for chloride. The phosphorus and phosphinate values were determined by the colorimetric vanadate and $HgCl_2$ procedures. Using these values and accounting for the presence of KCl, the respective equivalent weights of this compound were 88 and 89. The theoretical equivalent weight as shown by the formula is 87.

Another sample of this compound was isolated by ion exchange separation, and an ether determination was made by the standard reaction with HI in accordance with eq. 4.

$$HO\!-\!\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle H}{|}}{P}}\!-\!CH_2OCH_2\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle H}{|}}{P}}OH + 2HI \longrightarrow 2ICH_2\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle H}{\diagdown}}{P}}\!\!\nearrow^{OH} + H_2O \qquad (4)$$

The excess HI and KCl were determined by direct titration with $AgNO_3$ on one half of the sample. The other half of the sample was boiled with NaOH to liberate the iodine atom from iodomethylphosphinate as iodide ion. The total iodide was then titrated with $AgNO_3$. The difference in the $AgNO_3$ titration gave an equivalent weight of 91 for the compound.

3. Hydrolysis with Calcium Hydroxide.—To 57 g. (0.5 mole) of chloromethylphosphinic acid in 200 cc. water was added portionwise 41 g. (0.55 mole) of $Ca(OH)_2$. The mixture was heated to 100°. After 3 hours, 80% of the chlorine had become ionic. After a total of 5 hours, 97% of the chlorine atoms in the original chloromethylphosphinic acid had been ionized. If appreciable dibasic acid, such as methylphosphonic acid, has been formed, there would not

have been sufficient $Ca(OH)_2$ for the hydrolysis reaction to go to completion.

The reaction mixture was worked up by the addition of one mole of sodium hydroxide in 200 cc. of water at room temperature to convert the calcium salts to $Ca(OH)_2$. The solution was filtered to remove the precipitated $Ca(OH)_2$, and that $Ca(OH)_2$ remaining in solution was removed by bubbling CO_2 through the filtrate and filtering the $CaCO_3$ formed. The sodium salts were then converted to the free acid with concd. HCl and worked up as described in the NaOH hydrolysis.

The product thus obtained weighed 44 g. It contained 33.3% P and negligible amounts of Cl. Upon separation by a gradient elution ion exchange method, it was shown to contain 39% hydroxymethylphosphinic acid and 53.0% of α,α'-bis-(phosphinyl)-dimethyl ether, the remainder being a phosphonic acid which was assumed to be methylphosphonic acid.

4. Hydrolysis with $Mg(OH)_2$.—To 22.8 g. (0.2 mole) of chloromethylphosphinic acid dissolved in 200 cc. of H_2O was added 17.5 g. (0.3 mole) of magnesium hydroxide. The mixture was heated to reflux for 62 hours. A sample of the mixture was oxidized with $HgCl_2$ and results indicated that 96% of the phosphorus present was still in the tripositive state. The reaction mixture was worked up by adding 0.4 mole of NaOH dissolved in 100 cc. of H_2O to convert the magnesium salts to $Mg(OH)_2$, which was removed by filtration. The resulting solution was then worked up as described for the hydrolysis in $Ca(OH)_2$ solution. The product thus obtained weighed 16.8 g. Elemental analysis showed 29.3% P and 10.6% Cl. Paper chromatographic analysis showed that the following compounds were present. These compounds are given in the order in which they appeared in the chromatogram: $ClCH_2\!-$

$$\overset{\overset{\displaystyle O}{\|}}{P}HOH,\ 37.0\%;\quad HOCH_2\underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle O}{\|}}{P}}HOH,\ 39.1\%;\quad O\!\left(CH_2\overset{\overset{\displaystyle O}{\|}}{P}\!\!\diagup^{H}_{\diagdown OH}\right)_{\!\!2}$$

16.0%; $CH_3P(OH)_2$ and unidentified cmpd., 8.0%.

[CONTRIBUTION FROM TUFTS UNIVERSITY SCHOOL OF MEDICINE, BOSTON, MASS.]

The Incorporation of the Four Nitrogen Atoms of Purines into the Pyrimidine and Pyrazine Rings of Riboflavin[1]

BY WALTER S. MCNUTT

Experiments involving the use of N^{15}-adenine and xanthine labeled with N^{15} and C^{14} indicate that all the atoms of the purine ring except the carbon atom in position 8 are incorporated as a unit into riboflavin by the yeast, *Eremothecium ashbyii*.

Earlier studies suggested that naturally occurring purines may serve as donors of the pyrimidine ring in the biosynthesis of riboflavin and pteridines by *Eremothecium ashbyii*.[2-5] This direct biosynthetic relationship of purines to the pyrimidine rings of other molecules contrasts with the lack of a precursor relationship between the purines and pyrimidines of polynucleotides. As previous studies of this conversion dealt with purines labeled in the carbon atoms only, it was not evident whether any nitrogen in the imidazole ring of the purine was incorporated into riboflavin, and suggestions that a diaminopyrimidine derivative might arise biologically from the purine and serve as a precursor of riboflavin[2,6,7] were thus mere speculations. Although enzyme preparations from microorganisms

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