61. A New Synthesis of Riboflavin-5'-phosphate

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Some reports on the synthesis of riboflavin-5'-phosphate have been published by R. Kuhn et al.¹⁾, P. Karrer et al.²⁾ and others^{3,4)} since nearly twenty years ago, but the pure substance was not isolated.

Recently, L. A. Flexner and W. G. Farkas⁵⁾ have prepared this phosphate by the action of a mixed solution of phosphorousoxychloride and water to riboflavin, and separated it as crystalline diethanolamine salt. P. Karrer et al.⁶⁾ have also reported that they obtained pure crystalline phosphate by reacting metaphosphoric acid to riboflavin at 50~60°C.

The authors investigated a new synthetic method by using alkyl-dichlorophosphate ($R=CH_3$ or C_2H_5) instead of phosphorousoxychloride or metaphosphoric acid which is easily removed from the reaction system in vacuo. Riboflavin-5'-monoalkylphosphate which is considered as an intermediate could not be isolated in pure state, but, the reaction product includes undoubtedly methoxy- or ethoxy group and these groups are easily eliminated by acid hydrolysis^{7,8,9)}.

By this method, riboflavin-5'-phosphate is obtained more readily and is yielded better than by the methods mentioned above.

Experimental

Alkyldichlorophosphate^{10,11,12)} ($R=CH_3$ or C_2H_5) is prepared by mixing the same mols of phosphorousoxychloride and absolute methyl or ethyl alcohol under sufficient cooling. One part of riboflavin is dissolved in thirty parts of undistilled monoalkyl-dichlorophosphate heating at 40°C and the solvent is then removed in diminished pressure heating at 45~65°C. The residual hard syrup coloured in yellowish brown is rapidly crystallized by stir up with small amount of absolute alcohol. This yellow crystal is hydrolysed by normal hydrochloric acid heating at 95~100°C for 10 minutes and then treated in the same manner as P. Karrer et al.⁶⁾ The yield of crystalline crude phosphate is about 90%.

The salts of the phosphate and amino acid such as L-arginine, L-lysine and L-histidine have obtained. Each of these compositions showed the ratio of 1 mol of the phosphate to $\frac{1}{2}$ mol of L-arginine (Calcd. N; 15.5, P; 1.71, Found, N; 15.01, P; 5.75) and 1 mol of L-lysine (Calcd. N; 13.95, P; 5.15, Found, N; 14.45, P; 5.5) and

L-histidine (Calcd. N; 16.01, P; 5.06, Found, N; 15.85, P; 5.09) respectively.

These salts and the free phosphate showed no definite melting point and these are very soluble in water and more insoluble in alcohol than the sodium salt. These properties, therefore, are applicable to the separation and purification of riboflavin-5'-phosphate.

The syrup described above, on the paper chromatography⁶⁾¹³⁾ in water-isoamylalcohol system, shows three spots in which R_f values are 0.41~0.42 (I), 0.57~0.58 (II) and 0.84~0.86 (III) respectively. (I) is unchanged riboflavin and very small compared with the other spots. (II) may be riboflavin-5'-monoalkylphosphate. Being examined again with 5% aqueous disodium hydrogen phosphate-isoamyl alcohol system, (III) is divided in two spots in which R_f values are 0.65~0.66 and 0.79 respectively. The former is riboflavin-5'-phosphate and the latter may be diphosphate.

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References

- 1) R. Kuhn, H. Rudy, and F. Weygand: Ber., **69**, 1543 (1936). R. Kuhn and H. Rudy: Ber., **69**, 1974 (1936).
- 2) P. Karrer, P. Frei, and H. Meerwein: Helv. Chim. Acta, **20**, 79 (1937).
- 3) D. R. P. 809884, 11 Mar. 1937.
- 4) U. S. P. 2535385, 9 Jan. 1947.
- 5) L. A. Flexner and W. G. Farkas: Chem. Eng. News, **29**, 3947 (1951).
- 6) M. Viscontini, C. Ebnather, and P. Karrer: Helv. Chim. Acta, **35**, 457 (1952).
- 7) O. Bailly: Compt. rend., **208**, 443 (1939).
- 8) O. Bailly and J. Gaume: Compt. rend., **199**, 793 (1934); **198**, 2258 (1934).
- 9) A. Desjokert: Bull. soc. chim., 809 (1947).
- 10) H. Wichelhaus: Ann. Suppl., **6**, 257 (1868).
- 11) R. M. Calven: J. Chem. Soc., **81**, 1368 (1902).
- 12) B. C. Saunders, G. J. Stacey, F. Wild, and I. G. E. Wilding: J. Chem. Soc., 699 (1948).
- 13) It was performed by the ascending method using the No. 50 filter paper made by Toyo Filter Paper Co. Ltd.