[7] H. F. Deutch and J. I. Morton, *J. Biol. Chem.*, 231 (1958) 1107.
[8] R. K. Brown, W. H. Baker, A. Peterkovsky and D. L. Kauffman, *J. Am. Chem. Soc.* 76 (1954) 4244.
[9] O. Smithies, *Biochem. J.*, 61 (1955) 629.
[10] M. D. Poulik and O. Smithies, *Biochem. J.*, 68 (1958) 636.
[11] M. Schonenberger, R. Schmidtberger and H. E. Schultze, *Z. Naturforsch.*, 13b (1958) 761.
[12] H. Isliker, *Helv. Med. Acta*, 25 (1958) 41.
[13] O. Smithies, *Biochem. J.*, 71 (1959) 585.
[14] M. D. Poulik, *Nature*, 180 (1957) 1477.
[15] O. Smithies and G. E. Connell, in *Ciba Foundation Symposium on Biochemistry of Human Genetics*, Little Brown & Company, New York, 1959, p. 178.

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Polarographic identification of the reactive form in the hydrolysis of pyridoxal 5-phosphate

The monoanion of phosphoric acid monoalkyl and monoaryl esters undergoes hydrolysis more easily than the undissociated form or the dianion[1]. In view of its biochemical importance, the hydrolysis of pyridoxal 5-phosphate was studied, using

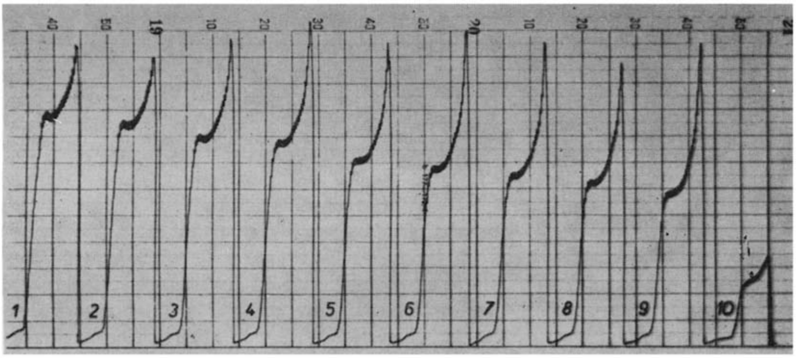


Fig. 1. Polarographic record of the course of hydrolysis of pyridoxal 5-phosphate in 0.01 M $HClO_4$ at $I = 1.0$, and at 79°. $5 \cdot 10^{-4}$ M pyridoxal 5-phosphate after: (1) 15; (2) 23; (3) 41; (4) 50.5; (5) 79; (6) 94.5; (7) 110; (8) 125 (9) 152 min. Curve 10, $5 \cdot 10^{-4}$ M pyridoxal. Curves were registered on a pen-recording polarograph, constructed by Institut für Gerätebau DAW, Berlin, D.D.R. Full scale deflection, 10 μA.

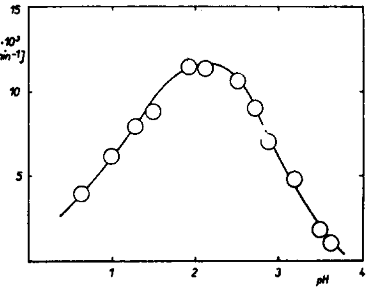
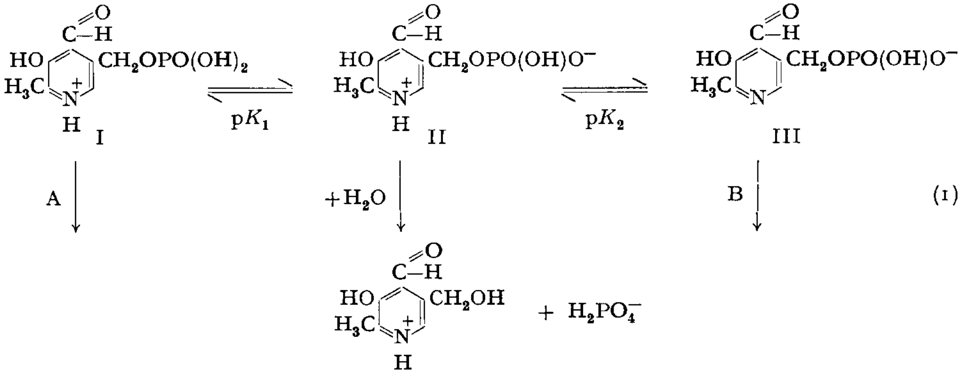


Fig. 2. The dependence of the rate constant of hydrolysis of pyridoxal 5-phosphate on pH at 87.8° and $I = 1.0$.

the polarographic method. In $HClO_4$ solutions and in buffers at pH 1-4 (I, 1.0) at 60-90° the decrease of the diffusion current of pyridoxal 5-phosphate was measured (Fig. 1). Pyridoxal formed in this reaction gives under these conditions a kinetic current[2,3] which represents only 10-30 % of the total wave height, and does not interfere with the measurements. The pH-dependence of the first-order rate constant (Fig. 2) shows a maximum at pH 2.1. From the rising part of the curve the value of the first dissociation constant $pK_1 = 1.5$, corresponding to the dissociation of the first proton from the phosphoric acid part of the molecule, was estimated. This value is in accordance with the pK_1 for monobenzyl phosphate[4]. The declining part of the curve lies in the region of $pK_2 = 3.65$ (determined potentiometrically). This value is in the region of pK values found with other pyridine derivatives. For phosphoric acid aryl esters no pK value has been found in this region[4,5]. The pK_2 value is attributed to the dissociation of a proton from the pyridine nitrogen, thus:



(1)

The reactive form is II. The increase of the rate of hydrolysis at pH < 2 is due to the formation of II from I; the decrease at pH > 3 is due to a diminishing concentration of II owing to formation of III. The independence of the hydrolysis rate on the type and concentration of the buffer and the small increase of the rate constant with increasing ionic strength are in agreement with this scheme. Reactions A and B are unimportant under the conditions used.

Full details of this reaction, important for understanding vitamin B_6 reactions, will be given elsewhere.

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[1] C. A. VERNON, *Chem. Soc. Special Publ. No. 8* (1957) 17.
[2] J. VOLKE, *Z. phys. Chem.*, Sonderheft (1958) 268.
[3] O. MANOUŠEK AND P. ZUMAN, *J. Electroanal. Chem.*, 1 (1960) 324.
[4] J. KUMAMOTO AND F. H. WESTHEIMER, *J. Am. Chem. Soc.*, 77 (1955) 2515.
[5] P. W. C. BERNARD, C. A. BUNTON, D. R. LLEWELLYN, K. G. OLDHAM, B. L. SILVER AND C. A. VERNON, *Chem. & Ind.*, (1955) 760.

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