and Schmidt and Levene.[4] The latter authors considered the thermostable pancreatic nuclease to have only a depolymerizing effect on nucleic acid without the production of Cellophane dialyzable products such as mononucleotides. Hence they named the enzyme "ribonucleodepolymerase." However, as mentioned before, the split products formed from yeast nucleic acid by the new crystalline protein were found to diffuse readily through collodion and Cellophane membranes; hence the more general term "ribonuclease" has been provisionally retained for this crystalline protein until more definite information becomes available about the chemical nature of the process.

The procedure for the isolation of the crystalline protein follows: Fresh beef pancreas is extracted with cold 0.25 N sulfuric acid, as described in the publications on the isolation of chymotrypsinogen and trypsinogen.[5] The acid extract is brought to 0.7 saturation with solid ammonium sulfate and filtered. The filtrate is then brought to 0.8 saturation with more ammonium sulfate and is refiltered with suction.

Crystallization: 10 gm of the semi-dry precipitate is dissolved in about 10 ml of water. The solution is filtered with the aid of about 0.5 gm of Standard Super-Cel (Celite Corporation) through soft filter paper on a small Büchner funnel; the residue on the paper is washed with water. The combined filtrate and washings are brought to a final volume of 20 ml. Saturated ammonium sulfate is then added slowly with stirring until a very faint turbidity appears. The pH of the solution is adjusted first to about pH 5.0 (brick red color with 0.01 per cent. methyl red solution on test plate) with the aid of a few drops of 1.0 N sodium hydroxide and then to pH 4.2 (orange color with 0.01 per cent. methyl orange) by means of 1.0 N sulfuric acid. The solution is allowed to stand at about 20° C. An amorphous precipitate rapidly forms. This changes within one or two days into a mass of fine needles or aggregates of long thin plates. The crystals are filtered after 2 or 3 days. The filtrate on further addition of saturated ammonium sulfate yields more crystals. The recrystallization is performed by adding saturated ammonium sulfate to an aqueous solution of the crystals until a very faint turbidity appears.

The crystalline material contains about 16 per cent. total nitrogen; 7 per cent. of the total nitrogen is free amino nitrogen. It does not diffuse through ordinary collodion membranes to any significant amount.

A solution of the crystalline material is precipitated completely in 10 per cent. trichloracetic acid and incompletely in 2.5 per cent. trichloracetic acid. It gives the usual protein color tests. The tyrosine-tryptophane content is about 15 per cent. of the total dry weight. The ribonuclease activity of the material becomes constant after the second crystallization.

Further investigation of the physical and chemical properties of the new crystalline protein and of the chemical changes brought about by its nuclease activity is in progress.

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[4] G. Schmidt and P. A. Levene, *Jour. Biol. Chem.*, 126: 423, 1938.

[5] M. Kunitz and J. H. Northrop, SCIENCE, 78: 558, 1933; 80: 505, 1934; *Jour. Gen. Physiol.*, 18: 433, 1935; 19: 991, 1936.

ISOSTERISM IN THE VITAMIN B COMPLEX

THE physiological effect of both nicotinic acid and thiamine is characterized by exceptional specificity. Numerous investigations in the recent literature show that even small changes in the loading of these two heterocycles greatly affect their physiological activity. It seemed of special interest, therefore, to investigate some isosters of these two vitamins.

The concept of isosterism as proposed by Grimm[1] and investigated by Erlenmeyer[2] and others has been applied with particular success to nuclear derivatives where ring sulfur and an aromatic –CH=CH– group are mutually replaceable. According to them a 3-substituted pyridine compound is isosteric with a 5-substituted thiazole compound. This permits consideration of a relation between nicotinic acid and thiamin which has hitherto been unsuspected.

We have found that thiazole-5-carboxylic acid, isosteric with nicotinic acid,[3] stimulates the growth of dysentery bacilli on Koser's synthetic medium.[4] Its activity is of the order of .1 per cent. of that of nicotinic acid. Its activity in curing black tongue in dogs has been investigated to some extent, but the results have been inconclusive.

2-methyl-3-β-hydroxyethyl-N-[(2-methyl-6-amino-pyrimidyl-(5))-methyl]-pyridinium bromide hydrobromide isosteric with Vitamin B_1 hydrobromide has been synthesized by condensation of 2-methyl-3-β-hydroxyethyl pyridine with 2-methyl-5-bromomethyl-6-amino pyrimidine hydrobromide. 2-methyl-3-acetyl pyridine was obtained from 2-methyl-3-cyanopyridine by a Grignard reaction. Bromination of the ketone followed by hydrolysis and Clemmensen reduction yielded the new 2-methyl-3-hydroxyethyl pyridine (picrate M.P. 129° uncorr.). Fed to a series of rats with medium or acute polyneuritic symptoms the new condensation product shows Vitamin B_1 activity. The experimental data do not as yet permit exact quantita-

[1] *Naturw.*, 17: 535–40, 557–64, 1929.

[2] *Helv. Chim. Acta.*, 16: 733–8, 1933.

[3] Erlenmeyer, *Helv. Chim. Acta.*, 20: 204, 1937.

[4] *Jour. Infectious Diseases*, 62: 212, 1938.

tive evaluation and suggest possibilities of modified action.

Further experiments are in progress.

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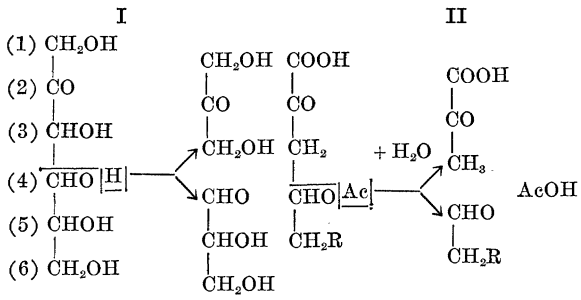
A REVERSED ALDOL CONDENSATION

DURING the past years many steps in the mechanism of glycolysis in muscle as well as in yeast have been cleared up. One of the remaining questions is: What causes the disruption of the easily fermentable hexose diphosphate ("primaeres Veresterungsproduct" of Meyerhof, etc.), to form two triose phosphates? Another open question is the position of the phosphoric acid rests in this hexose diphosphate. I would like to record an observation made a few years ago which may throw some light on these questions. Further work (which I am unable to undertake) is necessary to test whether this is a true or a false light.

The splitting of the hexose carbon chain to form two trioses is, chemically speaking, the reversal of an aldol condensation (I). I could observe a similar breakdown of a carbon chain in the case of α-keto γ-acetoxy acids. When incubating α-keto γ-acetoxy valeric acid [(II: R – H)] at 37° with water, the thick oil slowly dissolved within a few days and pyruvic acid, acetic acid and acetaldehyde could be isolated and identified in form of derivatives. The next higher homologue [(II: R – CH_3)] gave the same decomposition, while aldol, acetaldol, β-acetoxybutyric



acid and β-acetoxy δ-keto-pentane were quite stable under these conditions. It appears that an acid pH of the solution or an acid group in the molecule is an essential condition for this breakdown, as well as an oxo-group in β-position to an esterified alcoholic one; this latter alcoholic group forms the new aldehyde group after the rupture of the chain. In analogy one is tempted to suppose that the readily fermentable hexose diphosphate ("primaeres Veresterungsproduct") is a ketose, carrying one phosphoric acid group in position 4. This might weaken the C–C-link between carbon atoms 3 and 4 sufficiently to cause a spontaneous formation of two trioses under our experimental conditions and the zymohexase might only be needed to catalyze this reaction to proceed with the necessary speed.

HEINZ FRAENKEL-CONRAT

THE PRODUCTS OF THE CYCLIZING DEHYDRATION OF 1-BETA-PHENYLETHYLCYCLOHEXANOL-1 AND THE SYNTHESIS OF SPIROCYCLOHEXANE-1,1-INDANONE-3

BY a cyclizing dehydration of 1-*beta*-phenylethylcyclohexanol-1 with 85 per cent. sulfuric acid, Perlman, Davidson and Bogert[1] observed the simultaneous formation of octahydrophenanthrene and spirocyclohexane-1,1-indane.

Recently, Cook, Hewett and Lawrence,[2] and Cook, Hewett and Robinson,[3] by cold oxidation with chromic acid of a glacial acetic acid solution of the mixture of hydrocarbons resulting from the cyclization of *beta*-phenylethylcyclohexene-1, obtained a mixture of ketones, which they converted into the corresponding oximes, and then separated the latter by fractional crystallization. They thus isolated three oximes, which melted, respectively, at 187.5°, 175–177° and 124°. The first of these, they believed to be the oxime of spirocyclohexane-1,1-indanone, the second that of the *trans*-ketoöctahydrophenanthrene, and the third (m.p. 124°) that of its *cis*-isomer.

We have been occupied lately in the synthesis and study of the pure spirocyclohexane-1,1-indane, to learn more about its behavior when subjected to dehydrogenation reactions, and how its monacetyl derivative compares with those of the *cis*- and *trans*-octahydrophenanthrenes reported by Van de Kamp and Mosettig.[4]

In the course of this investigation, we synthesized the spirocyclohexane-1,1-indanone (VI), and found that its oxime melted at 137–137.8° (corr.). This wide divergence from the melting-point of 187.5° recorded by Cook *et al.* for their oxime, led us to reinvestigate the aforementioned hydrocarbon mixture, by a procedure essentially identical with that of our English colleagues with the result that we isolated the following oximes:

(1) An oxime, m.p. 187–188° (corr.).

(2) An oxime, m.p. 136.5–137° (corr.), which m.p. was not depressed when the compound was mixed with the oxime (m.p. 137–137.8°, corr.) of our synthetic spiroketone (VI). Each oxime was nitrated separately in cold concentrated sulfuric acid with powdered potas-

[1] Perlman, Davidson and Bogert, *Jour. Org. Chem.*, 1: 288, 300, 1936. See also Perlman and Bogert, *Jour. Am. Chem. Soc.*, 59: 2534, 1937.

[2] Cook, Hewett and Lawrence, *Jour. Chem. Soc.*, 1936, 71.

[3] Cook, Hewett and Robinson, *ibid.*, 1939, 168.

[4] Van de Kamp and Mosettig, *Jour. Am. Chem. Soc.*, 58: 1062, 1936.