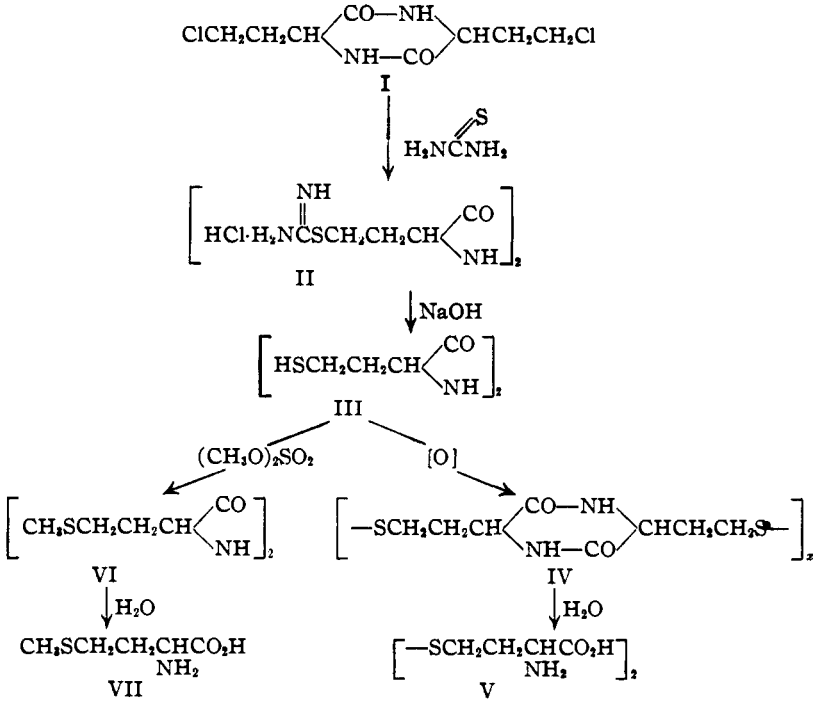
[CONTRIBUTION FROM THE NOYES LABORATORY OF CHEMISTRY, UNIVERSITY OF ILLINOIS]

A New Synthesis of Homocystine and a Further Improvement in the Synthesis of Methionine

BY H. R. SNYDER AND GEORGE W. CANNON

In a study of the utilization of 3,6-bis-(β-chloroethyl)-2,5-diketopiperazine[1] (I) in the synthesis of amino acids it has been found that the substance (I) reacts with thiourea to give nearly quantitative yields of the bis-isothiouronium chloride (II). The ready availability of this salt is of interest because it should provide a convenient source of a number of important derivatives related to homocysteine, such as the diketopiperazine (III), homocystine (V), S-benzylhomocysteine and methionine (VII). Except for methionine,[1] these substances have been prepared most conveniently from benzyl β-chloroethyl sulfide through the malonic ester synthesis.[2,3,4]



Some of these possible transformations have been tested. Homocystine (V) was obtained in excellent yields by subjecting the isothiouronium salt to mild alkaline hydrolysis, followed by air oxidation in the presence of ferric chloride and finally by acid hydrolysis. Neither the diketopiperazine of homocysteine (III) nor the polymeric anhydride (IV) of homocystine was isolated.

Treatment of the isothiouronium salt with aqueous alkali followed by methyl sulfate gave poor yields of the anhydride of methionine (VI). However, when a mixture of the salt and methyl sulfate, suspended in ice-water, was decomposed by the gradual addition of aqueous sodium hydroxide, the diketopiperazine VI was produced in high yields. The crude product was of suitable quality for use in the preparation of *dl*-methionine. This method of converting the dichlorodiketopiperazine (I) to *dl*-methionine is more convenient than the earlier procedure,[1] in which the reaction of I with sodium methylmercaptide was employed.

Experimental

(a) **3,6-Bis-(β-isothiouroniumethyl)-2,5-diketopiperazine Chloride** (II).—A mixture of 47.8 g. (0.2 mole) of 3,6-bis-(β-chloroethyl)-2,5-diketopiperazine,[1] 33.5 g. (0.44 mole) of thiourea, and 500 cc. of absolute ethanol was heated under reflux for twenty-four hours. The reaction mixture was cooled in an ice-bath and the product collected by filtration; weight (air-dried) 77 g. (98%). It could be recrystallized from water to give a white solid which darkened at 250° and melted with decomposition at 255°.

Anal. Calcd. for $C_{10}H_{20}O_2N_6S_2Cl_2$: C, 30.69; H, 5.15. Found: C, 30.61; H, 5.29.

(b) **Homocystine** (V).—A solution of 8 g. (0.2 mole) of sodium hydroxide in 40 cc. of water was added dropwise to a stirred mixture of 19.5 g. (0.05 mole) of the isothiouronium salt and 100 cc. of water at room temperature. The stirring was continued for a total of one hour. Two crystals of ferric chloride were added to the mixture and air was bubbled through it for forty-eight hours. The mixture then was concentrated to dryness under diminished pressure. The residue was dissolved in 250 cc. of concentrated hydrochloric acid, and the solution was heated under reflux for three hours. It was then again concentrated to dryness under diminished pressure. The residue was extracted with several portions of boiling absolute ethanol. The combined alcoholic extracts were treated with Norite and then with an excess of pyridine. The solution was allowed to stand in a refrigerator over-night and the product was collected by filtration; it weighed 10 g. (74.5%). After several recrystallizations from water it darkened at 250° and decomposed without melting at 258–263° (lit.[5] 260–265°).

(c) **Methionine Anhydride** (VI).—A mixture of 19.5 g. (0.05 mole) of the diisothiouronium salt and 100 cc. of water was placed in a 500-cc. three-necked flask fitted with a reflux condenser, stirrer, and dropping funnel. The flask was immersed in an ice-bath and 15.1 g. (0.12 mole) of dimethyl sulfate was added at once to the reaction mix-

(1) Snyder, Andreen, Cannon and Peters, THIS JOURNAL, **64**, 2082 (1942).

(2) Patterson and du Vigneaud, *J. Biol. Chem.*, **111**, 393 (1935).

(3) Riegel and du Vigneaud, *ibid.*, **112**, 149 (1935–1936).

(4) Du Vigneaud, Patterson and Hunt, *ibid.*, **126**, 217 (1938).

(5) Butz and du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932–1933).

ture. A solution of 11 g. (0.275 mole) of sodium hydroxide in 40 cc. of water was then added dropwise over a period of twenty minutes. The stirring was continued for a total of one hour, and the product was collected by filtration. The mother liquor was cooled overnight in a refrigerator and a small additional amount of product was obtained. The combined crops of the product weighed 13.5 g. (100%). After one recrystallization from ethanol, the methionine anhydride weighed 10.1 g. (75%) and melted at 226–227.5°. A mixed-melting point determination with an authentic sample of methionine anhydride was not depressed. It was of sufficient purity to give *dl*-methionine in 85–95% yields. Direct hydrolysis[1] of the crude product gave 10 g. (65%) of crude *dl*-methionine.

Summary

3,6-Bis-(β-chloroethyl)-2,5-diketopiperazine is converted quantitatively to the bis-isothiouronium salt by treatment with thiourea in boiling ethanol.

Treatment of the bis-isothiouronium salt with dilute alkali, followed by oxidation with air in the presence of ferric chloride and hydrolysis with acid, provides a convenient synthesis of homocystine.

Decomposition of the bis-isothiouronium salt with alkali in the presence of methyl sulfate produces *dl*-methionine. This method of converting the dichlorodiketopiperazine to *dl*-methionine is superior to that previously described.

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[FROM THE DEPARTMENT OF BIOCHEMISTRY AND PHARMACOLOGY, THE UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

Metabolism of Phosphoryl Choline. I. Synthesis of Calcium Phosphoryl Choline Chloride Containing the Radioactive Isotope, P^{32} [1]

BY RICHARD F. RILEY

Although monophosphoric acid esters of both choline[2] and ethanolamine[3,4] have been isolated from normal tissues, their place in metabolism has not been adequately described. An extension[5] of available information on phosphoryl choline (Smith,[6] Welch and Welch,[7] and Taurog[8]) therefore appeared of interest. Since an undue increase in tissue concentration of any substance is to be avoided where its normal metabolism is to be followed (ref. 9, p. 292) and since labeling this molecule with radiophosphorus would provide a convenient means of following the metabolic path of that portion of the ester, a highly radioactive preparation was desirable.

The published procedures for the synthesis of the monophosphoric acid ester of choline are not well suited to preparation of the ester containing radiophosphorus. They permit extensive isotope dilution[2,10,11] or yield products contaminated by the diester and inorganic phosphate.[12] Accordingly, the method of preparation has been adapted to efficient use with radioactive phosphorus and/or choline containing tracer elements, and to elimination of contaminating compounds. The phosphoryl choline containing radiophosphorus, of which the preparation is described here, was of satisfactory purity and, allowing for decay, possessed the same activity per mg. of phosphorus as the starting material.

The ester was prepared in most satisfactory yields by heating equimolar quantities of 100% phosphoric acid with dry choline chloride under reduced pressure for twelve hours at 165°, and isolating the product as the calcium chloride salt. In preliminary attempts at improvement of the synthesis, several new derivatives of the mono- and dicholine esters of phosphoric acid were prepared for purposes of identification and comparison. An attempt to form the ester by treating bromocholine bromide and trisilver phosphate or disilver monophenyl phosphate gave instead excellent yields of neurine. It was also found that choline hydroxide and phosphoric acid may be esterified in refluxing toluene to give a satisfactory product, although in rather low yield and with considerable decomposition.

Experimental

Calcium Phosphoryl Choline Chloride Containing P^{32}.—150 mg. of phosphoric acid containing P^{32} in 6 ml. of water was introduced into a pear-shaped flask of 35 ml. capacity. The flask was fitted with a standard taper 24 stopper and two outlets with glass stopcocks. The dilute acid was evaporated to 100% phosphoric acid by immersion in an oil-bath which was gradually raised to 180° as a stream of dry air was passed through the flask. Only 0.005% of the activity was lost by this evaporation. When complete, 214 mg. of pure dry choline chloride was well mixed with the phosphoric acid and about 1 g. of phosphorus pentoxide mixed with asbestos contained in a Soxhlet thimble,

(1) The substance of this paper was presented in part before the American Chemical Society at Pittsburgh, September, 1943. This investigation was supported by a grant from the Nutrition Foundation, Inc., of New York City.

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(4) S. P. Colowick and C. F. Cori, *Proc. Soc. Exp. Biol. and Med.*, **40**, 586 (1939).

(5) R. F. Riley, Abstracts, Pittsburgh Meeting, A. C. S., Sept., 1943, in press.

(6) M. I. Smith, *Nat. Inst. Health Bull.*, No. **165**, 11 (1936).

(7) A. DeM. Welch and M. S. Welch, *Proc. Soc. Exp. Biol. and Med.*, **39**, 7 (1938).

(8) Personal communication (1942).

(9) I. L. Chaikoff, *Physiol. Rev.*, **22**, 291 (1942).

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(12) E. L. Jackson, THIS JOURNAL, **57**, 1903 (1935).