A solution of 20 g. of benzenesulfonyl-*p*-nitrobenzhydrazide in 100 ml. of ethylene glycol was heated to 160°, 20 g. of anhydrous sodium carbonate added, the vigorous exothermic reaction allowed to go to completion, 250 ml. of water added to the cooled reaction mixture, the latter acidified with 5 *N* hydrochloric acid, the precipitate collected, and solid *p*-benzoquinone carefully added to the filtrate. The crystalline product thus formed was collected and recrystallized from aqueous ethanol to give 2,5-dioxodiphenylsulfone, orange rhombs, m. p. 202–203°.[11]

Anal. Calcd. for $C_{12}H_8O_4S$ (248.2): C, 58.1; H, 3.2. Found: C, 57.9; H, 3.6.

The precipitate obtained by acidification of the reaction mixture was dissolved in hot ethanol, the solution filtered, the precipitate discarded, and an equal volume of ether added to the filtrate. The precipitate was discarded, the filtrate evaporated to dryness, the residue taken up in ethanol, an equal volume of water added, the precipitate collected and dried to give 6 g. of crude *p*-nitrobenzoic acid. Recrystallization of the crude product from hot water gave *p*-nitrobenzoic acid, pale yellow irregular platelets, soluble in aqueous sodium bicarbonate, m. p. 238–239°.

Anal. Calcd. for $C_7H_5O_4N$ (167.1): C, 50.3; H, 3.0; N, 8.4. Found: C, 50.5; H, 3.1; N, 8.5.

(11) Hinsberg, *Ber.*, **27**, 3259 (1894).

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA RECEIVED NOVEMBER 9, 1942

The Absence of β-Alanine in Proteins

BY MAXWELL A. POLLACK[1]

Although β-amino acids have never been isolated from proteins, there is no evidence which rules out the possibility that they might be present in amounts too small to be detected in the ordinary procedures. Of all the possible β-amino acids, β-alanine is the simplest and at the same time the most likely to be present in proteins, in view of the fact that peptides of it, namely, carnosine and anserine, as well as pantothenic acid, have already been isolated from natural extracts.

It is possible to test for the presence of β-alanine by virtue of its strong growth-promoting action for yeast.[2,3] This phenomenon can be employed in the direct assay of hydrolysis products from proteins, for none of the other known amino acids will produce this growth response. The method as described below will show the presence of one β-alanyl unit in a protein of one million molecular weight.

Using this test, the hydrolysis products from silk fibroin, horse hemoglobin, egg albumin, gelatin, casein and lactoglobulin were assayed and found to be substantially free from β-alanine. It is very unlikely that this result can be accounted for by *destruction of β-alanine during the hydrolysis* of the proteins, for the amino acid was tested and found to be stable under these conditions, and it is not probable that peptides of β-alanine would be much more unstable than the amino acid.

The results indicate that β-alanine is not a general constituent of proteins. However, the possibility of its occurrence in special proteins still remains.

Experimental

Protein Hydrolyzates.—The lactoglobulin, egg albumin, and horse hemoglobin were all thrice-recrystallized materials. The silk fibroin had a nitrogen content of 18.97% (dry weight basis), while the gelatin was the commercial product "Knox U.S.P. Plain Sparkling Gelatine," and the casein was the Difco vitamin-free brand. These proteins were dried *in vacuo* over anhydrous calcium chloride before use.

The hydrolyzates were kindly furnished by Mr. J. R. McMahan, who used the following procedure in their preparation. One cc. of 10% hydrochloric acid was added to 100 mg. of the protein in a test-tube which was sealed and heated in an autoclave under a pressure of 15 pounds of steam for ten hours. The tube was opened, the contents rinsed out and brought to a *p*H of 6.6–7.0 by adding sodium hydroxide, and finally adjusted to the desired concentration. Pure β-alanine was put through this procedure without loss of growth-promoting potency.

Assay Method.—Varying amounts of the above solutions (filtered to remove humin) were added to short, wide-mouthed test-tubes so that the amounts in the tubes corresponded with 0.2, 1.0, 2.0, and 4.0 mg. of the original proteins. One sequence of tubes contained 0.1, 0.3, 0.5, and 1.0 microgram of pure β-alanine to serve as a standard. The volumes were adjusted to 2.0 ml. by the addition of distilled water where necessary.

The tubes were sterilized by heating in steam for ten minutes and were then cooled. To each tube was then added five ml. of medium containing ten micrograms of suspended Gebrüder Mayer (G.M.) yeast cells. The medium was the same as that of Snell, Eakin, and Williams,[4] except that the β-alanine was omitted and 0.2 microgram of biotin was added per liter of medium. The tubes were incubated at 30° for sixteen hours, following which the yeast productions were determined by shaking the tubes and measuring the turbidities of the resulting suspensions.[5]

Whereas the tubes containing added pure β-alanine showed a striking production of yeast, the turbidities of all the other tubes were approximately equal to those of the blanks, except in the case of the hydrolyzate from hemoglobin, which showed a slightly higher turbidity. The original solution in this case had been colored light

(1) Present address, E. F. Drew and Co., Inc., Boonton, N. J.

(2) R. J. Williams and E. Rohrmann, THIS JOURNAL, **58**, 695 (1936).

(3) E. E. Snell, *J. Biol. Chem.*, **141**, 121 (1941).

(4) E. E. Snell, R. E. Eakin and R. J. Williams, THIS JOURNAL, **62**, 175 (1940).

(5) R. J. Williams, E. D. McAlister and R. R. Roehm, *J. Biol. Chem.*, **83**, 315 (1929).

brown, and the coloring matter (hemin?) had precipitated during the growth test. Comparison of the turbidity in this case with that in a control tube containing the same amounts of hydrolyzed hemoglobin and medium but to which no yeast seeding had been added, showed that the turbidity was due almost entirely to the precipitated coloring matter. The difference between the two turbidities corresponded to 0.07 microgram of β-alanine per four mg. of original hemoglobin, which is much less than the minimum figure of 5.3 micrograms per 4 mg. which would be required by the presence of one β-alanyl unit in the hemoglobin molecule (molecular weight 66,700).

CLAYTON RESEARCH FOUNDATION AND
THE BIOCHEMICAL INSTITUTE
THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS RECEIVED DECEMBER 26, 1942

The Preparation of Desoxycholic Acid from Cholic Acid

ALEXANDER W. SCHNEIDER[1] AND WILLARD M. HOEHN

In a recent publication by Haslewood[2] the preparation of desoxycholic acid from cholic acid was presented. The details of the method were not given. It was stated the cholic acid was preferentially oxidized by means of chromic acid to 3,12-dihydroxy-7-ketocholanic acid and this product converted to the corresponding semicarbazone. We assume the semicarbazone was reduced according to the Wolff–Kishner technique to yield desoxycholic acid.

More than two years ago we carried out these particular reactions not only with cholic acid but also with derivatives of it. The data obtained from these investigations enabled Schmidt, Hughes and one of us (W. M. H.) to ascertain the course of the bacterial oxidation of cholic acid.[3] Cholic acid, methyl cholate, methyl 3-benzoxy-7,12-dihydroxycholanate[4] were some of the derivatives used. The compound was dissolved in either acetic acid or a mixture of acetic acid, benzene and water, and a solution of chromic acid in dilute acetic acid added. The mixture of oxidation products was converted to semicarbazones or hydrazones, which were then reduced according to the method of Wolff and Kishner (*cf.* ref. (3)). It was found that concentrated solutions of potassium or sodium hydroxide in methanol could be used instead of an alcoholic sodium alkoxide solution. The desoxycholic acid was isolated from the mixture of reduction products in accordance with known methods.

The controlled oxidation of methyl 3-benzoxy-7,12-dihydroxycholanate gave a mixture of products. From this mixture a monosemicarbazone was isolated by virtue of its solubility in methanol. The monosemicarbazone was heated with sodium methoxide in methanol (10 g. of sodium in 100 cc. of methanol) or with sodium or potassium hydroxide in methanol (10% solutions), to a temperature of 170–200° for periods up to six hours. The crude desoxycholic acid prepared by these reactions may be crystallized directly from acetic acid to yield the acetic-choleic acid. Desoxycholic acid is obtained from this complex in the usual manner.[5] The melting point[6] for the pure acid was 174–176°. The desoxycholic acid prepared by this procedure still gave a positive Gregory–Pascoe reaction[7] which is indicative of the presence of cholic acid. The melting point of a mixture of a sample of desoxycholic acid (m. p. 172–173°) (Gane and Ingram, N. Y.) and that prepared by the above method was 172–174°. The $[\alpha]^{26}D$ of a methanol solution of the desoxycholic acid was observed to be $+57 \pm 1°$.

(1) Present address: Central Soya Company, Decatur, Ind.

(2) Haslewood, *Nature*, **150**, 211 (1942); also *cf. C. A.*, **36**, 7029 (1942).

(3) The results of this investigation were presented at the A. C. S. meeting in Memphis, April, 1942.

(4) Hoehn and Mason, THIS JOURNAL, **62**, 569 (1940).

(5) Sobotka, "Chemistry of Sterids," Williams and Wilkins Co., Baltimore, Md., 1938, p. 77.

(6) Melting points were observed on the Johns melting point block.

(7) L. H. Schmidt, *Am. J. Physiol.*, **120**, 75 (1937).

RESEARCH LABORATORIES
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KANSAS CITY, MO. RECEIVED DECEMBER 17, 1942

Total and Partial Pressures of Binary Mixtures of Dioxane in Benzene at 25°

BY PEYTON C. TEAGUE[1] WITH W. A. FELSING

In an investigation with dioxane, it became necessary to know the partial vapor pressure of a solution of dioxane (diethylene dioxide) in benzene. Since the similarity in structure and polarity indicated solutions approaching ideality, it was decided to cover the entire composition range.

The method used was the differential method of Parks and Schwenck[2] as modified by Olsen and Washburn[3] and by Allen, Lingo and Felsing.[4] The benzene was purified as described previously[4] and the dioxane was purified by the method of

(1) Present address: Department of Chemistry, University of Alabama, Tuscaloosa, Ala.

(2) Parks and Schwenck, *J. Phys. Chem.*, **28**, 720 (1924).

(3) Olsen and Washburn, *ibid.*, **41**, 457 (1937).

(4) Allen, Lingo and Felsing, *ibid.*, **43**, 425 (1939).