a purple solution, which on dilution yielded white needles, m. p. 65–66°. This was the 2,5-dimethoxy-3,4,6-trimethyl-benzyl ester of acetic acid XI.

Anal. Calcd. for $C_{14}H_{20}O_4$: C, 66.70; H, 7.90. Found: C, 66.3; H, 7.62.

Trimethyl-nitrobenzoquinone (VII).—When the 85° aldehyde is warmed with concd. (d., 1.4) nitric acid a clear yellow solution results. This solution, on the addition of water, deposits glistening yellow platelets, which on recrystallization from dil. alcohol melt at 112–113°. The melting point of the substance agrees with that given by Nef[10] for trimethyl-nitrobenzoquinone. The latter was prepared by nitrating pseudocumoquinone (trimethyl-quinone). Upon taking a mixed melting point, the mixture showed no lowering.

Summary

1. Sodium malonic ester has been added to duroquinone, giving as the primary products the hydroquinone and a red sodium derivative, which with acids, gives a yellow lactone-ester.

2. The reaction is complicated by oxidation, and the quinone is the oxidizing agent.

3. The yellow lactone-ester forms colorless acetyl and methyl derivatives, and a structure change of the *para*-keto-enol type is involved in these reactions. The colorless compounds are benzene derivatives, while the colored compounds are analogous to the oxyanthranols. The mechanism of the primary reaction has not yet been determined.

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THE SEPARATION OF THE DICARBOXYLIC AMINO ACIDS FROM CERTAIN PROTEIN HYDROLYSATES BY ELECTRICAL TRANSPORT

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RECEIVED MARCH 2, 1926 PUBLISHED JUNE 5, 1926

Some years ago Ikeda and Suzuki[1] patented a process for the separation of protein hydrolysates into three groups of amino acids by electrical transport. We became interested in this process during a survey of the methods for the preparation of amino acids in relatively large quantities, and we have described elsewhere the conditions under which the hexone bases may be separated from certain protein hydrolysates.[2] In the present paper experiments dealing with the transport of the predominantly acid amino acids are reported, and the conditions for their separation from certain protein hydrolysates are outlined. Data relative to the preparation of arginine and lysine by the method of electrical transport are also included.

[1] Ikeda and Suzuki, U. S. pat. 15,891, Jan. 30, 1912.

[2] Foster and Schmidt, *J. Biol. Chem.*, **56**, 545 (1923).

The magnitude of the acid dissociation constants of aspartic, glutamic[3] and pyrrolidone-α-carboxylic[4] acids indicates that at hydrogen-ion concentrations less than P_H 5.0 these substances are completely dissociated as acids. Although the dissociation constant for hydroxyglutamic acid has not been determined, it seems probable that it is at least as great as that of glutamic acid. Tyrosine, although a dibasic acid,[5] is very much weaker than the above-mentioned amino acids, and in the region of P_H 4.0–7.5 it is iso-electric. We should, therefore, expect that at P_H 5.0 only aspartic, glutamic, hydroxyglutamic and pyrrolidone-α-carboxylic acids would be transported from protein hydrolysates to the anode compartment by the electrical current. Our experiments confirm this prediction.

The experiments were carried out with the aid of the three-compartment cell and according to the same method as was described in the previous paper.[2] Unless otherwise stated the hydrogen-ion concentration of the solution of amino acids in the center compartment was maintained at approximately P_H 5.5. In the preliminary experiments mixtures of purified amino acids were employed in order to simplify the analytical procedures.

Experiment I

Amino acid mixture employed: 2.42 g. of aspartic acid (0.254 g. of nitrogen) plus 0.050 g. of tyrosine (0.004 g. of nitrogen). At the end of the experiment the solutions in each of the three compartments were found to contain the following amounts of nitrogen: anode, 0.224 g.; cathode, 0.002 g.; center 0.014 g. Tests for tyrosine with the phenol reagent of Folin and Denis showed that only a trace was present in the anode and none in the cathode. The results show a recovery of 88% of the aspartic acid in the anode compartment.

Experiment II

Amino acid mixture employed: 1.20 g. of glutamic acid (0.114 g. of nitrogen), 1.21 g. of aspartic acid (0.127 g. of nitrogen) and 0.048 g. of tyrosine (0.004 g. of nitrogen). The three compartments were found to contain the following amounts of nitrogen: anode, 0.232 g.; cathode, 0.001 g.; center, 0.008 g. The cathode solution gave no test for tyrosine while a trace was found in the anode; 96% of the dicarboxylic acids was recovered in the anode solution.

Experiment III

Amino acid mixture employed: 2.35 g. of pyrrolidone-α-carboxylic acid (0.254 g. of nitrogen) plus 0.046 g. of tryptophan (0.0032 g. of nitro-

[3] Levene and Simms, *J. Biol. Chem.*, **55**, 801 (1923). Holmberg, *Z. physik. Chem.*, **62**, 726 (1908).

[4] McCay and Schmidt, *J. Gen. Physiol.*, **9**, 333 (1926).

[5] Harris, *Proc. Roy. Soc.*, [B] **95**, 440 (1923). Hitchcock, *J. Gen. Physiol.*, **6**, 747 (1924).

gen). Analyses of the solutions in each of the compartments gave the following nitrogen values: cathode, none; anode, 0.244 g.; center, 0.014 g. Tests for tryptophan gave negative results with the anode and cathode solutions. The original solution of pyrrolidone-α-carboxylic acid contained 5 mg. of amino nitrogen; 2.3 mg. was found in the anode and 0.7 mg. in the center compartment. The experiment indicates that 96% of the pyrrolidone-α-carboxylic nitrogen was recovered in the anode.

Experiment IV

Amino acid mixture employed: 1.73 g. of pyrrolidone-α-carboxylic acid (0.187 g. of nitrogen) plus 0.685 g. of nitrogen made up from the following amounts of amino acids: 1.3 g. of glycine, 1.6 g. of alanine, 2.1 g. of norleucine, 0.70 g. of leucine and 0.70 g. of phenylalanine. The following values were found for each of the solutions in the cell: anode, 0.201 g. of total nitrogen, 0.044 g. of amino nitrogen; cathode, 0.018 g. of total nitrogen, 0.018 g. of amino nitrogen; center, 0.617 g. of total nitrogen, 0.593 g. of amino nitrogen. Although this experiment indicates that practically all of the pyrrolidone-α-carboxylic acid nitrogen had migrated to the anode compartment, it also indicates an appreciable wandering of other amino acids to the anode. In order to minimize the amount of the latter, the anode solution was placed in the center compartment and the amino acids were again subjected to migration. At the end of the experiment the content of the amino nitrogen in the anode solution was found to be 1.4 mg. This probably was due to the presence of glutamic acid since, as noted above, our pyrrolidone-α-carboxylic acid contained a small amount of amino nitrogen.

Experiment V

Since no data relative to the dissociation constants of proline or oxyproline were available when our experiments were carried out[6] it appeared of interest to determine experimentally whether these amino acids interfere with the separation of the dicarboxylic amino acids. Since no oxyproline could be obtained the experiment was limited to proline. It is probable, however, that the values of the dissociation constants of oxyproline do not differ materially from those of proline. The experimental results are probably representative of both proline and oxyproline. The amino acid mixture which was employed consisted of 0.75 g. of proline (total nitrogen 0.088 g., amino nitrogen 0.0014 g.) and 0.92 g. of glutamic acid (amino nitrogen, 0.099 g.). On completion of the experiment the anode compartment contained 0.098 g. of nitrogen of which 0.092 g. was

[6] The dissociation constants of racemic proline have since been determined by McCay and Schmidt, Ref. 4, to have the values $K_a = 2.5 \times 10^{-11}$, $K_b = 1 \times 10^{-12}$; iso-electric point, P_H 6.3. No data relative to the dissociation constants of oxyproline are available.

amino nitrogen. The experiment indicates that the current is not to any appreciable extent carried by proline.

Experiment VI

On account of its content of hydroxyglutamic acid in addition to glutamic and aspartic acids the hydrolysate from casein was employed to test further the separation of the dicarboxylic amino acids, especially hydroxyglutamic acid. Two separate preparations of hydrolyzed casein were made observing the special precautions which have been suggested by Foreman[7] to avoid the formation of pyrrolidone-α-carboxylic acid during the preparation of the hydrolysate.

The dicarboxylic amino acids were transported twice to the anode compartment to minimize the amount of other amino acids which are carried in small quantities along with the dicarboxylic acids. In the analytical procedure glutamic acid was separated by twice saturating the solution containing the dicarboxylic amino acid with hydrochloric acid. After concentration in a vacuum the filtrate was saturated with calcium hydroxide and the calcium salts of the dicarboxylic acids were precipitated by alcohol in the manner suggested by Foreman.[7] Aspartic and hydroxyglutamic acids were estimated together in the precipitate and the non-dicarboxylic amino nitrogen was estimated in the filtrate. For the present purpose these procedures were considered sufficiently accurate. On addition of calcium hydroxide to the solution from which glutamic acid had been removed, a considerable evolution of ammonia was observed. It was estimated that ammonium nitrogen constituted about 6% of the total nitrogen in the anode solution. It seems probable that in the attempt to transport the amino acids to the anode a higher current density than usual was employed and, as a result, oxidation took place which led to the liberation of ammonia. The anode solution contained 1.978 g. of nitrogen. This was distributed as follows: glutamic acid, 57.5%; ammonia, 6.4%; non-dicarboxylic nitrogen, 1.5%; aspartic plus hydroxyglutamic acid, 34.6%. A similar experiment was carried out on another casein hydrolysate. The anode solution contained 0.872 g. of nitrogen. This was distributed as follows: glutamic acid, 63%; ammonia, 6%; aspartic plus hydroxyglutamic acid, 28%; non-dicarboxylic acids, 3%. Aspartic and hydroxyglutamic acids were qualitatively identified by employing the method of separation described by Dakin.[8] The values for the distribution of the dicarboxylic amino acids in the anode solution compare favorably with those reported for casein by Dakin.

We have also been interested in testing the applicability of the method of transport for the separation of relatively large quantities of amino acids,

[7] Foreman, *Biochem. J.*, **8**, 463 (1914).

[8] Dakin, *Biochem. J.*, **12**, 290 (1918).

particularly lysine and arginine. A large cell constructed of Bakelite[9] (external dimension, 19 × 28 × 33 cm.) was used to carry out the transport experiment and gelatin was used as a source of these amino acids. The gelatin hydrolysate contained 240 g. of nitrogen of which 59 g. was basic nitrogen. After completion of the transport at P_H 7.5–8.0 the cathode solution was found to contain essentially all of the basic nitrogen and in addition 28 g. of non-basic nitrogen. This solution was then subjected to transport at P_H 7.5–8.0 in order to effect a more thorough separation of the basic from the non-basic nitrogen. The new cathode solution was found to contain 42.7 g. of basic and 2.3 g. of non-basic nitrogen. An aliquot part of this solution which contained 21.8 g. of arginine nitrogen and (by difference) 10.3 g. of nitrogen in the form of lysine was employed for the isolation of arginine and lysine. Arginine was separated as the picrolonate. The yield of arginine picrolonate was 175 g. which corresponds to 21.3 g. of arginine nitrogen or 98% of that which was present in the solution. The yield of recrystallized lysine picrate was 98 g. which corresponds to 7.3 g. of lysine nitrogen. Using as a basis for computation the figures for the distribution of nitrogen in gelatin which were obtained by Van Slyke[10] the yield of arginine picrolonate was 85% and the yield of lysine picrate was 67% of that which would have been expected. The losses are probably due to incompleteness of transport and incompleteness in precipitation and recrystallization. To this must be added the uncertainty in the analytical method for the estimation of lysine.

After removal of lysine picrate the filtrate was found to contain 1.10 g. of nitrogen which could be precipitated by means of phosphotungstic acid. It was estimated that there were present 0.22 g. of arginine nitrogen and approximately 0.25 g. of lysine nitrogen. Histidine was not present. Our attempts at determining the remainder of the nitrogen, with particular reference to the amino acid recently described by Van Slyke and Robson,[11] were negative. The pyrrole tests were negative and it was not found possible to prepare a crystalline copper salt.

Summary

1. Experiments have been carried out which indicate that the dicarboxylic amino acids may be separated from protein hydrolysates by transport to the anode chamber of a 3-compartment cell. The results of this and former work indicate that at P_H 5.5 protein hydrolysates may be separated into three fractions containing (a) the basic amino acids which migrate to the cathode, (b) the dicarboxylic acids (including pyrrolidone-α-carboxylic acid) which migrate to the anode and (c) those amino acids

[9] We are indebted to the General Bakelite Company for the donation of the cell.

[10] Van Slyke, *J. Biol. Chem.*, **10**, 48 (1911–12).

[11] Van Slyke and Robson, *Proc. Soc. Exptl. Biol. Med.*, **23**, 23 (1925).

possessing approximately equally acid and basic properties which remain in the center compartment.

2. The method of transport was found to be applicable to the preparation of arginine and lysine in relatively large quantities.

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THE OXIDATION OF CHAULMOOGRIC ACID BY PERMANGANATE[1]

BY GRANVILLE A. PERKINS

RECEIVED MARCH 5, 1926 PUBLISHED JUNE 5, 1926

Derivatives of the peculiar fatty acids of chaulmoogra oil are being used for the treatment of leprosy with marked success. Bacteriological experiments have shown that these fatty acids have a strong selective toxic and growth-inhibiting action on acid-fast bacteria, and we have reason to believe that this accounts in part for the clinical effects.

Neverthless, the curative effects of the medicines now in use leave much to be desired, and work is in progress in this Laboratory to prepare, if possible, compounds which will be more bactericidal, less toxic to the patient, and more readily diffusible to the bacteria *in situ*. Chaulmoogric acid was chosen as one of the starting points for this work, and its oxidation products were among the first derivatives studied.[2]

The oxidation of chaulmoogric acid by permanganate was studied by Power and Gornall[3] and by Barrowcliff and Power[4] in the course of an extensive pioneer investigation of the structure of this interesting acid. The present study covers a portion of the field in greater detail, dealing especially with the structure and properties of the glycols formed in the first step of the reaction.

The Structure of Chaulmoogric Acid

The question of the structure of chaulmoogric acid is also here involved, but unfortunately the results to date are not decisive on this point. For this reason publication has been deferred for some time. Recently, however, Shriner and Adams[5] have brought the subject to general attention and, therefore, the data at hand may be of interest.

From the work of Barrowcliff and Power[4] one of the courses of oxidation

[1] Published with the permission of the Director of Health upon recommendation of the Philippine Leprosy Research Board.

[2] The bacteriological testing of these products is being done by Dr. Otto Schöbl, Bureau of Science, Manila, and is to be reported in the *Philippine Journal of Science.*

[3] Power and Gornall, *J. Chem. Soc.*, **85**, 859 (1904).

[4] Barrowcliff and Power, *ibid.*, **91**, 557–8 (1907).

[5] Shriner and Adams, THIS JOURNAL, **47**, 2727 (1925).