tion of 0.34 g. of recrystallized dibutyldixanthyl in 25 cc. of ether was filtered into a small flask which was then closed with a calcium chloride tube and allowed to remain overnight. By morning, a few colorless crystals of peroxide had formed. Shaking separated more crystals, smaller in size. After another day, the light yellow ether solution was decanted from the crystals which were then washed with a small amount of ether and dried; yield 0.19 g., or 52%; m. p., 182–183.5°, with evolution of gas at 183.5°.

Evaporation of the ether solution gave 0.02 g. of inferior product melting at 158–160°.

The peroxide was found to be quite stable in air, its melting point not changing after a week's exposure.

Anal. Calcd.: C, 80.6; H, 6.8. Found: C, 80.3; H, 6.9.

Summary

1. Dibenzyldixanthyl has been prepared by reducing benzylxanthydrol with vanadous chloride. It behaves as though it dissociated into a free radical in solution, rapidly absorbing oxygen to form a peroxide and giving the usual color changes when heated and cooled. Its molecular weight in freezing benzene corresponds to a dissociation of less than 4%.

2. Dibutyldixanthyl has been prepared by reducing butylxanthyl perchlorate with vanadous chloride. It is bimolecular in freezing benzene within the limits of error of the cryoscopic method. It absorbs oxygen slowly, forming a peroxide. It is probably slightly dissociated in solution.

3. Solutions of dibenzyldixanthyl and dibutyldixanthyl are decomposed by heat. The change apparently involves the formation of monomolecular saturated and unsaturated hydrocarbons.

4. The effectiveness of the benzyl group as compared with hydrogen or an alkyl group in causing the dissociation of an ethane does not accord with certain theories of valence.

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EVIDENCE OF A NEW AMINO ACID IN PROTEINS[1]

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While determining the nitrogen distribution of a series of proteins (Hoffman and Gortner,[2] 1924) according to the Van Slyke method,[3] the phosphotungstates of the basic amino acids, arginine, histidine, lysine

[1] Published with the approval of the Director, as paper No. 508, Journal Series, Minnesota Agricultural Experiment Station.

[2] Hoffman and Gortner, "Physico-chemical Studies on Proteins. I. The Prolamines—Their Chemical Composition in Relation to Acid and Alkali Binding." Second Colloid Symposium Monograph, Chemical Catalog Company, New York (in press).

[3] Van Slyke, *J. Biol. Chem.*, 10, 15 (1911).

and cystine, were precipitated at room temperature and then set aside near a partly open window for 48 hours. In some instances, a part of the solution froze, indicating a precipitation temperature below zero. Under such conditions it was not always possible to obtain good agreement between duplicated analyses. In several instances, one flask indicated approximately the same amount of basic nitrogen that had been reported by other workers while the duplicate flask showed much higher basic nitrogen values, the increase invariably occurring in the lysine fraction.

During the analysis of the protein teozein, it was noted that in the partly frozen solution were large (1–4 mm.), red-brown, apparently isometric crystals formed on top of the usual, cream-colored phosphotungstates. These crystals undoubtedly formed subsequent to the usual phosphotungstate precipitate. When the precipitation was carried out in an ice-cooled refrigerator, good duplicate determinations were obtained for total basic nitrogen values. In the case of gliadin and hordein such values agreed very well with those of other workers. A comparison of the amounts of total basic nitrogen precipitated when the solutions were cooled to their freezing temperatures as compared to that precipitated when the solutions were cooled in an ice-cooled refrigerator are shown below.

Total basic nitrogen as percentage of total nitrogen

Protein	Precipitated at about 10° for 48 hours[a] %	Precipitated when cooled to freezing temperature of solution for about 48 hours %
Teozein	8.68	16.48
Gliadin	11.98	17.84
Hordein	18.90	22.69

[a] These values are not corrected for solubility, according to Van Slyke, Ref. 3.

From these data, it appeared that some amino acid other than arginine, histidine, lysine and cystine is precipitated with phosphotungstic acid when the solution is cooled to below zero and somewhat concentrated by the separation of ice. Approximately 7.80% of the total nitrogen of teozein, 5.8% of the gliadin nitrogen and 3.79% of the hordein nitrogen appears to be present in this amino acid.

With the exceptions of arginine, histidine, lysine, cystine and prolein[4] none of the known amino acids forms insoluble phosphotungstates from dilute solution. Other amino acids may precipitate phosphotungstates when in concentrated solutions but such phosphotungstates are readily soluble in water or dilute acids. After the phosphotungstate of the "new" amino acid has crystallized, it appears to be relatively insoluble and may be washed with phosphotungstic acid in dil. hydrochloric acid solution, without excessive loss. The fact that a stable, well-crystallized phosphotungstate is formed argues that the unknown amino acid must possess more or less basic properties.

[4] *Compt. rend. trav. lab. Carlsberg*, **6**, 168 (1905).

Of the newer amino acids, β-hydroxy-glutamic acid reported by Dakin,[5] and the new sulfur-containing amino acid reported by Mueller[6] do not possess the properties exhibited by this compound.

It is a well-known fact that when lysine is separated as the picrate from the lysine fraction of the Kossel separation, some amino nitrogen remains in the mother liquors. It has been assumed that this is due to traces of mono-amino acids that have not been separated from the bases. Van Slyke and Hiller[7] reported an unidentified base among the hydrolytic products of gelatin. The base was isolated from the products of acid hydrolysis of gelatin by precipitating the bases with phosphotungstic acid, redissolving and freeing from the acid in the usual manner. Histidine and arginine were precipitated with silver sulfate and barium hydroxide, and lysine was precipitated as the picrate. The residual solution contained non-amino nitrogen equal to that present in excess of the arginine and histidine non-amino nitrogen as determined by the Van Slyke method. On recrystallization, the phosphotungstate gave a ratio of total nitrogen to amino nitrogen of two to one. As will be seen later, the fact that their base contains non-amino nitrogen precludes the possibility that it is the compound we have isolated.

Inasmuch as evidence for the presence of this compound was found in the analysis of at least three of the prolamines, attempts were made to isolate it in sufficient quantity to determine some of its properties.

Preparation.—Fifty g. of teozein[8] was hydrolyzed by boiling for 24 hours with 600 cc. of 20% hydrochloric acid. As much of the acid as possible was driven off by distilling in a vacuum over a boiling water-bath. The ammonia was completely removed by adding 200 cc. of water, 200 cc. of ethyl alcohol and enough calcium hydroxide suspension to the residue to make it alkaline to litmus, and distilling the ammonia in a vacuum at 40–50°. The humins were then filtered off and 180 cc. of concd. hydrochloric acid, 200 g. of phosphotungstic acid and enough water to make a final volume of about 2000 cc. were added.

This mixture was placed in an ice-cooled refrigerator (at about 10°) for 48 hours, under which conditions, according to Van Slyke a practically complete precipitation of the basic amino acids as the phosphotungstates takes place. These phosphotungstates were filtered off in the usual manner except that the precipitate was not washed, in order to avoid increasing the quantity of the known basic phosphotungstates in the mother liquor.

[5] Dakin, *Biochem. J.*, **12**, 290 (1918); **13**, 398 (1919).

[6] Mueller, *Proc. Soc. Exptl. Biol. Med.*, **19**, 161 (1921–22); *J. Biol. Chem.*, **56**, 157 (1923).

[7] Van Slyke and Hiller, *Proc. Nat. Acad. Sci.*, **7**, 185 (1921).

[8] A new prolamine from seeds of *Euchlaena Mexicana* Schrad. See Ref. 2 for the method of preparation, elementary analysis and the distribution of the nitrogen according to the Van Slyke method of analysis.

The filtrate was again placed in an ice-cooled refrigerator for 72 hours but no more precipitate formed. It was then placed in a cold storage room (at about —15°) until partly frozen. The mixture was then removed to a refrigerator room at 0° until the ice was melted and was again partly frozen and again melted. This process was repeated several times. After the first freezing, a precipitate was formed which at first separated as a heavy sirup that later crystallized. The succeeding freezings brought down more crystals, some attaining a considerable size. These crystals appeared to be of the isometric system with sides up to about 2–3 mm.

The filtrate was poured off and the crystals were washed by decantation with a cold solution of hydrochloric acid and phosphotungstic acid as in the regular Van Slyke analysis. No difficulty in washing was experienced inasmuch as the crystals are very heavy and show no tendency to remain suspended in the wash solution. The amino acid was separated from the phosphotungstic acid by dissolving the crystals in 2 liters of water and making the solution slightly alkaline with barium hydroxide. After filtering off the precipitated barium phosphotungstate, the filtrate was neutralized to litmus with sulfuric acid, and silver hydroxide was added in excess to remove the hydrochloric acid. The silver chloride was filtered off and the excess of silver removed by means of hydrogen sulfide. Barium carbonate was then added in excess to remove any sulfuric acid and the barium carbonate and barium sulfate were filtered off.

The filtrate was then concentrated in a vacuum to about 300 cc., the precipitated barium carbonate again removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in 150 cc. of 40% alcohol and the solution decolorized with Norite. On concentrating the clear filtrate, the amino acid separated in stellate groups of needles. The crystals were filtered off and washed with 80% alcohol. The yield of this supposedly pure product was 1.4 g., or 2.8% of the original protein. When the mother liquor was evaporated to dryness it yielded about 1.5 g. of an impure product.

An analysis of the crystals gave 28.75% of carbon, 6.55% of hydrogen and 8.64% of nitrogen.

This compound which was thought to be the pure amino acid was apparently a salt, for about 28% of the weight of the crystals remained in the platinum boat as ash from the carbon and hydrogen analysis. A qualitative analysis of the ash showed that it contained a large amount of barium. The presence of so great an amount of ash may vitiate conclusions drawn from the elementary analysis. However, correcting for 28% of ash in this product, the elementary analysis would show 39.93 of carbon, 9.09% of hydrogen, 12.00% of nitrogen and 38.98% of oxygen, corresponding to an empirical formula of $C_4H_{11}O_3N$.

A sample of the amino acid was dissolved in 100 cc. of water, and 2 cc.

of this solution gave 0.718 mg. of amino nitrogen as determined by the Van Slyke micro-amino acid apparatus, or 39.90 mg. of nitrogen in the entire sample; 10.00 cc. of the solution gave 3.55 mg. of total nitrogen by a Kjeldahl analysis, or 35.5 mg. of total nitrogen in the entire sample. Thus all of the nitrogen is amino nitrogen. From these analytical data, the structural formula of this amino acid cannot be determined. The data would indicate an amino-hydroxybutyric acid as a possibility, although it is difficult to see why such a compound should readily form a phosphotungstate.

Due to the small amount of the purified product (possibly the barium salt) obtained the physical characteristics could not be accurately determined. It was soluble in hot water, soluble with difficulty in cold water, and insoluble in alcohol. It showed slight optical activity in water, being levorotatory.

Phenyl Isocyanate Derivative.—The phenyl isocyanate derivative was prepared as follows. To a solution of 0.75 g. (calcd. ash-free) of the amino acid in 20 cc. of water and 15 cc. of *N* sodium hydroxide solution was added 1 cc. of phenyl isocyanate and the mixture shaken vigorously until the odor of phenylisocyanate had disappeared. During the reaction a white precipitate separated, but at the completion of the reaction the solution was distinctly alkaline to litmus. The precipitate (diphenyl-urea) was filtered off and the filtrate acidified with hydrochloric acid. The phenyl isocyanate precipitated in a more or less gelatinous mass. After several hours, the precipitate was filtered off and dissolved in a small amount of 90% alcohol, and then enough water was added to form a turbid solution. Several hours later, the product had crystallized. The crystals appeared to be prisms; m. p., 141° (uncorr.). After the material had again been recrystallized as described above, the crystals melted at 140° (uncorr.).

Anal. Calcd. for $C_{11}H_{14}O_4N_2$: C, 55.69; H, 5.48; N, 11.81. Found: C, 55.40; H, 5.25; N, 11.93.

These results agree very well with the empirical formula of a phenyl isocyanate of a mono-amino acid, $C_4H_{11}O_3N$, such as was suggested by the elementary analysis of the amino acid. This derivative is readily soluble in alcohol and insoluble in water.

Work is in progress on the preparation of a larger amount of the pure amino acid. We hope to carry out further work on its structure and on its quantitative determination and also to determine to what extent it is found in the different classes of proteins.

Summary

A new amino acid has been isolated from the protein teozein. This amino acid may be precipitated as the phosphotungstate under the conditions of the Van Slyke method of protein analysis when the solution is cooled to its freezing temperature. The pure amino acid was not prepared, as the crystalline preparation contained considerable ash in which barium predominated. Our present data indicate an empirical formula of $C_4H_{11}O_3N$ or some multiple of this. The phenyl isocyanate was prepared; m. p., 140° (uncorr.). The analysis of this derivative agrees with the apparent empirical formula. The work is being continued.

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