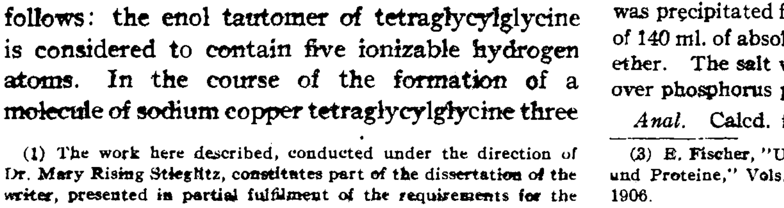
[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

The Biuret Reaction of the Pentapeptide Tetraglycylglycine[1]

BY PAUL E. WENAAS

As a continuation of the study of the biuret reaction of the polypeptides of the glycine series in preparation for a study of the biuret reaction of the proteins,[2] the biuret salt of the pentapeptide tetraglycylglycine, $NH_3^+(CH_2CONH)_4CH_2CO_2^-$, has been isolated and examined.

The biuret salt of tetraglycylglycine was prepared by the saturation with cupric hydroxide of a solution of the pentapeptide in aqueous sodium hydroxide. The analytical data for the completely dehydrated salt obtained from this aqueous solution by precipitation with an absolute alcohol–absolute ether mixture agree quite favorably with the empirical formula $[Na_3^+Cu^{++}(NH_2(CH_2CO^-{=}N)_4CH_2CO^-_2)]$ for the salt. The amount of sodium found, however, was lower than that calculated by a greater amount than would result from any possible analytical errors, and at the same time the hydrogen found was correspondingly higher than that required by theory. These results suggest two possibilities: (1) that the trisodium salt was hydrolyzed in part during its preparation, or (2) that not all the acid hydrogen atoms in the pentapeptide molecule were neutralized by the alkali used. The molecular weight of the salt was not determined. If its molecular and empirical formulas are identical the structure of the salt may be



The structure assigned may be interpreted as follows: the enol tautomer of tetraglycylglycine is considered to contain five ionizable hydrogen atoms. In the course of the formation of a molecule of sodium copper tetraglycylglycine three of these are considered to be neutralized by the alkali used. When the acid strength of the peptide molecule is thus reduced by the neutralization of three acid groups, the amine character of the peptide is enhanced and cupric ion then may enter a complex of the cupri-ammonium type with four of the strongly basic nitrogen atoms of the peptide molecule. The two remaining acid groups of the enolate serve as the negative radical of the "complex salt," as sulfate ion is the negative ion of copper ammonium sulfate. The term "complex salt" as here used signifies that part of the biuret salt molecule which is placed within the brackets in the structure given. The negative radical of the cupri-peptide complex is not free to ionize, since this radical is an integral part of the peptide molecule.

Experimental

Synthesis of Peptides.—The synthesis of tetraglycylglycine was accomplished according to the procedure described by Fischer.[3] Glycine was converted into its ester hydrochloride from which was obtained glycine anhydride. The anhydride yielded upon hydrolysis and treatment with chloroacetyl chloride in one operation chloroacetylglycylglycine, which was converted by the use of aqueous ammonia into diglycylglycine. The treatment of the tripeptide successively with chloroacetyl chloride and ammonia yielded triglycylglycine, which on treatment successively with chloroacetyl chloride and aqueous ammonia gave tetraglycylglycine.

Sodium Copper Tetraglycylglycine, $Na_3CuC_{10}H_{12}N_5O_6$.—The salt was prepared as follows: a solution of 0.1 g. of tetraglycylglycine in 7.16 ml. of water was treated with 2.84 ml. of a 1.754% solution of carbonate free sodium hydroxide. The resultant solution was shaken with an excess of cupric hydroxide for fifteen minutes.[4] After removal of the excess cupric hydroxide on a Neubauer micro-platinum filtering crucible having a porous iridium bottom, the biuret salt was precipitated from the reaction mixture by the addition of 140 ml. of absolute ethyl alcohol and 100 ml. of absolute ether. The salt was collected, dried at room temperature over phosphorus pentoxide and analyzed.

Anal. Calcd. for $Na_3CuC_{10}H_{12}N_5O_6$: Na, 16.02; Cu,

---

(1) The work here described, conducted under the direction of Dr. Mary Rising Stieglitz, constitutes part of the dissertation of the writer, presented in partial fulfilment of the requirements for the doctorate degree at the University of Chicago.

(2) Rising, Parker and Gaston, THIS JOURNAL, **56**, 1178 (1934).

(3) E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vols. I and II, Verlag von Julius Springer, Berlin, 1906.

(4) The cupric hydroxide was prepared by the method described by Böttger, *J. prakt. Chem.*, **73**, 491 (1858).

14.76; N, 16.26; C, 27.86; H, 2.81. Found: Na, 13.20, 13.62; Cu, 14.35, 14.85; N, 15.47, 15.55; C, 27.14, 27.30; H, 3.73, 3.69.

The salt was dehydrated completely at 78° over phosphorus pentoxide. A sample of the salt weighing 25.270 mg. dried at room temperature over phosphorus pentoxide when completely dehydrated at 78° lost 0.378 mg. weight, or 1.50%. This dehydrated salt was analyzed for nitrogen.

Anal. Calcd. for $Na_3CuC_{10}H_{12}N_5O_6$: N, 16.26. Found: N, 15.81, 15.98.

The analytical data of the product dried at room temperature were corrected for the loss of weight on complete dehydration at 78°.

Anal. Calcd. for $Na_3CuC_{10}H_{12}N_5O_6$: Na, 16.02; Cu, 14.76; N, 16.26; C, 27.86; H, 2.81. Found (corr.): Na, 13.41, 13.83; Cu, 14.57, 15.07; N, 15.71, 15.79; C, 27.56, 27.72; H, 3.62, 3.58.

Sodium copper tetraglycylglycine is a hygroscopic pink powder. When heated in a capillary-size melting point tube it begins to decompose with a blackening of the sample at 268°, shrinks to a considerable extent at 272°, and evolves gas at 279–281° (all temp. corr.).

Summary

The sodium biuret salt of the pentapeptide tetraglycylglycine has been isolated and analyzed. The character of this salt is discussed in terms of the theory of the biuret reaction so far developed.

Received May 6, 1937

[Contribution from the Department of Chemistry of Stanford University]

The Composition of Grignard Reagents as Determined by Precipitation with Dioxane

By C. R. Noller and W. R. White

Schlenk and Schlenk, Jr.,[1] who first investigated the action of dioxane on Grignard solutions explained their results by postulating that Grignard solutions are equilibrium mixtures represented by equation I or II. They favored equation I

$$2RMgX \rightleftarrows R_2Mg + MgX_2 \qquad (I)$$

$$R_2Mg_2X_2 \rightleftarrows R_2Mg + MgX_2 \qquad (II)$$

since eight-fold dilution of a solution of ethylmagnesium iodide did not affect the position of equilibrium as determined by precipitation of the halogen-containing compounds with dioxane. This result has been confirmed for Grignard solutions from normal, secondary and isobutyl bromides.[2] One would expect from this to be able to calculate equilibrium constants for Grignard solutions which would hold not only for variations in concentration but also for different ratios of halogen to alkyl radical. Miss Probert[2] determined the equilibrium compositions of solutions in which the ratio of halogen to alkyl radical varied widely for the normal, secondary and isobutyl compounds but no equilibrium constants could be obtained satisfying either I or II. In this work the equilibrium composition was determined by precipitation of the halogen compounds with dioxane and immediately removing the precipitate by filtration.

Although duplicate determinations by the same worker usually check fairly well, the values for dialkylmagnesium content obtained by different workers frequently vary considerably and it was thought that this and possibly also the inability to obtain satisfactory equilibrium constants might be due to occlusion of varying amounts of dialkylmagnesium under different conditions of manipulation and concentrations. It was thought also that occlusion might explain the results of Cope[3] who reported that the amount of diphenylmagnesium left in solution after precipitation of the halogen-containing compounds from a solution of phenylmagnesium bromide increased from 67 to 75% on standing in contact with the precipitate. Accordingly solutions of Grignard reagents from ethyl bromide containing varying concentrations and ratios of halogen to ethyl radical were precipitated with dioxane and shaken with the precipitate for eighteen to twenty hours before centrifuging and analyzing the supernatant liquid. The reproducibility of determinations under these conditions was increased but the data, given in Table I, again do not give satisfactory equilibrium constants on the basis of either I or II. It can be seen, however, that in general the more halogen present in the solution, the greater the amount of alkyl radical precipitated. This is illustrated better in Fig. 1 where the equivalents of ethyl radical precipitated are plotted against the equivalents of halogen in the orginal solution. While it is highly questionable whether one is justified in attempt-

---

(1) Schlenk and Schlenk, Jr., *Ber.*, **62**, 920 (1929).

(2) Probert, Master's Thesis, Stanford University, 1933.

(3) Cope, This Journal, **57**, 2238 (1935).