as shown by hydrolysis with 85% acetic acid.[12] This hydrolysis gave two fractions, one acidic and one neutral. The acidic fraction formed a semicarbazone, m. p. 161–162°, corresponding to the semicarbazone of α-methyl-δ-keto-iso-octanic acid.[13] The neutral fraction proved to be the ethyl ester of this acid. It is obvious that both of these compounds result from the ketonic hydrolysis of the ester IX. No trace of a cyclic ketone could be detected.

It seems reasonably certain that fraction 4 also contains some unchanged ester IX.

The products boiling higher than fraction 4 appear to have been formed by the condensation of diethyl α-methylglutarate with itself. This conclusion seems likely in view of the low yield of this ester (relative to ethyl isobutyrate) from the reaction. In fact when diethyl α-methylglutarate was heated alone with sodium ethoxide under the conditions described above for the reaction of IX, alcohol distilled from the reaction mixture and when the reaction product was worked up and fractionated, the same series of higher boiling fractions as were obtained in the case of IX resulted.

(12) This reagent has been shown by Dieckmann and Kron (Ref. 3, p. 1266) to decarboxylate the cyclic β-ketonic esters to yield the cyclic ketones.

(13) Wallach, *Ann.*, **327**, 138 (1903).

Summary

A study of the acetoacetic ester condensation between a carbethoxy group and an alpha carbon atom carrying a single hydrogen atom has been made. It has been found that such a condensation takes place only when the condensing carbethoxy group is a part of a monosubstituted malonic ester structure. A mechanism, which explains the facts now known, is suggested for the acetoacetic ester condensation.

MADISON, WISCONSIN RECEIVED JANUARY 25, 1934

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

The Biuret Reaction. IV. (a) A Biuret Salt of the Tetrapeptide Triglycylglycine. (b) A Biuret Salt of Glycine Anhydride. (c) The Barium Biuret Salt of Succinimide[1,2]

BY MARY M. RISING, FRANCIS M. PARKER AND DOROTHY R. GASTON

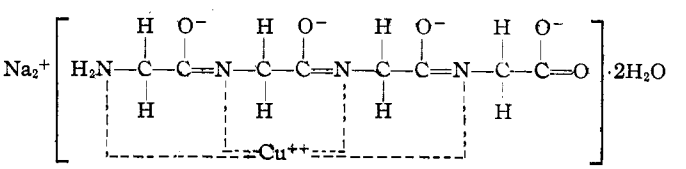
Following the investigation of the biuret reaction of amino acid amides,[3] and in preparation for a study of the biuret reaction of the proteins, the biuret salt of the tetrapeptide triglycylglycine, $NH_3^+ (CH_2CONH)_3CH_2CO_2^-$ has been isolated and examined.

The synthesis of triglycylglycine was accomplished in a series of reactions described by Fischer.[4] Glycine was converted into its ester hydrochloride, from which there 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 ammonia into diglycylglycine. By treatment of the tripeptide successively with chloroacetyl chloride and ammonia, triglycylglycine was obtained. It was found that liquid ammonia acts more efficiently in the ammonolysis of certain of the chloroacetyl peptides than does the aqueous ammonia recommended by Fischer.

The biuret salt sodium copper triglycylglycine

$$Na_2^+[Cu^{++}(NH_2(CH_2CO^- = N)_3CH_2CO_2^-)]\cdot 2H_2O$$

was prepared by treatment of the tetrapeptide with copper acetate and sodium hydroxide. The analytical data for the salt point to the empirical formula just given. It is of peculiar interest to note that the molecule of the salt contains only one peptide molecule, which furnishes the four basic nitrogen atoms and the four acid hydrogen atoms which have already been found requisite for the occurrence of a number of typical biuret reactions. The molecular weight of the salt has not been ascertained. If its molecular and empirical formulas are identical the structure of the salt may be



Sodium copper triglycylglycine

It will be noted that formation of a salt molecule of this structure involves (1) neutraliza-

(1) The contents of this paper were reported at the National Meeting of the American Chemical Society held at Chicago, September, 1933.

(2) The work here described constitutes part of the dissertations of Francis M. Parker and Dorothy R. Gaston, presented in partial fulfilment of the requirements for the doctorate degree at the University of Chicago.

(3) Rising and Yang, *J. Biol. Chem.*, **99**, 755 (1933).

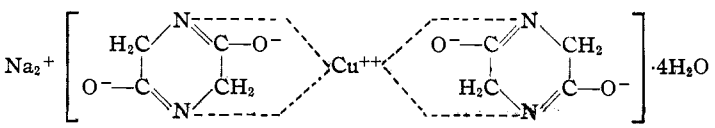
(4) E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vols. I and II, Verlag von Julius Springer, Berlin, 1906.

tion by alkali of two of the acid groups of an enol tautomer of the peptide molecule. The other two acid groups serve as the negative radical in the complex salt, as does the sulfate ion in copper ammonium sulfate. The formation of the salt molecule involves (2) complex ion formation of copper with four amine nitrogen atoms.

Incidental to the synthesis of the peptides of the glycine series, the biuret reaction of glycine anhydride was investigated. While glycine itself shows no biuret reaction, its anhydride, $\underline{CONHCH_2CONHCH_2}$ does so readily, behaving as an acyl disubstituted di-acid amide. The biuret salt of the anhydride was isolated, analyzed and its probable empirical formula shown to be

$$Na_2^+\left[\begin{array}{l} O^- \quad O^- \\ Cu^{++}\ (C{=}NCH_2C{=}N(CH_2)_2 \end{array}\right]\cdot 4H_2O$$

The structure of the salt may be



Sodium copper glycine anhydride

It is significant that the formula of the compound agrees with that shown previously to be correct for the biuret salts of other di-acid amides, *e. g.*, $Me_2^+[Cu(\text{di-acid amide})_2]^{=}\cdot xH_2O$.

It is a curious fact that the first peptide to show the biuret reaction in the glycine series is triglycylglycine. The cause of the failure of many di- and tripeptides to react with cupric ion and alkali should be investigated, since there is no apparent reason for this sharp difference in behavior between the tetrapeptides and their precursors. Di-*l*-alanyl-*l*-alanine is said to show a "weak" biuret reaction and the isolation of its biuret salt is now being attempted.

The pentapeptide tetraglycylglycine has been prepared from chloroacetyltriglycylglycine and liquid ammonia and converted into its biuret salt by Paul E. Wenaas. Analytical data and other facts concerning this salt will be reported at a later time.

For the adequate development of the theory of the biuret reaction there must be uncovered facts regarding the nature of the hypothetical "biuret acids," the parent acids of biuret salts. There should exist, for example, however unstable they may be, the acids $H_2^+[Cu(\text{malonamide})_2]^{=}$, corresponding to the salt $Na_2^+[Cu(\text{malonamide})_2]^{=}$, and $H_2^+[Cu(\text{succinimide})_4]^{=}$, the parent acid of the salt $K_2^+[Cu(\text{succinimide})_4]^{=}$. The acids should be, in certain instances at least, obtainable from their salts, and it is anticipated that the barium salts may prove particularly suitable for this purpose. Accordingly, the biuret salt barium copper succinimide was prepared, of formula $Ba^{++}\ [Cu^{++}(\underline{CH_2CH_2CON{=}CO^-})_4]\cdot H_2O$. This salt is comparable in formula and doubtless in structure with potassium copper succinimide, $K_2^+\ [Cu^{++}(\underline{CH_2CH_2CON{=}CO^-})_4]\cdot 6H_2O$.

Experimental

Synthesis of Peptides.—The procedure described by Fischer for the preparation of triglycylglycine was followed faithfully with only one notable change: in the ammonolysis of chloroacetylglycylglycine and chloroacetyldiglycylglycine, liquid, rather than aqueous, ammonia was used by us. The tri- and tetrapeptides so obtained were purer and formed in about 8% larger yields than was the case when Fischer used aqueous ammonia.

Sodium Copper Triglycylglycine, $Na_2CuC_8H_{14}N_4O_7$.—The salt was prepared as follows: a filtered solution of 1.5 g. of triglycylglycine in 90 cc. of water was treated with 30 cc. of 2% sodium hydroxide solution and sufficient freshly precipitated copper hydroxide so that some of the latter base remained undissolved at the end of the reaction. The red reaction solution was shaken for five minutes and then filtered into 2000 cc. of a 2–1 absolute alcohol–ether mixture. Presently a pink precipitate formed, which was collected, dried at room temperature over phosphorus pentoxide and analyzed.

Anal. Calcd. for $Na_2CuC_8H_{14}N_4O_7$: Na, 11.86; Cu, 16.40; C, 24.76; H, 3.64; N, 14.45. Found: Na, 12.39, 12.82; Cu, 16.47, 16.73; C, 24.87, 24.44; H, 4.24, 4.39; N, 14.23, 14.00.

Dehydration of this salt was carried out at 80° and 15 mm. over phosphorus pentoxide. The dehydrated substance was analyzed for nitrogen.

Anal. Calcd. for $Na_2CuC_8H_{10}N_4O_5$: N, 15.93. Found: N, 15.64, 15.70.

The analytical data indicate that dehydration of the compound *in vacuo* at 80° causes a loss of two molecules of water. The salt is a deep pink powder which chars at 278° (corr.) and melts with decomposition at 280° (corr.).

Sodium Copper Glycine Anhydride, $Na_2CuC_8H_{16}N_4O_8$.—The instability of this salt led to considerable difficulty in its isolation. The method used follows: 1 g. of glycine anhydride and 0.428 g. of freshly precipitated and washed copper hydroxide were suspended together in about 40 cc. of water. A solution of 0.7 g. of carbonate-free sodium hydroxide in 8 cc. of water was brought into this reaction mixture, whereupon a deep blue color developed. After ten minutes all of the anhydride and copper hydroxide had dissolved, and the solution was then concentrated under reduced pressure until its volume was about 17 cc. The

colored liquid was added drop by drop with stirring to a mixture of 700 cc. of absolute ethyl alcohol and 350 cc. of absolute ether, whereupon a deep lavender, finely divided precipitate of sodium copper glycine anhydride formed; this was collected as rapidly as possible on a filter. The extremely hygroscopic nature of the salt made its handling under a protective layer of dry alcohol and ether a necessity. The compound was stable in a desiccator over fresh phosphorus pentoxide.

Anal. Calcd. for $Na_2CuC_8H_{16}N_4O_8$: Na, 11.33; Cu, 15.67; C, 23.66; H, 3.97; N, 13.80. Found: Na, 11.48, 11.40; Cu, 14.52, 14.57; C, 24.82, 25.10; H, 4.16, 4.34; N, 13.63, 13.66.

The results of dehydration experiments indicated the presence of four molecules of water in the salt molecule. The substance decomposes when heated above 120°.

Barium Copper Succinimide, $BaCuC_{16}H_{16}N_4O_8$.—The salt was prepared as follows: 50 cc. of a saturated aqueous solution of barium hydroxide was filtered into 250 cc. of 90% alcohol which contained 2.5 g. of succinimide. Then 15 cc. of a saturated aqueous solution of copper acetate was filtered into this mixture, which was shaken meanwhile. After about two minutes a pink precipitate formed which was collected and dried to constant weight over phosphorus pentoxide at 100° and 15 mm. The analytical data for the dehydrated salt follow:

Anal. Calcd. for $BaCuC_{16}H_{16}N_4O_8$: Ba, 23.10; Cu, 10.72; C, 32.37; H, 2.71; N. 9.44. Found: Ba, 23.67, 23.66; Cu. 10.74, 10.61; C, 30.10, 29.92; H, 3.12, 3.05; N, 9.54, 9.37.

The salt, which is a brownish-red powder, melts with decomposition at 257° (corr.).

Summary

1. The sodium biuret salt of the tetrapeptide triglycylglycine, the barium biuret salt of succinimide and the sodium biuret salt of the acyl disubstituted di-acid amide glycine anhydride have been isolated and analyzed.

2. The theory and facts of the biuret reaction as so far developed are in agreement with the character of these salts.

CHICAGO, ILLINOIS RECEIVED JANUARY 29, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Hydrogenation of Cyclic Ureides under Elevated Temperatures and Pressures. I.[1] 2-Keto-1,2,3,4-tetrahydropyrimidines

BY KARL FOLKERS[2] AND TREAT B. JOHNSON

There are a vast number of glyoxalone, pyrimidone, quinazolone, xanthine, etc., derivatives which are valueless as therapeutics. However, if they could be converted, first, into their desoxy derivatives, and, second, into more saturated, if not perhydro, structures, their melting points would be lowered, their general solubilities greatly increased, and their basicity established or increased. These much desired changes in properties would be recognized to enhance their physiological activities and so to increase their chemical and pharmacological interest and possibilities. On the other hand, certain of these compounds, such as pilocarpine and caffeine, are valuable drugs and it would be of interest to know what such constitutional changes would do to their physiological actions.

The most noteworthy approach to desoxy derivatives of numerous uric acids, xanthines and barbituric acids was made by Tafel and his coworkers[3] by electrolytic reduction. The ureido group, —NHCONH—, has often been converted into —NHC(Cl)=N— and thence to —NHCH=N—, but such indirect procedures have numerous objections.

Catalytic methods of attaining the above objectives would possess many advantages. A course to reach the desoxy derivatives might result through study of the action of hydrogen on these cyclic ureides over copper–barium–chromium oxide catalysts at approximately 250° and 200 atmospheres pressure. So far as known, such ureides have not been subjected to this particular study.[4]

Hydrogen over nickel at 150–250° and 200 atmospheres pressure has been expected to hydrogenate the cyclic double bonds of ureides in a manner far more satisfactory than past attempts[5]

(1) Researches on Pyrimidines, No. CXLII.

(2) Eli Lilly and Co. Post-doctorate Research Fellow, 1933–1934.

(3) These papers, too numerous to cite here, were published in the *Berichte der Deutschen Chemischen Gesellschaft* during 1899–1911.

(4) N-Caproylpiperidine has been converted to N-*n*-hexylpiperidine and probably N-ethyl-3-methyl-2-piperidone to N-ethyl-3-methyl-piperidine by a technique for hydrogenation of esters to alcohols [Folkers and Adkins, THIS JOURNAL, **54**, 1145 (1932)]. By amalgam reduction, diethyl thiobarbituric acid has been reduced to desoxyveronal [Einhorn, *Ann.*, **359**, 176 (1908)]. The hydrogenation of numerous amides to amines has just been published [Adkins and Wojcik, THIS JOURNAL, **56**, 247 (1934)].

(5) Exemplary references on the reduction of the 5,6-double bond of pyrimidines by platinum and palladium catalysts have been cited with recent such experimental studies [Folkers and Johnson, *ibid.*, **55**, 1140 (1933)]. In the glyoxaline series, benziminazole, histidine, lysidine, 2,4,5-trimethylglyoxaline and glyoxaline failed to be reduced by a platinum catalgst, whereas amarin and lophine were