[CONTRIBUTION FROM THE ORGANIC LABORATORY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

A THIRD PHENYL-BIURET

FIFTH PAPER ON PURINES

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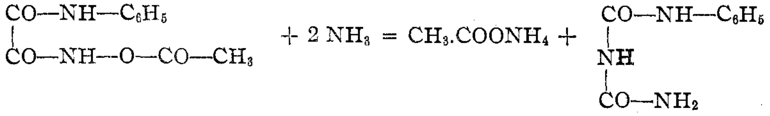
Received July 10, 1922

Two phenyl-biurets are already known. Of these, that melting at 165° is generally believed to be the unsymmetrical compound. It was discovered by Pickard and Carter[1] who allowed ammonia to react with several phenyl derivatives of oxamide, and obtained the best yields by the use of acetyl-phenyl-hydroxy-oxamide.



$$\begin{matrix}\mathrm{CO{-}NH{-}C_6H_5}\\ |\\ \mathrm{CO{-}NH{-}O{-}CO{-}CH_3}\end{matrix} + 2\,\mathrm{NH_3} = \mathrm{CH_3.COONH_4} + \begin{matrix}\mathrm{CO{-}NH{-}C_6H_5}\\ |\\ \mathrm{NH}\\ |\\ \mathrm{CO{-}NH_2}\end{matrix}$$

As might be expected numerous by-products are formed, and the reaction is no convincing argument for structure. Better evidence is furnished by the recent work of Fromm[2] who has prepared this substance by the hydrolysis of phenylcarbamide cyanide, $C_6H_5.NH.CO.NH.CN$. It is also formed by the action of aniline upon biuret which in the hands of Hofmann[3] yielded *sym*-diphenyl biuret. He apparently overlooked the simultaneous formation of the simpler compound. This method of preparation, of course, throws no light upon the structure. *Asym*-phenyl-biuret does not give the biuret reaction.

The second phenyl-biuret melts at 190° and shows a strong biuret reaction. It was first prepared by Weith[4] by heating phenyl-urea with phosphorus trichloride, a reaction which cannot be used for the determination of constitution. In 1907, Schiff[5] made a careful comparison of the two substances and assigned the symmetrical structure to the compound melting at 190°, relying mainly upon the biuret reaction. For some years he had been studying the elements of structure which are essential conditions of this test, and he had come to the conclusion that only those substituted biurets give a violet color with copper sulfate and alkali which have both of the original amino-groups intact. It may be added that Emil Fischer,[6] on the basis of his experience with polypeptides, etc., has commented favorably upon the general validity of Schiff's rules.

Schiff also calls attention to a difference in the chemical behavior of

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[1] Pickard and Carter, *J. Chem. Soc.*, **79**, 841 (1900); **81**, 1563 (1902).

[2] Fromm, *Ber.*, **55**, 1911 (1922).

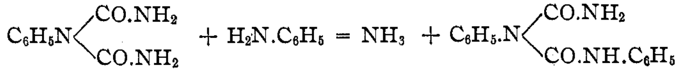
[3] Hofmann, *ibid.*, **4**, 265 (1871).

[4] Weith, *ibid.*, **10**, 1743 (1877).

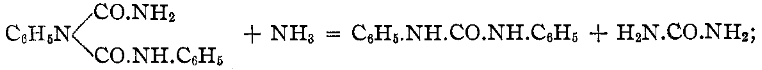
[5] Schiff, *Ann.*, **352**, 72 (1907).

[6] E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Berlin, 1906, p. 301.

the two compounds which may not be without significance. *asym*-Phenyl-biuret when warmed with aniline, even in water, yields *sym*-diphenyl-biuret. The phenyl-biuret melting at 190°, however, yields carbanilide. He assumes that *asym*-diphenyl-biuret is first formed, but that this is decomposed by the ammonia to carbanilide and urea.

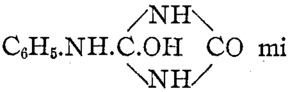
I 

$$\text{I}\quad C_6H_5N\left\langle\begin{matrix}CO.NH_2\\CO.NH_2\end{matrix}\right. + H_2N.C_6H_5 = NH_3 + C_6H_5.N\left\langle\begin{matrix}CO.NH_2\\CO.NH.C_6H_5\end{matrix}\right.$$

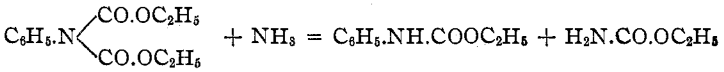
II 

$$\text{II}\quad C_6H_5N\left\langle\begin{matrix}CO.NH_2\\CO.NH.C_6H_5\end{matrix}\right. + NH_3 = C_6H_5.NH.CO.NH.C_6H_5 + H_2N.CO.NH_2;$$

in other words, that centrally substituted biurets tend to split off the carbon and nitrogen at the unsubstituted end of the molecule. Schiff, however, gives no experimental evidence for the presence of urea in his reacting mixture.

It was shown in the preceding paper that a third phenyl-biuret can be obtained by the action of hydrogen peroxide upon 9-phenyl-uric acid. This has the same percentage composition and molecular weight as the foregoing, but differs from both in crystalline form and in optical properties. When pure it melts at 198° and shows the biuret reaction, though not so strongly as the compound of Weith. It seems not to be an enol, such as $R.NH.C(OH):NH.CO.NH_2$, since it gives no color with ferric chloride. A methyl-phenyl-isobiuret is indeed known[7] but it is very unstable toward hydrochloric acid, splitting off methyl chloride and yielding *asym*-phenyl-biuret. The new phenyl-biuret, however, is obtained from a hydrochloric acid solution. Greater stability toward acids might be consistent with a formula such as $HN:C(OC_6H_5).NH.CO.NH_2$, but this seems excluded by the formation from 9-phenyl-uric acid, which must have its phenyl group upon nitrogen. Ring formulas such as  $C_6H_5.NH.\underset{\diagdown NH\diagup}{\overset{\diagup NH\diagdown}{C.OH\quad CO}}$ might be set up, but they are improbable especially in the case of a compound which is so clearly a biuret.

In the preceding paper, certain theoretical considerations were set forth which made it seem desirable to appropriate for this compound the symmetrical formula now assigned to the compound of Weith. It was hoped to settle the question by the synthesis of a true symmetrical biuret, and for this purpose aniline-N-dicarboxylic ester was treated with ammonia. Unfortunately, the aqueous reagent had no action, while liquid ammonia and alcoholic ammonia caused splitting to phenyl-urethane, apparently in accordance with the following equation.



$$C_6H_5.N\left\langle\begin{matrix}CO.OC_2H_5\\CO.OC_2H_5\end{matrix}\right. + NH_3 = C_6H_5.NH.COOC_2H_5 + H_2N.CO.OC_2H_5$$

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[7] McKee, *Am. Chem. J.*, **26,** 209 (1901).

In view of this disappointment, it will be necessary to leave the question of the structure of these substances open for the present. Meantime, the most interesting fact about the new compound is its curious relation to *asym*-phenyl-biuret. Even in dilute aqueous solution, it can be transformed into the latter by the action of ammonia and a great variety of organic bases. The list thus far includes: methyl amine, dimethyl amine, ethyl amine, tetramethyl-ammonium hydroxide, tetramethyl-phosphonium hydroxide, trimethyl-sulfonium hydroxide and diphenyl-iodonium hydroxide. That this is not an hydroxyl-ion reaction is shown by the fact that it is not brought about by caustic potash, caustic soda, barium hydroxide or moist silver oxide, although caustic potash effects the change incompletely, if hydrogen peroxide is present. Pyridine also fails to cause the reaction and benzyl amine apparently reacts in some other sense. Aniline yields *sym*-diphenyl-biuret, obviously in consequence of a preliminary isomerization to *asym*-phenyl-biuret.

This suggests the inquiry whether the new phenyl-biuret is not a "physical isomer" of the unsymmetrical compound. Against this must be urged the fact that *asym*-phenyl-biuret here appears as the more stable substance although it melts lower and is more soluble. Further, all attempts to effect the reverse transformation have hitherto failed. These have included inoculation of the saturated solutions, inoculation of the melts, and the action of ultra-violet light upon both the solid substances and their solutions.

Experimental

***asym*-Phenyl-biuret.**—This compound melts at 165°. It is soluble in water, in alcohol and in ether. It crystallizes in shining scales. It dissolves readily in *N* alkali and with difficulty in dil. hydrochloric acid. Habit, thin plates; indices, $\alpha = 1.572$, $\gamma = 1.67$.

Weith's Phenyl-biuret.—Great difficulty was experienced in following the procedure of Schiff, who felt that he had improved that of Weith. It was only after several trials that any of the product was obtained, but this showed the properties described by Schiff. It melts at 190°, and is readily soluble in hot water and in alcohol. Treatment with ammonia causes *no* isomerization. Habit, short thick prisms; extinction, 32–34°; indexes, $\alpha = 1.532$, $\gamma = 1.649+$.

New Phenyl-biuret.—The preparation of the new phenyl-biuret from 9-phenyl-uric acid has been described in the preceding paper (p. 146). The substance melts at 196–198° with decomposition. It is soluble in hot water and in alcohol, but not as soluble as *asym*-phenyl-biuret. Habit, long needles; extinction, 32–33°; indices, $\alpha = 1.559$, $\gamma = 1.73+$.

Analyses. Calc. for $C_8H_9O_2N_3$: C, 53.63; H, 5.03; N, 23.46. Found: C, 53.73, 53.43; H, 4.66, 4.54; N, 23.19, 23.21.

Molecular weight: by freezing-point method in glacial acetic acid. Calc. for $C_8H_9O_2N_3$: 179. Found: 146, 158.

Isomerization.—Parallel tests were made as follows. A little of the powdered substance melting at 197–198° was suspended in water and warmed. Then a few drops of the base were added, the solution was heated once to boiling, and filtered onto a watch glass. The crystalline product formed as the solution cooled was collected and its melting point determined. A mixed melting point determination was then made with whichever phenyl-biuret melted nearest to it. The results are tabulated below. It should be added that an effort was made to see that the nitrogen bases were free from ammonia.

Base	M. p. °C.	M. p. °C.	When mixed with phenyl-biuret
NH_4OH	164	163–164	asym
CH_3NH_2	164	163–164	asym
$C_2H_5NH_2$	163	163–164	asym
$(CH_3)_2NH$	164–165	165–166	asym
$(CH_3)_4NOH$	165	164–165	asym
NaOH	197–199	198–200	new
KOH	198–200	198–200	new
Ag_2O	198–200	198–199	new
$Ba(OH)_2$	198	198	new
$C_6H_5NH_2$	203	205	α,α'-diphenyl-biuret
$C_6H_5CH_2NH_2$	80–85	. . .	
$(CH_3)_3SOH$	162–164	163–164	asym
$(CH_3)_4POH$	164–166	165–166	asym
$(C_6H_5)_2IOH$	165	165–166	asym
C_5H_5N	196–200	198	new

The new phenyl-biuret was also warmed with dil. hydrochloric acid but was recovered unchanged.

Although special tests showed that neither caustic potash nor hydrogen peroxide caused the isomerization alone, they did together, that is, under the conditions obtaining in the oxidation of phenyl-uric acid.

Five tenths g. of the new phenyl-biuret was dissolved in 9 cc. of water containing 0.8 g. of potassium hydroxide. It was cooled with ice water, and 28 cc. of hydrogen peroxide solution was added. A precipitate formed immediately. A little of it was collected and found to melt at 165° and when mixed with *asym*-phenyl-biuret at 164°. The solution was then allowed to stand for 2 days longer. By that time 0.2 g. of the same material had been formed. The filtrate from this on acidification yielded 0.01 g. of the unchanged new phenyl-biuret melting at 198–200°.

Experiments with Ultra-violet Light.—(1) Dry, powdered samples of both biurets were separately exposed for 8 hours to the action of the rays from a mercury arc (110 volts) at a distance of 20 cm. from the source. Both remained unchanged.

(2) Samples of each weighing 0.5 g. were separately dissolved in 2 cc. of alcohol and exposed in small quartz tubes to the radiation from the same arc for 24 hours. No change was noticed in either.

Action of Ammonia upon Aniline-N-dicarboxylic Ester.—The diethyl ester of aniline-N-dicarboxylic acid was prepared by the method of Diels and Narviosky[8] by the action of ethyl chloroformate upon phenyl-urethane. A sample weighing 0.5 g. was allowed to stand with concentrated aqueous ammonia (15 *N*) for several days. Oily drops formed. On evaporation of the water a thick sirup was obtained from which

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[8] Diels and Narviosky, *Ber.*, **42**, 3681 (1909).

crystals melting at 60–65° were obtained. Mixed with the original ester they melted at 60°.

Another sample was allowed to stand overnight in a Dewar bulb with liquid ammonia until all the latter had evaporated. A yellow oil remained which solidified after several days. From it crystals were obtained which melted at 51°, the melting point of phenyl-urethane.

Two more samples (0.5 g.) were sealed in glass tubes with alcoholic ammonia and heated for 48 hours, one at 80° and the other at 160–180°. The only substance isolated from either was phenyl-urethane.

Summary

1. A third phenyl-biuret has been prepared by the action of alkaline hydrogen peroxide upon 9-phenyl-uric acid, and clearly distinguished from the 2 isomers previously known.

2. This substance can be transformed to *asym*-phenyl-biuret, a compound of lower melting point and greater solubility, by the action of ammonia and a great variety of organic bases, but not by alkalies. No method of reversing this reaction has been discovered.

3. Although the structure theory contemplates the existence of but 2 nitrogen-substituted biurets, there is as yet no evidence that any one of the 3 existing compounds is an enol or an oxygen ether, or that it has a ring structure.

4. Certain theoretical considerations, set forth in the preceding paper, tempt one to assume the structure of symmetrical N-phenyl-biuret for the new compound.

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THE QUANTITATIVE ESTIMATION OF THE GRIGNARD REAGENT[1]

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Received July 20, 1922

Introduction

In connection with a series of quantitative studies of the Grignard reagent it was necessary to devise methods for the rapid and exact estimation of this reagent in ether, the commonly employed solvent. A search of the literature disclosed that only one method has been tried. This work by Jolibois[2] was repeated, and the method indicated has been found quite unsatisfactory and unreliable. The present report is an account of the study of several different methods: titration with iodine, gravimetric analysis, a so-called indirect analysis, gas analysis and titra-

[1] A preliminary report of this work was made at the Rochester meeting of the American Chemical Society, April 28, 1921.

[2] Jolibois, *Compt. rend.*, 155, 213 (1912).