

ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY OF THE UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, CALIF.]

The Dipole Moment and Structure of Biuret¹

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RECEIVED MAY 31, 1960

The titration curve of biuret with base is indistinguishable from that of water which rules out the presence of enol forms. The infrared spectrum also indicates no enol forms are present. The dipole moment of the biuret is 3.27 D. which is consistent with the molecule being coplanar with an intramolecular hydrogen bond. The negative dielectric increment of biuret compared with that of urea suggests that the intramolecular hydrogen bond is present when biuret is in water solution.

Introduction

Calvalca and Nardelli² have obtained indications from X-ray studies on divalent metal chloride complexes of biuret that this molecule is flat. Kogon³ obtained evidence from infrared studies in benzene that unsymmetrical tetraalkyl biurets have an intramolecular hydrogen bond.

If biuret is not flat, there are a large number of possible conformations, but these are reduced to a few in a flat molecule. The possibilities involve two enol forms I and II, one with an O-H...O and the other with an N-H...O hydrogen bond, and three keto forms, one with an N-H...O, one with a N-H...N hydrogen bond and one with no hydrogen bond, IV, V and III.

These forms I to IV, Fig. 1, would be flat if there were appreciable contribution from resonating forms with a separation of charge. Dissociation constant, dipole moment and infrared spectra should give evidence as to which of these forms is present.

Results

If the compound exists as an enol such as I or II, it should have an acid dissociation constant larger than 10^{-10} for these contain a structure analogous to a carboxylic acid, *i.e.*, -C-OH as



compared to -C-OH . While the =N is not as electronegative as =O , the presence of the nitrogen atoms and the other oxygen would give such a structure an acidity at least as strong as a K_a of 10^{-10} .

We have titrated biuret with sodium hydroxide and obtained a titration curve identical with that of distilled water. The K_a of the compound is therefore no stronger than that of water which has a K_a of 1.8×10^{-16} and this rules out the possibility that the structure of the compound is I or II.

The dipole moment can be used to distinguish between structure III and IV or V. As a first approximation, one can think of biuret as being made up of two urea molecules which have the dipoles in the direction of the carbonyl groups. If we neglect the effect of the hydrogen bond, we can consider the two urea dipoles to be at an angle of 120° in structures IV and V and at an angle of 0° in structure III. The dipole moment of urea is about 4.6 D.⁴ and since the resultant of two like

vectors at 120° is the same as the value of the individual vectors, the value for conformations IV and V would be 4.6 D. while that of III where the moments add up would be 9.2 D. These are maximum values, for the central nitrogen in biuret is not equivalent to the other nitrogens so the contribution of the forms with separation of charge is less here than in urea. The magnitude of this effect probably would reduce the moment by less than 1 D.

The dipole moment of the compound has been measured in dioxane at 30° and the results are given in Table I. The high dilution used in the dipole moment measurements would tend to prevent association of the solute molecules with each other.

TABLE I

DIPOLE MOMENT DATA FOR BIURET IN DIOXANE AT 30°

ω_2	ϵ_{12}	ν_{12}
0.0000000	2.19707	0.97867
.0009093	2.20875	.97834
.0015969	2.21773	.97808
.0019769	2.22130	.97789
.0024750	2.22828	.97769
.0029347	2.23534	.97744

ϵ_1 2.196931 α 12.8313 P_2 237.07 μ 3.27 \pm 0.02
 ν_1 0.976773 β - 0.41689 P_E 21.90 calcd.

The observed value of 3.27 ± 0.02 D. definitely rules out conformation III and is consistent with structure IV or V. Dipole repulsion would also cause III to be highly improbable. The fact that the observed moment is 1.33 D. less than 4.6 D. is in keeping with a hydrogen bond being present to cause a further lowering of the moment.

Conformation V is inconsistent with the molecule being coplanar because the hydrogens of one amino group would have to be out of the plane to enable the lone pair to be in a position to form the hydrogen bond with a hydrogen on the other amino group. Also with V, one would expect one carbonyl frequency in the infrared and not two, for both carbonyl groups are free. The infrared spectrum of the compound, Fig. 2, shows two carbonyl bands which is in keeping with structure IV where one carbonyl oxygen would be hydrogen bonded and the other one would not.

As a coupling of carbonyl vibrations in certain cyclic dicarbonyl compounds⁵ sometimes gives rise to two bands without any possibility of an

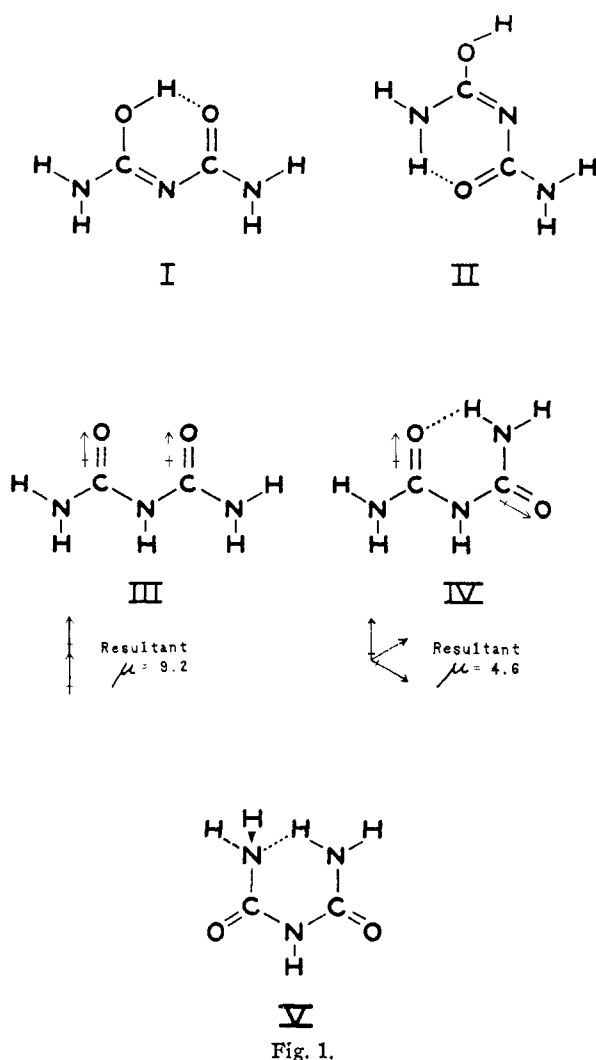
(1) Presented at the 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960.

(2) L. Calvalca and M. Nardelli, private communication.

(3) I. C. Kogon, *THIS JOURNAL*, **79**, 2253 (1957).

(4) W. D. Kumler and G. M. Fohlin, *ibid.*, **64**, 1944 (1942).

(5) K. H. Hall, *ibid.*, **80**, 6428 (1958).



intramolecular hydrogen bond being present, it seemed desirable to see whether two carbonyl bands were present in similar compounds that did not have the possibility of hydrogen bonding. N-Methyl-diacetamide is such a compound as is diacetamide and their infrared spectra show the presence of only one carbonyl band. The fact that biuret shows two carbonyl bands is then evidence for structure IV.

The N-H region shows the presence of both a free N-H at 3380 cm.^{-1} and a hydrogen bonded N-H at 3185 cm.^{-1} . These frequencies are lower than those reported for dipeptides⁶ where bands for a free NH are given at 3461 cm.^{-1} and for intramolecular hydrogen bonded NH at 3361 cm.^{-1} . Two factors probably contribute to this: the different environment about the NH in biuret as compared to the dipeptides and the fact that the former

(6) S. Mizushima, M. Tsuboi, T. Shimanouchi and M. Asai, *THIS JOURNAL*, **76**, 6003 (1954).

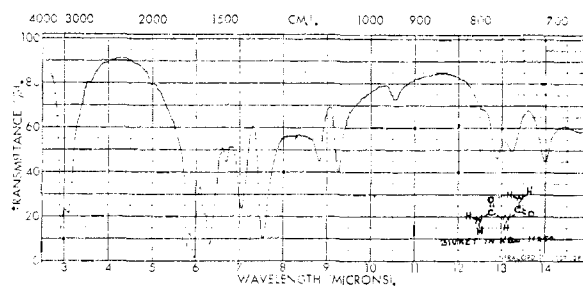


Fig. 2.—Spectra of biuret in KBr.

was measured in KBr and the latter in carbon tetrachloride. The difference in frequency between the hydrogen bonded and free N-H in biuret is much larger ($3380 - 3185 = 195\text{ cm.}^{-1}$) than that in the dipeptides ($3461 - 3361 = 100\text{ cm.}^{-1}$) which suggests that the hydrogen bond in biuret is stronger than that in the dipeptides. The six-membered ring, connecting the hydrogen, in biuret would be expected to have a stronger hydrogen bond than the seven-membered ring in the dipeptides. The bands are too far separated to arise from symmetrical and asymmetrical vibrations of the NH_2 groups.

The oxygen atoms in the solvent dioxane might have broken up the intramolecular hydrogen bond in biuret by forming intermolecular hydrogen bonds, but the evidence indicates that this does not happen. Water would be expected to be a more potent solvent for breaking up the intramolecular hydrogen bonds. Evidence with regard to this point can be obtained from the dielectric increment of biuret and urea in water solutions. These compounds contain the same kind of atoms and groups and should have approximately the same dielectric increment. Actually, biuret has a negative dielectric increment of -4.37 and urea has a positive increment of $+3.4$. This suggests that the intramolecular hydrogen bond is still present when biuret is dissolved in water. The evidence then indicates the presence of an intramolecular hydrogen bond in biuret regardless of whether it is in the form of the solid or in dioxane or water solutions.

Experimental

Eastman biuret was recrystallized several times from ethanol until a constant melting point was obtained. The dipole moment was measured in dioxane at 30° and the calculations made using the equation and method of Halverstadt and Kumler.⁸ The dioxane was purified by refluxing for several hours with concentrated hydrochloric acid neutralizing and drying with solid potassium hydroxide, then refluxing over molten sodium and twice fractionally distilling from molten sodium through a 1-meter column packed with glass helices until a product boiling within a 0.1 degree range was obtained.

We wish to thank Mr. M. K. Hrenoff of the Spectrographic Laboratory for running the spectra.

(7) J. Wyman, Jr., *Chem. Revs.*, **19**, 213 (1936).

(8) I. F. Halverstadt and W. D. Kumler, *THIS JOURNAL*, **64**, 2988 (1942).