Acta Chemica Scandinavica B 31 (1977) 251—255

Crystal and Molecular Structure of 1-Hydroxybiuret

INGRID KJØLLER LARSEN

Royal Danish School of Pharmacy, Department of Chemistry BC, Universitetsparken 2, DK-2100 Copenhagen, Denmark

1-Hydroxybiuret, $H_2NCONHCONHOH$, has been prepared and isolated in the pure state. The melting point of the compound is 146 – 147 °C (decomp.), and the pK_A value was determined to 9.58(1). The structure of 1-hydroxybiuret has been determined using three-dimensional diffractometer-collected X-ray data. The compound crystallizes in the space group $P\bar{1}$ with four formula units ($C_2H_5N_3O_3$) in the unit cell with the dimensions $a = 7.054(2)$, $b = 6.958(2)$, $c = 10.793(2)$ Å, $\alpha = 106.65(2)°$, $\beta = 96.34(2)°$, $\gamma = 71.03(3)°$. The structure was solved by direct phasing techniques, and was refined by full-matrix least-squares calculations to an R-value of 0.035.

Both of the two crystallographically unrelated molecules form an *intra*-molecular NH···O bond. Significant differences were found in the dimensions of the two molecules. The crystals are built up of layers of hydrogen bonded molecules.

1-Hydroxybiuret, $H_2NCONHCONHOH$, has been shown to inhibit the DNA synthesis in an *in vitro* Ehrlich ascites tumor test system.[1] The substance used in the experiments was prepared by reaction of methyl allophanate with hydroxylamine [eqn. (1)], but no physical data of the compound, obtained by evaporation of the reaction mixture to dryness, were given.

$$H_2NCONHCOOCH_3 + H_2NOH,HCl + NaOH \rightarrow H_2NCONHCONHOH + CH_3OH + NaCl \quad (1)$$

This work presents the preparation and the crystal structure determination of the compound. The structure determination is part of an X-ray study of hydroxylamine derivatives, which inhibit the DNA synthesis in different cell systems, probably by preventing the bioreduction of ribonucleotides. Hydroxyurea, the parent compound in this series, has been shown to inhibit the enzyme system ribonucleotide reductase of *E. coli* by destroying the organic free radical of protein B2, a subunit of the enzyme system.[2] A series of hydroxyurea analogs, including 1-hydroxybiuret in the pure state, has recently been prepared in our laboratory and tested for inhibitory action on the same enzyme system. 1-Hydroxybiuret was found to be a less potent inhibitor than is hydroxyurea.[3]

Preliminary investigations in our laboratory seem to indicate a correlation between the ability of the hydroxyurea analogs to inactivate protein B2 and the ability of the compounds to destroy (reduce) the nitroxide radical in Fremy's salt [$(KO_3S)_2NO\cdot$]. Hydroxylamine and easily oxidizable substituted hydroxylamines have been reported to be oxidized by Fremy's salt.[4] Further investigations in this field are in progress.

EXPERIMENTAL

Synthesis. 1-Hydroxybiuret was prepared from methyl allophanate and hydroxylamine as earlier proposed.[1] To a solution of 10.0 g (0.085 mol) of methyl allophanate[5] in anhydrous methanol (800 ml) was added 5.9 g (0.085 mol) of hydroxylammonium chloride and 5.2 g (0.130 mol) of sodium hydroxide. After standing at room temperature for about 14 days the solution was adjusted to pH 7 with a methanolic solution of hydrogen chloride and evaporated to dryness *in vacuo*. The residue was extracted with six 25 ml portions of ethanol. The combined ethanol extracts were evaporated to dryness *in vacuo* and the residue (5.3 g), which by TLC was shown to consist of several compounds, was subjected to column chromatography [silica gel: 200 g; eluent: benzene – methanol – ethyl acetate (4:2:1) containing 1 %

Table 1. Crystal data for 1-hydroxybiuret.

Mol. formula	$C_2H_5N_3O_3$
Mol. weight	119.1
Melting point, °C	146 – 147 (decomp.)
Space group	$P\bar{1}$
a, Å	7.054(2)
b, Å	6.958(2)
c, Å	10.793(2)
α,°	106.65(2)
β,°	96.34(2)
γ,°	71.03(3)
V, Å^3	479.9
Z	4
D_x, g cm^{-3}	1.65
D_m, g cm^{-3}	1.64
μ Mo$K\alpha$ cm^{-1}	1.64

of formic acid]. The fractions giving a blue colour reaction with iron(III) chloride on TLC were collected and evaporated to dryness *in vacuo* to give 1.0 g of crude 1-hydroxybiuret. Recrystallization of the product from methanol gave 0.5 g (5 %) of slightly coloured crystals, m.p. 146 – 147 °C (decomp.). Anal. $C_2H_5N_3O_3$: C, H, N. The pK_A value of the compound was determined to 9.58(1) by electrometrical titration in aqueous solution at 21 °C, according to the method described by Albert and Serjeant.[6] The pH values were measured on a Radiometer pH meter 26.

X-Ray crystallography. Single crystals suitable for the X-ray work were obtained by slow evaporation from a solution of the compound in methanol. The size of the crystal chosen for data collection was $0.14 \times 0.20 \times 0.30$ mm^3. Some crystal data of 1-hydroxybiuret are given in Table 1. The density was measured by flotation in a mixture of carbon tetrachloride and methyl iodide. The melting point was determined with a hot stage microscope (Mikroskop Heiztisch Ernst Leitz G.m.b.H., Wetzlar).

The lattice parameters of the compound were calculated from 32 diffractometer-measured θ-values ($\lambda = 0.71069$ Å). The crystal was mounted in a glass capillary and oriented with the b-axis parallel to the ϕ-axis of the goniostat. The intensity data were collected on a NONIUS three-circle automatic diffractometer, using the same technique as described earlier.[7] Intensities of reflexions were measured in the range $2.5° \leq \theta \leq 25.0°$; of the 1578 independent reflexions 1170 had $I(\text{net}) \geq 2.5\sigma(I)$, and were considered observed. The data were corrected for Lorentz and polarization effects, but no corrections for absorption or extinction were made.

STRUCTURE DETERMINATION

The structure was solved by direct methods using the automatic phasing program MULTAN.[8] Refinement of the positional and thermal parameters of the non-hydrogen atoms, using the full-matrix least-squares method and anisotropic temperature factors, led to an R-value of 0.062. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$, where the weights were initially taken as unity.

All hydrogen atoms were located in the difference electron density map. The final cycles of refinement included all positional parameters, as well as anisotropic temperature factors for the non-hydrogen atoms. The isotropic thermal parameters of the hydrogen atoms were fixed at the values of the parameters of the atoms to which they are bonded. The weighting scheme used in the last cycles of refinement was of the form $w = 1/\{1 + [(|F_o| - 7.0)/8.0]^2\}$.

The final R-value is 0.035 for all observed reflexions. The scattering factors used for hydrogen were those of Stewart, Davidson and Simpson,[9] and for oxygen, nitrogen and carbon

Table 2. Fractional coordinates and thermal parameters for the non-hydrogen atoms. The thermal parameters ($\times 10^2$) are of the form: $\exp[-2\pi^2 (U_{11}h^2a^{*2} + \cdots 2U_{12}hka^*b^* \cdots)]$

ATOM	X/A	Y/B	Z/C	U11	U22	U33	U12	U13	U23
N11	0.5443(3)	0.2186(4)	0.4504(2)	2.7(1)	6.1(1)	2.5(1)	-1.9(1)	0.0(1)	1.0(1)
C12	0.3721(3)	0.2608(4)	0.5040(2)	2.8(1)	3.5(1)	2.5(1)	-1.1(1)	-0.1(1)	1.3(1)
O12	0.3482(2)	0.3269(3)	0.6237(1)	3.0(1)	5.7(1)	2.0(1)	-1.6(1)	-0.7(1)	1.1(1)
N13	0.2094(3)	0.2295(3)	0.4261(2)	2.1(1)	4.5(1)	2.2(1)	-1.2(1)	0.1(1)	1.2(1)
C14	0.1952(3)	0.1991(4)	0.2942(2)	2.9(1)	3.0(1)	2.3(1)	-0.9(1)	0.2(1)	0.9(1)
O14	0.3423(2)	0.1573(3)	0.2259(1)	2.9(1)	5.8(1)	2.5(1)	-1.3(1)	0.4(1)	1.2(1)
N15	0.0153(3)	0.2145(4)	0.2396(2)	2.7(1)	5.9(1)	1.8(1)	-1.4(1)	0.1(1)	1.0(1)
O16	-.1499(2)	0.2554(3)	0.3120(2)	3.0(1)	5.7(1)	3.1(1)	-1.2(1)	0.6(1)	1.2(1)
N21	0.7200(3)	0.3313(4)	0.7834(2)	2.7(1)	7.1(2)	3.1(1)	-1.8(1)	-0.4(1)	2.3(1)
C22	0.8827(3)	0.3048(4)	0.7226(2)	2.9(1)	3.7(1)	2.4(1)	-1.5(1)	-0.4(1)	1.3(1)
O22	0.8839(2)	0.3095(3)	0.6092(2)	3.2(1)	7.1(1)	2.5(1)	-2.2(1)	-0.4(1)	2.1(1)
N23	1.0636(3)	0.2717(3)	0.7924(2)	2.6(1)	4.7(1)	2.4(1)	-1.6(1)	0.0(1)	1.4(1)
C24	1.0952(3)	0.2416(4)	0.9138(2)	3.2(1)	3.5(1)	2.2(1)	-1.0(1)	0.0(1)	0.9(1)
O24	0.9618(2)	0.2482(3)	0.9809(2)	3.3(9)	7.4(1)	2.8(1)	-1.9(1)	0.0(1)	2.3(1)
N25	1.2873(3)	0.1952(4)	0.9567(2)	2.9(1)	6.3(2)	2.4(1)	-1.3(1)	-0.1(1)	2.0(1)
O26	1.4256(3)	0.2392(3)	0.8955(2)	3.3(1)	6.0(1)	3.9(1)	-1.3(1)	0.6(1)	1.7(1)

Table 3. Fractional coordinates and thermal parameters for the hydrogen atoms.

ATOM	X/A	Y/B	Z/C	B
H111	0.548(4)	0.186(4)	0.367(3)	2.7
H112	0.643(4)	0.245(4)	0.496(3)	2.7
H13	0.113(4)	0.254(4)	0.464(2)	2.2
H15	0.000(4)	0.200(4)	0.157(3)	2.4
H16	-.216(4)	0.417(5)	0.335(3)	3.0
H211	0.728(4)	0.321(4)	0.869(3)	3.0
H212	0.611(4)	0.352(4)	0.742(3)	3.0
H23	1.158(4)	0.263(4)	0.754(2)	2.4
H25	1.306(4)	0.206(4)	1.035(3)	2.8
H26	1.507(4)	0.108(5)	0.855(3)	3.3

those of Cromer and Mann.[10] Final parameters for the non-hydrogen atoms are given in Table 2, and the hydrogen atom parameters are given in Table 3. Notation of the atoms is given in Fig. 1. The observed and calculated structure factor data are available from the author on request. The programs used in the crystallographic analysis were the same as mentioned earlier.[7]

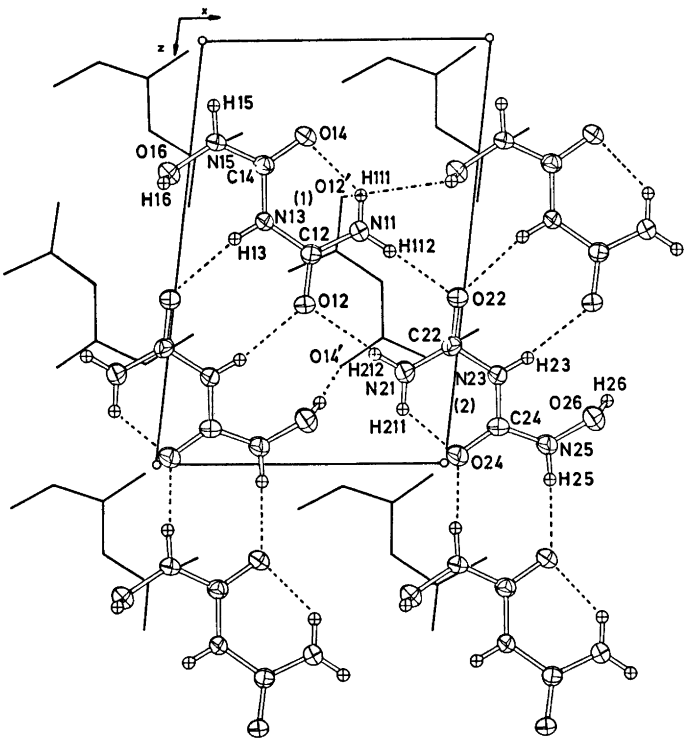

Fig. 1. The structure of 1-hydroxybiuret viewed along the b^*-axis. Dashed lines indicate hydrogen bonds within a layer, ····· indicate hydrogen bonds to a molecule above or below this layer.

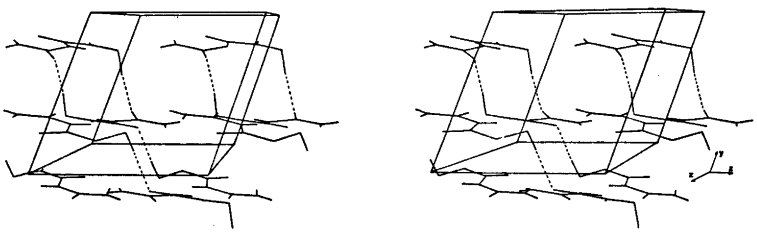

Fig. 2. Stereoscopic diagram of the structure. Only hydrogen bonds between the layers are indicated.

Table 4. Distances and angles concerning the hydrogen bonding system.

X–H···Y	Distances (Å) X···Y	H···Y	Angle (°) X–H···Y
N11–H111···O14	2.669(3)	2.00(3)	134(2)
N11–H112···O22	2.921(3)	2.08(3)	179(3)
N13–H13···$O22_{x-1,y,z}$	3.005(3)	2.24(3)	167(3)
N15–H15···$O24_{x-1,y,z-1}$	2.842(3)	2.00(3)	165(3)
O16–H16···$O12_{-x,1-y,1-z}$	2.702(3)	1.67(3)	173(3)
N21–H211···O24	2.655(3)	1.95(3)	130(2)
N21–H212···O12	2.979(3)	2.16(3)	167(3)
N23–H23···$O12_{x+1,y,z}$	3.033(3)	2.26(3)	161(2)
N25–H25···$O14_{x+1,y,z+1}$	2.964(3)	2.16(3)	165(3)
O26–H26···$O14_{-x+2,-y,-z+1}$	2.735(3)	1.82(3)	177(3)
Non-bonded short distances			
O16···$N11_{x-1,y,z}$	2.871(3)		
O26···$N21_{x+1,y,z}$	2.838(4)		

Table 5. Deviations of atoms (Å) from the least-squares planes through the indicated atoms. The equations of the planes are in direct (unit cell) space. Atoms defining the planes are asterisked.

Molecule (1)

Urea plane I: $0.4818x + 6.7034y - 3.6449z - 0.0872 = 0$
Urea plane II: $1.1184x + 6.8041y - 1.7094z - 1.0678 = 0$

Molecule (2)

Urea plane I: $1.1294x + 6.5463y + 0.2415z - 3.1701 = 0$
Urea plane II: $1.1306x + 6.2935y + 1.3904z - 4.0177 = 0$

Molecule (1) Atom	I	II	Molecule (2) Atom	I	II
N11	−.001*		N21	.001*	
C12	.004*		C22	−.003*	
O12	−.001*		O22	.001*	
N13	−.001*	−.001*	N23	.001*	−.004*
C14		.002*	C24		.012*
O14		−.001*	O24		−.004*
N15		−.001*	N25		−.004*
O16		−.031	O26		.345
H111	.09		H211	−.03	
H112	.06		H212	.00	
H13	−.02	−.01	H23	.05	.00
H15		.03	H25		.20
H16		.96	H26		−.44
Angle I:II	12.3°		Angle I:II	6.2°	

RESULTS AND DISCUSSION

The crystal structure of 1-hydroxybiuret consists of roughly planar layers of molecules that are extensively hydrogen bonded to one another, *cf.* Figs. 1–2. The dimensions of the hydrogen bonds are listed in Table 4. Each of the two crystallographically unrelated molecules is connected to three neighbouring molecules by pairs of NH···O bonds. The terminal hydroxyl groups connect adjacent layers by forming OH···O bonds. The hydroxyl groups

Table 6. Bond lengths (Å) and angles (°) for the two crystallographically non-equivalent molecules of 1-hydroxybiuret.

N11-C12	1.312(3)	N21-C22	1.320(4)
C12-O12	1.252(3)	C22-O22	1.234(3)
C12-N13	1.384(3)	C22-N23	1.395(3)
N13-C14	1.375(3)	N23-C24	1.369(3)
C14-O14	1.247(3)	C24-O24	1.231(3)
N15-O16	1.381(3)	N25-O26	1.391(4)
N11-H111	0.86(3)	N21-H211	0.94(3)
N11-H112	0.84(3)	N21-H212	0.84(3)
N13-H13	0.78(3)	N23-H23	0.80(3)
N15-H15	0.86(3)	N25-H25	0.82(3)
O16-H16	1.04(3)	O26-H26	0.91(3)
N11-C12-O12	122.7(2)	N21-C22-O22	124.2(2)
N11-C12-N13	119.3(2)	N21-C22-N23	117.3(2)
O12-C12-N13	118.0(2)	O22-C22-N23	118.5(2)
C12-N13-C14	125.8(2)	C22-N23-C24	126.9(2)
N13-C14-O14	123.1(2)	N23-C24-O24	124.0(2)
N13-C14-N15	117.1(2)	N23-C24-N25	115.8(2)
O14-C14-N15	119.8(2)	O24-C24-N25	120.2(2)
C14-N15-O16	121.3(2)	C24-N25-O26	118.5(2)
H111-N11-C12	118(2)	H211-N21-C22	121(2)
H111-N11-H112	120(3)	H211-N21-H212	121(3)
H112-N11-C12	121(2)	H212-N21-C22	118(2)
H13-N13-C12	114(2)	H23-N23-C22	115(2)
H13-N13-C14	119(2)	H23-N23-C24	118(2)
H15-N15-C14	120(2)	H25-N25-C24	117(2)
H15-N15-O16	119(2)	H25-N25-O26	116(2)
H16-O16-N15	106(2)	H26-O26-N25	103(2)

of both molecules are hydrogen bonded to oxygen atoms (O14′ and O12′) of a molecule symmetry related to molecule (1). The environments of the two unrelated molecules are thus most different in the *y* direction. The short distances between the hydroxyl oxygen atoms and nitrogen atoms of neighbouring NH_2-groups within the same layer are not considered as hydrogen bonds. The lengths of these non-bonded distances are also given in Table 4.

As 3-hydroxybiuret [7] the molecule of 1-hydroxybiuret can be considered to be composed of two overlapping urea moieties. Each of these individual moieties is planar within experimental error, *cf.* Table 5, while each of the two molecules as a whole is rather non-planar. The angles between the two planes are 12.3 and 6.2° for molecule (1) and (2), respectively. The terminal N – O bond is twisted out of the plane of the urea moiety in both molecules. The torsion angle N13 – C14 – N15 – O16 is – 1.2(4)°, and N23 – C24 – N25 – O26 is 18.0(3)°. These small conformational variations are probably due to the different environments of the two molecules in the *y* direction.

The overall conformation of the 1-hydroxybiuret molecules is the same, *i.e.* the conformation that is stabilized by an *intra*-molecular NH···O hydrogen bond. A similar *intra*-molecular contact was also found in the three crystal structures of 3-hydroxybiuret,[7] in perdeuterated biuret,[11] triuret,[12] and in the oxalurate ion, $H_2NCONHCOCOO^-$.[13] None of these molecules were found to be planar. The angles between the two urea moieties (or urea moiety and peptide unit) [13] are of the same magnitude as found in the 1-hydroxybiuret molecules, *i.e.* 5 – 15°.

Bond lengths and angles of the 1-hydroxybiuret molecules are given in Table 6. Some differences between corresponding bonds and angles are found. In fact greater variations in the dimensions of the two crystallographically unrelated molecules in the crystal structure of 1-hydroxybiuret are observed than in the dimensions of the 3-hydroxybiuret molecules in the three different crystal structures of this compound.[7]

Acknowledgement. The author wishes to thank Søren Munk Madsen for skillful technical assistance in preparation of the compound.

REFERENCES

1. Gale, G. R., Smith, A. B. and Hynes, J. B. *Proc. Soc. Exp. Biol. Med. 127* (1968) 1191.
2. Atkin, C. L., Thelander, L., Reichard, P. and Lang, G. *J. Biol. Chem. 248* (1973) 7464.
3. Thelander, L. *Personal communication.*
4. Schlude, H. *Tetrahedron 29* (1973) 4007.
5. Dains, F. B. and Wertheim, E. *J. Am. Chem. Soc. 42* (1920) 2303.
6. Albert, A. and Serjeant, E. P. *The Determination of Ionization Constants*, Chapman and Hall, London 1971, p. 28.
7. Larsen, I. K. *Acta Chem. Scand. B30* (1976) 533.
8. Germain, G., Main, P. and Woolfson, M. M. *Acta Crystallogr. A 27* (1971) 368. Updated MULTAN version of Dec. 1974.
9. Stewart, R. F., Davidson, E. R. and Simpson, W. T. *J. Phys. Chem. 42* (1965) 3175.
10. Cromer, D. and Mann. J. *Acta Crystallogr. A24* (1968) 321.
11. Craven, B. M. *Acta Crystallogr. B 29* (1973) 1525.
12. Ringertz, H. *Acta Crystallogr. 20* (1966) 932.
13. Shieh, H.-S. and Voet, D. *Acta Crystallogr. B 31* (1975) 2192.

Received October 15, 1976.