Barbiturates and Adenine Derivatives. Molecular Structure of a Hydrogen-Bonded Complex

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Abstract: A crystalline 1:1 intermolecular complex has been formed between 9-ethyladenine and 5-isopropyl-5-bromoallylbarbituric acid. The complex crystallizes in a triclinic unit cell, the molecular structure of which has been solved by X-ray diffraction analysis. It was found that the molecules form linear hydrogen-bonded arrays in which the adenine derivative forms a pair of hydrogen bonds with one barbituric acid molecule using the Watson–Crick bonding that is found in DNA. In addition, the adenine forms another pair of hydrogen bonds with a second barbituric acid molecule using the imidazole bonding that has often been observed in crystalline complexes of derivatives of adenine and uracil. Each molecule in the complex thus participates in four different hydrogen bonds.

Barbiturates have diverse actions in biological systems. In addition to their widely known sedative effects, they are also regarded as general metabolic depressants because they inhibit a large number of enzyme systems, including those involved in oxidative phosphorylation.[1] A great deal of information concerning the complex pharmacological activities of the barbiturates has been accumulated, but very little is known concerning the molecular basis of their activities.

The barbiturates are derivatives of uracil. Solution studies of the specificity of hydrogen bonding among purines, pyrimidines, and barbiturates have shown that the barbiturates have a selective affinity for forming strongly hydrogen-bonded complexes with a variety of adenine derivatives. This strong association has been demonstrated by infrared studies,[2] and by the formation of a number of crystalline intermolecular complexes containing derivatives of adenine and the barbiturates.[3] The molecular basis of the extremely varied pharmacological effects of the barbiturates might therefore be understood in terms of the strong association of the barbiturates with adenine-containing coenzymes as well as with molecules, such as cyclic AMP, that play an important role in controlling various metabolic activities. Thus, it is of considerable interest to determine the detailed molecular geometry of the complexes between barbiturates and adenine derivatives.

The crystallographic analysis of one complex containing two molecules of an adenine derivative and one of a barbiturate has previously been reported.[4] In the present report we describe the structure of an intermolecular complex containing a commercially available barbiturate, 5-isopropyl-5-bromoallylbarbituric acid, and 9-ethyladenine. In this 1:1 complex the two types of molecules are organized into infinite ribbons of alternating adenine and barbiturate residues in which adjacent adenine and barbiturate molecules interact through a pair of hydrogen bonds.

9-Ethyladenine (Cyclo Chemical Corp.) and 5-isopropyl-5-bromoallylbarbituric acid (Riedel-deHaen, A. G., Hanover, West Germany) were mixed together in equimolar amounts and dissolved in 50% aqueous ethanol. The solution was allowed to evaporate to dryness, yielding a number of flat prismatic crystals with well-developed faces approximately 0.2–0.4 mm in diameter. The ultraviolet spectrum of an aqueous solution of one of these crystals revealed that the adenine and the barbiturate derivative were present in the crystal in equimolar amounts.

Precession and Weissenberg X-ray diffraction photographs showed that the complex crystallized in a triclinic lattice. The unit cell parameters of this lattice, as measured on a four-circle diffractometer, are presented in Table I. The buoyant density of the crystals was measured in a CCl_4–cyclohexane mixture. A statistical analysis of the diffraction data revealed that the space group contained a center of symmetry. Therefore, the space group of the crystal is $P\bar{1}$ and the asymmetric unit of the unit cell contains two sets of complexed molecules. Diffraction data were collected with a G.E. XRD-490 automatic diffractometer, using the stationary counter–stationary crystal method and Cu Kα X-radiation. Observable reflections (1718) were measured out to a value of $2\theta = 120°$.[5] After correcting the intensities for the Lorentz polarization factors, a three-dimensional sharpened Patterson function was calculated. The position of the bromine

Table I. Unit Cell and Space Group Data for 9-Ethyladenine–5-Isopropyl-5-bromoallylbarbituric Acid[a]

a = 9.094 (6) Å	α = 107.05 (5)°
b = 10.056 (8) Å	β = 106.71 (5)°
c = 14.271 (7) Å	γ = 114.08 (5)°
Space group, $P\bar{1}$	
Z = 2	
Density (obsd) = 1.501 g/cm³	Density (calcd) = 1.496 g/cm³

[a] The quantities in parentheses are the estimated standard deviations of the least significant figure of the tabulated values.

(1) S. K. Sharpless in "The Pharmacological Basis of Therapeutics," 3rd ed, L. S. Goodman and A. Gilman, Ed., Macmillan, New York, N. Y., 1965, p 105.
(2) Y. Kyogoku, R. C. Lord, and A. Rich, *Nature (London)*, 218, 69 (1968).
(3) D. Voet, S. H. Kim, and A. Rich, unpublished results.
(4) S. H. Kim and A. Rich, *Proc. Nat. Acad. Sci.*, 60, 402 (1968).

(5) Structure factors and thermal parameters will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-5888. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

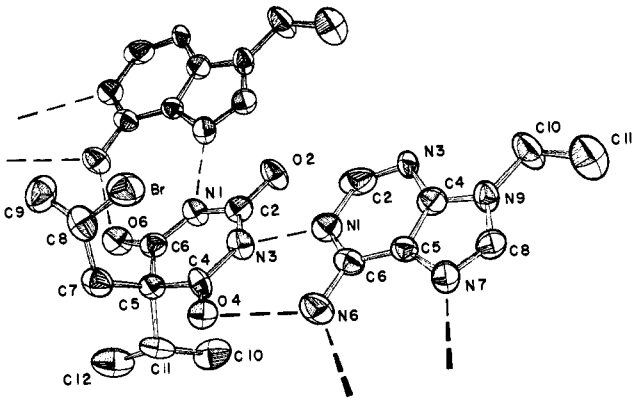


Figure 1. A perspective drawing of the hydrogen-bonded intermolecular complex 9-ethyladenine–5-isopropyl-5-bromallylbarbituric acid. The atoms are represented as thermal ellipsoids of a size such that the centers of the vibrating atoms have a 50% probability of being found within them.[7] The numbering system for the two molecules is shown in the figure.

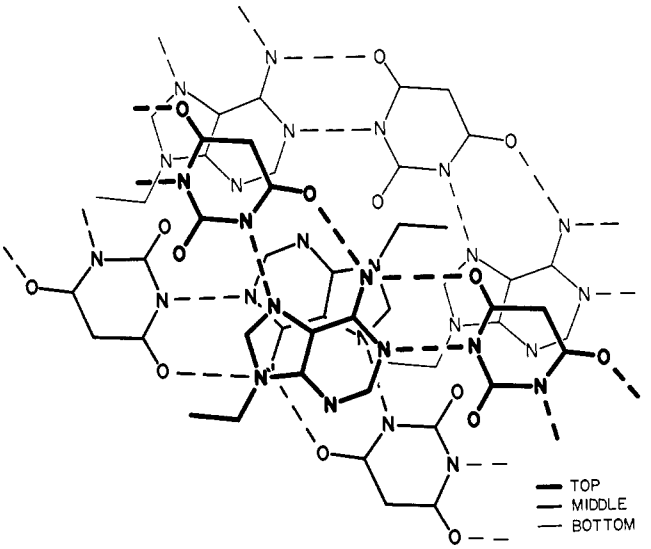


Figure 2. A projection of the crystal structure onto the plane of the aromatic rings. This shows the packing relationship among the adenine and the barbiturate rings. The side groups of the barbiturate which do not lie in the plane have been left out for the sake of clarity.

atom was located directly from analysis of the Patterson map. This information was used with the Woolfson weighting technique[6] to determine the phases and the amplitudes of the Fourier coefficients of an electron density map. This heavy-atom Fourier map revealed all 28 nonhydrogen atoms in the asymmetric unit of the unit cell. The structure was refined using full-matrix least-squares refinement, initially employing isotropic temperature factors and finally anisotropic temperature factors. The data converged to a final error residual of $R = 0.097$. Hydrogen atoms were not evident in the final difference Fourier map.

The structure of the complex together with the atomic numbering scheme used here is shown by the perspective drawing in Figure 1.[7] It can be seen that each barbiturate molecule participates in a cyclic dimer of hydrogen bonds with two neighboring adenine molecules. Thus, adenine atoms N-1 and N-6 hydrogen bond with barbituric acid atoms N-3 and O-4, respectively. This pair of hydrogen bonds resembles the Watson–Crick pairing between adenine and thymine in DNA. The second set of hydrogen bonds associates the imidazole nitrogen, N-7, and the amino group, N-6, on the second adenine molecule with barbituric acid atoms N-1 and O-6, respectively. The two adenine molecules are related by a unit cell translation. Thus, the structure forms an infinite hydrogen-bonded array of alternating adenine and barbiturate residues.

Many crystalline intermolecular complexes of adenine and uracil derivatives have been found in which the adenine forms a hydrogen-bonded cyclic dimer utilizing either its N-1 or its N-7 position as the hydrogen bond acceptor.[8] Only in the cases in which the stoichiometry of the complex is two uracil derivatives to one adenine derivative are both hydrogen bonding sites used.[8] The present structure is the only intermolecular complex of 1:1 stoichiometry reported to date in which both adenine sites are occupied. This is made possible because the barbituric acid derivative has a proton rather than some other substituent at the N-1 position, and this gives it the capability of forming a second pair of hydrogen bonds. In this respect barbituric acid derivatives are somewhat analogous to pseudouridine, a nucleoside that is found in all transfer RNA molecules. In pseudouridine the uracil ring is connected to ribose with a carbon–carbon bond to uracil atom C-5, leaving atom N-1 of the ring free to form a second set of hydrogen bonds. The barbiturate molecule in the present structure could thus be considered to be a model for a pseudouridine molecule in transfer RNA because both have the potential of forming a pair of hydrogen bonds to two different adenine bases. In the intermolecular complex of phenobarbital with two molecules of 8-bromo-9-ethyladenine[4] the barbiturate ring forms two sets of hydrogen bonds to two crystallographically independent adenine derivatives. In that crystal structure the formation of an additional pair of hydrogen bonds between symmetry-related adenine molecules exhausts the hydrogen bonding capability of the complex.

Figure 2 illustrates the mode of packing of successive layers in the lattice. As is shown in this figure, the arrays of hydrogen-bonded adenine–barbiturate complexes form an elongated flat zigzag chain. These are packed side by side in the crystal to form a layer structure. The hydrogen-bonded array at one level lies largely over and under the spaces between neighboring linear arrays of hydrogen-bonded molecules in the adjacent levels. The side chains of the barbiturate derivatives project above and below the plane of the hydrogen-bonded array to fill the space between neighboring layers in adjacent levels.

In the perspective drawing of Figure 1 the atoms are represented as thermal ellipsoids with a size such that the centers of the vibrating atoms have a 50% probability of being found within them.[7] The thermal ellipsoids are therefore indicative of the mobility of the different parts of the molecule in the crystalline lattice. It can be seen that the amino group (N-6) and the ethyl

(6) M. M. Woolfson, *Acta Crystallogr.*, 9, 804 (1956).
(7) C. K. Johnson, "ORTEP: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations," ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
(8) D. Voet and A. Rich, *Progr. Nucl. Acid Res. Mol. Biol.*, 10, 183 (1970).

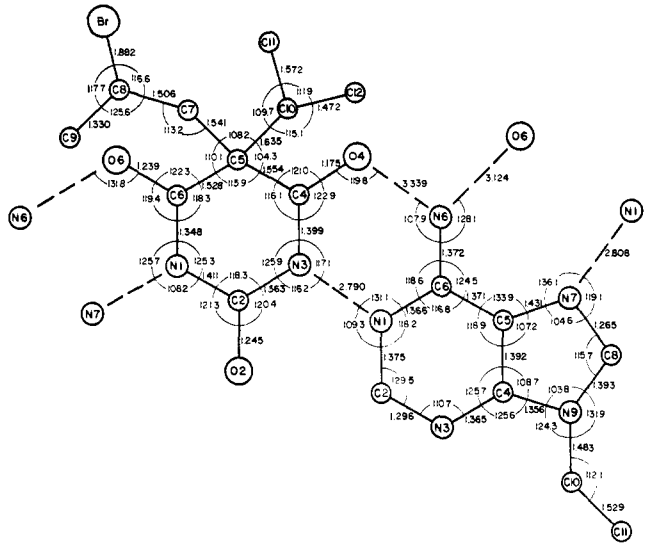


Figure 3. A schematic drawing of the complex showing the bond distances (in ångströms) and the bond angles (in degrees). Hydrogen bonds are represented by dashed lines.

group (C-10–C-11) of adenine vibrate in a direction out of the plane of the purine ring. In a similar fashion, the isopropyl group of the barbiturate (C-10, C-11, C-12) can be seen to vibrate about the C-5–C-11 bond.

The atomic coordinates of the intermolecular complex are listed in Table II. The bond distances and angles are given in Figure 3. The estimated standard deviations of these quantities are 0.02 Å and 1.0°, respectively. The bond distances and angles are generally similar to those which have been obtained from averaging the corresponding quantities in a number of crystal structures containing adenine or barbiturate derivatives.[8] An exception is found in those bonds involving atom C-10 in the barbiturate. Since no significant peaks could be detected in the difference Fourier map in that region, it is likely that the least-squares refinement did not position this atom appropriately. It has been noted by Hughes[9] that the estimated standard deviations obtained from least-squares refinement are often underestimates.

Table II. Atomic Parameters in Fractions of a Unit Cell Edge of the Crystal Structure 9-Ethyladenine–5-Isopropyl-5-bromoallylbarbituric Acid[a]

Atom	*x*	*y*	*z*
	9-Ethyladenine		
N-1	0.0240	0.2102	0.3869
C-2	0.0557	0.3408	0.4744
N-3	−0.0070	0.3405	0.5452
C-4	−0.1136	0.1832	0.5265
C-5	−0.1618	0.0403	0.4389
C-6	−0.0937	0.0541	0.3657
N-7	−0.2775	−0.0965	0.4493
C-8	−0.2918	−0.0315	0.5340
N-9	−0.1964	0.1403	0.5873
N-6	−0.1299	−0.0770	0.2768
C-10	−0.1743	0.2598	0.6888
C-11	−0.2802	0.1741	0.7411
	5-Isopropyl-5-Bromoallylbarbituric Acid		
N-1	0.5198	0.5584	0.3245
C-2	0.4017	0.4917	0.3668
N-3	0.2778	0.3258	0.3119
C-4	0.2496	0.2137	0.2128
C-5	0.3804	0.2857	0.1664
C-6	0.5155	0.4724	0.2303
O-2	0.4054	0.5818	0.4499
O-4	0.1385	0.0719	0.1683
O-6	0.6167	0.5469	0.1969
C-7	0.2654	0.2330	0.0441
C-8	0.1824	0.3329	0.0300
C-9	0.2260	0.4375	−0.0113
Br	−0.0050	0.2998	0.0699
C-10	0.4922	0.1932	0.1705
C-11	0.6001	0.2337	0.2934
C-12	0.6095	0.2239	0.1166

[a] The numbering system is the same as that shown in Figure 1. Carbonyl oxygen and amino nitrogen atoms have the same number as the carbon atom to which they are covalently bound.

The two NH–N hydrogen bond lengths (2.790, 2.808 Å) are in the normal range, whereas the two NH–O hydrogen bonds (3.124, 3.339 Å) are significantly longer than normal.[8,10] The reason for these longer hydrogen bonds is not entirely clear. Model building studies show that the two adenine molecules that are hydrogen bonded to a single barbiturate residue could not be parallel if all of their hydrogen bonds were of normal length. However, since these adenine molecules are related by the translational symmetry of the unit cell, they must be parallel. Hence the increased length of the longer hydrogen bonds may be due to constraints arising from the packing of molecules in the unit cell.

The closest nonbonding intermolecular approaches are between barbiturate atom O-2 and atoms O-2 and C-2 of the barbiturate molecule related to the first by a center of symmetry. These distances, which are 3.159 and 3.165 Å, respectively, are slightly larger than the minimal van der Waals contact for such interactions.[10]

Both the adenine and the barbiturate rings are very close to being planar. The root-mean-square deviation from planarity of the adenine atoms, excluding the side-chain atom C-11, is 0.022 Å. The corresponding quantity for the barbiturate molecule is 0.037 Å, excluding the side-chain atoms. The dihedral angle between these planes is 10.5°. In intermolecular complexes of this type, the hydrogen-bonded molecules usually have dihedral angles which are less than 5°.[8] However, the majority of these hydrogen-bonded purine–pyrimidine complexes contain only two molecules and do not form infinite chains. The small size of such a hydrogen-bonded complex may enable it to act as a more rigid unit in response to crystal packing forces than is possible for an infinite hydrogen-bonded array. Thus it seems likely that in the present case the deviation of the rings from coplanarity is the result of a compromise between the tendency of the infinite arrays of hydrogen-bonded molecules to be perfectly coplanar and the requirement that the bulky substituents of the barbiturate molecules be efficiently packed in a regular crystalline lattice.

Adjoining levels in the layered structure are separated by a distance of 3.2 Å. This is somewhat shorter than the 3.4 Å which is the van der Waals thickness of aromatic rings.[10] However, as is seen in Figure 2, there is very little overlapping of the rings in successive layers so that a closer approach is possible between successive layers of the structure than if there had been more overlap.

(9) E. W. Hughes, "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. A. Freeman, San Francisco, Calif., 1968, p 628.

(10) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960.

Our interest in intermolecular complexes between derivatives of purines and pyrimidines is related to the central role of hydrogen bonding in the transfer of information in biological systems. In the present case, however, the intermolecular complexes between adenine derivatives and the barbiturates are of interest because these interactions may provide some insight into the varied and profound biochemical effects of the barbiturates when they are present in biological systems. The present structure shows a somewhat unusual and extensive system of hydrogen bonding which is a reflection in the solid state of the highly selective hydrogen bonding affinity that is observed between the barbiturates and adenine-containing compounds.

Acknowledgment. This research was supported by grants from the National Science Foundation and the National Institutes of Health. We thank the General Electric Corporation for allowing us to use their XRD-490 diffractometer and are grateful to Mr. Howard Pickett for his assistance with that instrument.

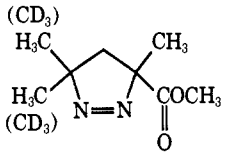
Communications to the Editor

Decomposition of Azo Compounds *via* Cationic Intermediates. Elucidation of the Mechanism of Ionization and Nitrogen Elimination

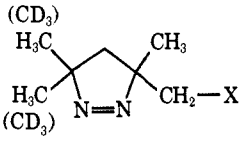
Sir:

Recently we reported the first two cases of azo compounds which eliminate nitrogen by mechanisms involving cationic intermediates.[1] The results clearly showed that for a structurally suitable system the -N=N- group anchimerically assists ionization.[1] However, the preliminary work did not provide for a definitive mechanistic description of nitrogen loss. This has stimulated us to begin a general investigation of the scope and mechanistic details of such reactions. In this communication we wish to report the acetolysis of the new system IIb-OBs and a detailed analysis of ionization–nitrogen elimination for this reaction.

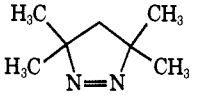
Addition of 2-diazopropane[2] to methyl methacrylate afforded I in excellent yield, bp 67–73° (1.5 mm).[3]



I



IIa, X = OH
b, X = OBs
c, X = OAc

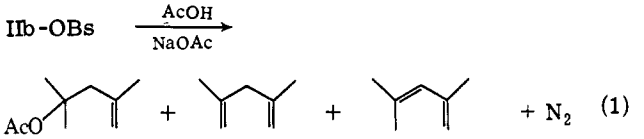


III

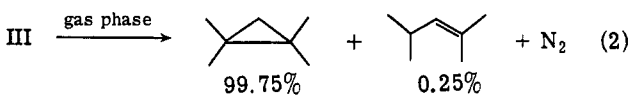
Reduction of I with lithium aluminum hydride followed by oxidation with yellow mercuric oxide gave IIa-OH, bp 88–90° (1.2 mm).[3] Reaction of IIa-OH with sodium hydride followed by treatment with *p*-bromobenzenesulfonyl chloride produced IIb-OBs, mp 109–110°. 2-Diazopropane-d_6[4] was converted in an analogous manner to IIb-OBs-d_6. Treatment of IIa-OH with acetic anhydride and pyridine afforded IIc-OAc.

Azo *p*-bromobenzenesulfonate IIb-OBs was solvolyzed in dry acetic acid buffered with sodium acetate. Rate measurements were made by the usual sealed ampoule technique. Titration of the developing *p*-bromobenzenesulfonic acid was performed with a Metrohm Potentiograph Model E-436 high-precision automatic titrator. All of the rate constants were nicely first order. The kinetic data are summarized in Table I. A reactivity comparison of IIb-OBs acetolysis with the gas-phase thermolysis of structurally related III also is included. Another reactivity comparison was made by subjecting IIc-OAc to the acetolysis conditions at 130°. The azo acetate was stable for at least 25 acetolysis half-lives of IIb-OBs.

p-Bromobenzenesulfonate IIb-OBs produced a quantitative yield of nitrogen and a mixture of the acetate and dienes shown in eq 1; no IIc-OAc was detected.



IV V VI



99.75% 0.25%

Product identification was based on glpc and nmr comparisons with authentic samples of IV,[5] V,[6] and VI.[7] Product yields and ratios are summarized in Table II. Equations 1 and 2 compare the products of IIb-OBs acetolysis and the gas-phase thermolysis of III.[8]

The best available evidence indicates that decomposition of III occurs by a mechanism which involves a

(1) E. L. Allred and C. R. Flynn, *J. Amer. Chem. Soc.*, **92**, 1064 (1970).

(2) A. C. Day, P. Raymond, R. M. Southam, and M. C. Whiting, *J. Chem. Soc. C*, 467 (1966).

(3) Satisfactory elemental analyses were obtained for all of the new compounds. The nmr, ir, and uv spectral data were in complete agreement with the structural assignments.

(4) 2-Diazopropane-d_6 was prepared from acetone-d_6 of 99.5% minimum isotopic purity.[2] Nmr analysis showed I-d_6 and II-d_6 to be of greater than 98% deuterium incorporation.

(5) Prepared from 2,4-dimethyl-4-penten-2-ol obtained from Chemical Samples Co.

(6) Obtained from Chemical Samples Co.

(7) T. L. Jacobs and R. A. Meyers, *J. Amer. Chem. Soc.*, **86**, 5244 (1964).

(8) R. J. Crawford and A. Mishra, *ibid.*, **88**, 3963 (1966).