and R. C. Sherwood for making measurements on our samples.

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Received November 1, 1967

Crystal Structure of Bullvalene at 25°[1]

Sir:

The self-interconversion of bullvalene has been studied by nmr in solution,[2] where it was found that at 120° all protons are magnetically indistinguishable, while at −85° the spectrum corresponds to that for a single valence tautomer. A solid-state nmr investigation of bullvalene indicated that the line width decreases with increase of temperature above 0°.[3] This effect was interpreted[3] either as indicating reorientation about a molecular axis or as valence tautomerism occurring in the crystal. Crystal structure analyses on two bullvalene–$AgBF_4$ complexes[4,5] have provided evidence for only a small amount of valence tautomerism occurring in these solid complexes. The Ag^+ ions complex to several double bonds and the resulting "freezing" of these bonds in definite positions reduces the number of self-rearrangement pathways. We have investigated the crystal structure of bullvalene itself at 25 ± 3° to determine whether there are unique positions for the bullvalene molecules in the crystal at this temperature and to investigate the extent of valence tautomerism in the solid.

Bullvalene ($C_{10}H_{10}$) crystallizes as transparent plates belonging to the monoclinic system. The most obvious choice of unit cell is virtually orthogonal ($\beta' = 90° 20'$), but this cell does not correspond to a conventional description for any space group. A simple transformation of reciprocal and real lattice axes leads to the following cell dimensions (Mo Kα, λ 0.7107 Å) at 25°: $a = 6.21 \pm 0.03$, $b = 20.73 \pm 0.05$, and $c = 10.52 \pm 0.04$ Å; and $\beta = 148° 18' \pm 30'$. The transformed cell belongs to the space group $P2_1/c$, with four molecules of $C_{10}H_{10}$ in the unit cell. Intensity data were obtained by equiinclination Weissenberg photographs and visual estimates. A total of 988 independent structure amplitudes was obtained. The crystal structure was solved by the symbolic addition technique,[6,7] and at the present stage of refinement, the crystallographic R factor is 0.14 on 988 reflections.

A view of the crystal structure looking down the b axis is shown in Figure 1. The packing is highly efficient and the definition of individual atoms indicates that motion of an entire bullvalene molecule about a molecular axis is not an important facet of the structure at this temperature. The agreement among the dimensions of chemically equivalent features within the bullvalene molecule is in accord with the estimated standard deviations (C–C distance ±0.02 Å, C–C–C angles ±1°). The mean C=C, C(sp^2)—C(apex), C(sp^2)—C(three-membered ring), and C—C (within the three-membered ring) distances are 1.33, 1.51, 1.45, and 1.54 Å, respectively. The angles at the apical carbon atom are close to tetrahedral, and those at the carbon atoms participating in the double bonds are all significantly greater than 120° (mean 126°); the average internal angle in the three-membered ring is 60° and the average external angle at the three-membered ring is 122°. There are no anomalous features in a three-dimensional difference map to indicate unusual atom vibrations, and indeed, well-defined positions can be found for most hydrogen atoms.

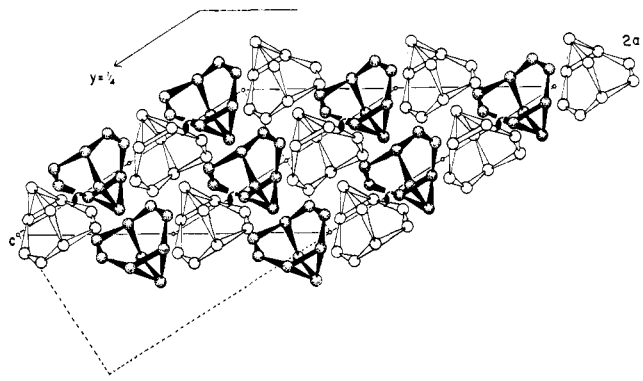

Figure 1. A view of the contents of two unit cells looking down the b axis. The bonds in the bullvalene molecules above $y = 1/2$ are shaded in black. In this projection, one of the atoms in the three-membered ring completely obscures another atom in one-half of the molecules and is completely obscured by that atom in the other half. The a' and c' axes of the pseudo-orthogonal cell are marked by discontinuous lines at the lower portion of the drawing.

We conclude that the packing forces prevent substantial valence tautomerism from occurring in the bullvalene crystal at 25°. The rearrangement observed in the 3:1 bullvalene–$AgBF_4$ complex[4] must be due to less restrictive packing forces in that crystal.[7a]

Acknowledgment. The bullvalene used in this study was obtained through the courtesy of Union Carbide Research Institute.

(1) Work supported by U. S. Public Health Service Grant GM 12470-03.

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(7a) NOTE ADDED IN PROOF. An electron diffraction study of bullvalene vapor reveals molecular dimensions in very good agreement with those found in the X-ray analysis: B. Andersen and A. Marstrander, *Acta Chem. Scand.*, **21**, 1676 (1967).

(8) Author to whom inquiry should be addressed.

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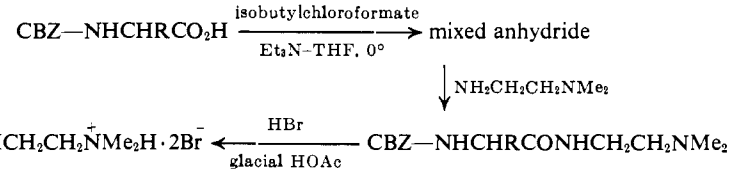
Topography of Nucleic Acid Helices in Solutions. V. The Interactions of L-, D-, and DL-Amino Acid Derivatives with Nucleic Acid Helices. Demonstration of an Asymmetric Surface[1]

Sir:

We wish to report the synthesis and the interactions of several amino acid amides of the general structure I,

(1) For part IV in this series see E. J. Gabbay and R. R. Shimshak, *Biopolymers*, in press.

Chart I



$^{+}NH_3CHRCONHCH_2CH_2N^{+}Me_2H \cdot 2Br^{-}$, with nucleic acid helices. These particular diammonium salts containing two positively charged groups at neutral pH[2] were selected since it is well known that diammonium salts II, $R_1R_2R_3N^{+}(CH_2)_nN^{+}R_1R_2R_3 \cdot 2X^{-}$, interact strongly with nucleic acids by binding to adjacent phosphate anions of each strand.[3] The synthetic methods used are outlined in Chart I.[4]

The effect of the various derivatives on the melting temperature of the polyriboinosinic-polyribocytidylic acid helix (rI–rC) is reported in Table I along with the specific rotations, $[\alpha]^{25}D$, of the salts I.

Several interesting points may be made. (1) The degree of stabilization of the double-stranded helix rI–rC depends on the nature of the amino acid. For example, increasing the size of the hydrophobic substituent, R, of the salts $^{+}NH_3CHRCONHCH_2CH_2N^{+}Me_2H \cdot 2Br^{-}$ lowers the degree of stabilization of the rI–rC helix. On the other hand, if R is a polar substituent, as in the case of methionine and lysine, substantial stabilization of the helix occurs which is completely in line with the earlier work on nucleic acid–amino acid and nucleic acid–protein interactions.[5] The extent of stabilization of the helical structure by these salts as measured by T_m depends on the relative stabilization of the double-stranded helix with respect to the random coils.[6] (2) The L-amino acid derivatives interact with the rI–rC helix to a greater extent than the corresponding D enantiomers. This effect appears to be general for all four enantiomeric pairs of amino acid derivatives studied (Table I). The results may be interpreted in terms of relative stabilization of the double-stranded nucleic acid helices by the L-amino acid amides I or in terms of relative stabilization of the random coils by the D-amino acid amides I, and/or both. It is noted that increasing the size of the alkyl substituent, R, of the salts, I, from methyl to ethyl, *i.e.*, alanine to α-aminobutyric acid derivative, has very little effect on ΔT_m, the difference between the T_m of the rI–rC helix in the presence of L and D enantiomers ($T_m(L) - T_m(D)$). Interestingly, a much greater difference in T_m of the rI–rC helix is observed in the presence of the proline derivatives **7** and **8**; this is not surprising and in fact is anticipated since the proline derivatives **7** and **8** contain a rigid cyclic structure. Moreover, a change in the nature of the substituent R of the salts I from a nonpolar to a polar substituent, *e.g*, alanine derivatives **2** and **3** to lysine derivatives **9** and **10**, does not alter the relative order of stabilization of the rI–rC helix by the L and D enantiomers.

The exact nature of the difference between the interaction of the L and D enantiomers is difficult to understand

Table I. The Effect of Various Concentrations of I, $^{+}NH_3CHRCONHCH_2CH_2N^{+}Me_2H \cdot 2Br^{-}$, on the T_m of the Helix–Coil Transition of rI–rC in 0.025 *M* Sodium Phosphate Buffer (0.025 *M* in Na^{+}), pH 6.15[a,b]

			T_m,[a,b,d] deg, at			
Compd	Deriv of	$[\alpha]^{25}D$[c]	0.5×10^{-3} *M*	1.0×10^{-3} *M*	3.0×10^{-3} *M*	5.0×10^{-3} *M*
1	Glycine			62.8	68.0	69.8
2	L-Alanine	+14.6	58.4	61.6	66.5	69.2
3	D-Alanine	−15.8	56.5	59.8	64.0	66.8
4	DL-Alanine			60.6	65.6	67.4
5	L-α-Amino-butyric	+30.4	57.2	59.8	65.6	67.5
6	D-α-Amino-butyric	−32.6	55.8	57.4	62.8	64.6
5 and **6**	DL-α-Amino butyric			60.0	63.8	
7	L-Proline	−14.6	59.6	62.6	67.6	69.2
8	D-Proline	+15.5	54.6	55.6	61.6	63.0
7 and **8**	DL-Proline			61.8	66.0	
9	L-Lysine	+23.2	77.2	79.2	82.5	82.2
10	D-Lysine	−24.0	75.8	77.7	80.4	80.4
9 and **10**	DL-Lysine			78.6	80.7	
11	DL-Leucine			55.0	59.0	60.3
12	DL-Phenylalanine			51.7	54.4	55.4
13	DL-Methionine			66.8	72.2	74.5

[a] Melting curves were measured in 1-ml cuvettes thermostated with a Haake constant-temperature water circulator equipped with a Neslab temperature programmer. A Beckman DU equipped with the Gilford automated accessories was used and the temperature of the cell compartment was measured directly by using an iron–constantan thermocouple connected to a Leeds Northrup Model 8290 potentiometer. [b] The T_m of the rI–rC helix in the absence of the salts I is found to be 48.8 ± 0.4° (average of ten determinations). [c] In H_2O; measured in degrees. [d] T_m curves at any one concentration of the salts I were run concurrently for each enantiomeric pair. Under these conditions ΔT_m ($T_m(L) - T_m(D)$) is found to be reproducible to within ±0.3°.

(2) Titration curves of the amino acid amides I, *i.e.*, glycyl, alanyl, α-aminobutyryl, and prolyl derivatives, indicate that they exist 100% in the diprotonated form at pH 6.15 and 30° in 0.025 *M* sodium chloride solution. At 50°, the pK_a values are lower but these salts still exist mainly in the diprotonated form (90–95%). The polar amino acid derivatives, *i.e.*, methionine and lysine, as well as the nonpolar amino acid derivative, *i.e.*, phenylalanine, show a greater dependence of pK_a values on temperature; *e.g.*, at 50°, the lysine, methionine, and phenylalanine derivatives contain approximately 2.60, 1.50, and 1.60 positive charges per molecule.

(3) (a) H. Tabor, *Biochemistry*, 1, 496 (1962); (b) H. R. Mahler and B. D. Mehrotra, *Biochim. Biophys. Acta*, 68, 211 (1963); (c) B. D. Mehrotra and H. R. Mahler, *ibid.*, 91, 78 (1964); (d) W. Szer, *J. Mol. Biol.*, 16, 585 (1966); (e) W. Szer, *Biochem. Biophys. Res Commun.*, 22, 559 (1966); (f) K. Matsuo and M. Tsuboi, *Bull. Chem. Soc. Japan*, 39, 347 (1966); (g) S. Higuchi and M. Tsuboi, *ibid.*, 39, 1886 (1966); (h) E. J. Gabbay, *Biochemistry*, 5, 3036 (1966); (i) E. J. Gabbay, *Biopolymers*, 5, 727 (1967); (j) E. J. Gabbay and R. R. Shimshak, *ibid.*, in press; (k) R. Glaser and E. J. Gabbay, *ibid.*, in press.

(4) All new compounds were analyzed by infrared, nmr, and elemental analyses and found to be totally consistent with the assigned structure.

(5) G. Zubay and P. Doty, *Biochim. Biophys. Acta*, 29, 47 (1958); C. D. Jardetzky, *J. Am. Chem. Soc.*, 80, 1125 (1958); S. Higuchi and M. Tsuboi, *Biopolymers*, 5, 837 (1966); M. Tsuboi, K. Matsuo, and P. O. P. Ts'o, *J. Mol. Biol.*, 15, 256 (1966); H. A. Sober, S. Schlossman, A. Yaron, S. A. Latt, and G. W. Rushizky, *Biochemistry*, 5, 3608 (1966); D. W. Olins, A. L. Olins, and P. H. Von Hippel, *J. Mol. Biol.*, 24, 157 (1967); S. A. Latt and H. A. Sober, *Biochemistry*, 6, 3293, 3307 (1967).

(6) A referee has pointed out that there are at least three types of interactions in the present system: (a) the interaction of the charged amino group with the nucleic acids (both coil and helix); (b) the interaction of the R group (the amino acid side chain) with the nucleic acids; and (c) the interaction of the R group with the α-amino group.

with or without three-dimensional models.[7] It appears, however, that a general phenomenon is being observed, namely, a higher affinity of the L-amino acid derivatives I to nucleic acid helices, *e.g.*, rI–rC, rA–rU$_2$,[8] and calf thymus DNA.[9] Whether or not the more specific interaction exhibited by the L-amino acid derivatives *vs.* the D enantiomers I for nucleic acid helices is peculiar only to these types of salts is not yet determined. It is felt that a general phenomenon is being observed; *i.e.*, other L-amino acid derivatives, *e.g.*, peptides, etc., have a higher affinity than the corresponding D-amino acid derivatives for nucleic acid helices. Further work along these lines is in progress.

Acknowledgment. This work was supported by the Rutgers Research Council and by Grants GM-13597 and GM-15308 from the U. S. Public Health Service.

(7) It has been suggested by a referee that the difference may be due to a stereochemical effect of the R group in blocking the interaction of the amino group with the phosphate group of the nucleic acids (coil and/or helix).

(8) The stabilization of the triple-stranded helix rA–rU$_2$ by the L-proline derivative 7 is greater than by the D-proline derivative **8** (ΔT_M = 1.0 and 0.9° at 3.0 × 10^{-3} and 5 × 10^{-3} *M*); however there is no difference between the T_m of the rA–rU$_2$ in the presence of compounds **2** and **3**.

(9) The L-amino acid derivatives, *i.e.*, L-proline derivative 7 and L-lysine derivative **9**, stabilize the double-stranded helix of calf thymus DNA to a greater degree than the corresponding D enantiomers, compounds **8** and **10**, respectively.

(10) National Science Foundation Predoctoral Trainee, 1965–1968.

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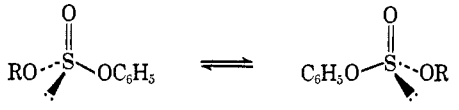
The Pepsin-Catalyzed Hydrolysis of Sulfite Esters. II. Resolution of Alkyl Phenyl Sulfites

Sir:

In recent years optically active enantiomers of sulfoxides,[1] sulfinate esters,[1] and sulfonium salts[2] have been reported, as well as rates of inversion for compounds containing a pyramidal sulfur atom.[3–5] Sulfite esters with two different groups should be resolvable into two enantiomers if the inversion barrier of the pyramidal sulfur atom is sufficiently high.



We wish to report resolution by the stereospecific pepsin-catalyzed hydrolysis of certain alkyl phenyl sulfites. This is the first report of the resolution of sulfite esters.[6] The finding that the hydrolysis of methyl phenyl sulfite is catalyzed by pepsin and that the salient features of this reaction are characteristic of enzymic reactions was reported in a previous communication in which the question of stereospecificity was not considered.[7]

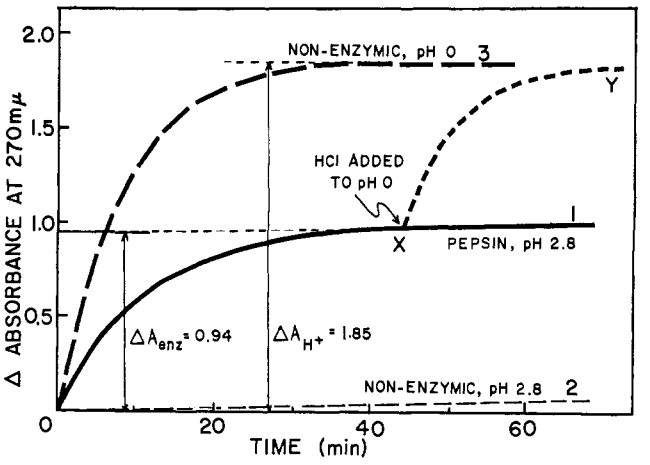


Figure 1. Time course of the enzymic and nonenzymic hydrolysis of phenyl tetrahydrofurfuryl sulfite (1.48 × 10^{-3} *M* in 0.1 *M* glycine and 0.5 *M* $LiClO_4$) at 25°: curve 1, in the presence of 8.2 × 10^{-6} *M* pepsin at pH 2.8; curve 2, in the absence of enzyme at pH 2.8; curve 3, in the absence of enzyme at pH 0; curve XY, addition of concentrated HCl at point X to lower pH of the reaction solution to pH 0.

Phenyl tetrahydrofurfuryl sulfite is an excellent substrate for pepsin.[8] Rates are measured by following the release of phenol spectrophotometrically at 270 mμ. Initial rates of hydrolysis conform to the Michaelis–Menten equation $v_0 = k_{cat}[S]_0[E_T]/(K_m + [S]_0)$.[9] Values of the steady-state kinetic parameters are k_{cat} = 3 min^{-1} and K_m = 1 × 10^{-3} *M* (25°; pH 2.8). However, the enzymic hydrolysis proceeds only to 50% completion (curve 1 of Figure 1), after correction for nonenzymic hydrolysis. The rate of the pepsin-catalyzed hydrolysis of the unreacted ester remaining after point X is not measurable, even at higher enzyme concentrations than that used in Figure 1. If the pH of the reaction solution is lowered to 0 by addition of concentrated HCl at point X, the remaining sulfite ester hydrolyzes (curve XY). Unreacted sulfite ester extracted at point X is optically

(1) K. Mislow, M. Green, P. Laur, J. Melillo, T. Simmons, and A. Ternay, *J. Am. Chem. Soc.*, 87, 1958 (1965).

(2) M. P. Balfe, J. Kenyon, and H. Phillips, *J. Chem. Soc.*, 2554 (1930).

(3) D. R. Rayner, E. G. Miller, P. Bickart, A. J. Gordon, and K. Mislow, *J. Am. Chem. Soc.*, 88, 3138 (1966).

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(8) This research was supported in part by Grant GM 13446 and by a predoctoral traineeship (T. W. R.), Training Grant T1 GM-463, U. S. Public Health Service. Sulfite esters were prepared by the method of P. Carré and D. Liberman, *Compt. Rend.*, 195, 799 (1926). *Anal.* Calcd for $C_{11}H_{14}O_4S$ (phenyl tetrahydrofurfuryl sulfite): C, 54.54; H, 5.83. Found: C, 54.95; H, 5.95. *Anal.* Calcd for $C_{12}H_{16}O_3S$ (phenylcyclohexyl sulfite): C, 59.99; H, 6.85. Found: C, 60.05; H, 6.71. These compounds were purified by low-temperature recrystallization from pentane. Their melting points were below −20°. Phenyl chlorosulfite (1.05 equiv) was allowed to react with racemic tetrahydrofurfuryl alcohol (1 equiv) in the presence of pyridine (1 equiv) at −20°. The product is a mixture of two pairs of enantiomers in equal proportions and showed no measurable optical rotation in a Cary 60 spectropolarimeter (240–450 mμ; 250 mg/ml, acetonitrile). The data presented in this communication clearly demonstrate that the presence of a second asymmetric center is not a necessary requirement for the stereospecific pepsin-catalyzed hydrolysis of this compound.

Kinetic constants were obtained by following the rate of production of phenol at 270 mμ with a Gilford 2000 spectrophotometer. The concentration of pepsin (twice recrystallized, Worthington Biochemical Corp.) was estimated from the absorbance at 278 mμ assuming a molar absorptivity of 50,900 l. mole^{-1} cm^{-1} determined by G. E. Perlmann, *J. Biol. Chem.*, 241, 153 (1966).

(9) $[E_T]$ is the total concentration of enzyme, $[S]_0$ is the concentration of the reactive enantiomer at zero time, and K_m is the Michaelis (apparent dissociation) constant of the enzyme–substrate complex.