A Conformational Assignment of 1,5,9-Tribromo-*cis,cis,cis*-1,5,9-cyclododecatriene by Nuclear Magnetic Resonance Analysis

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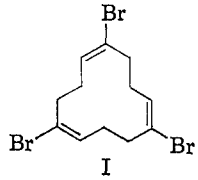
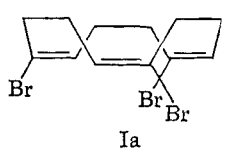
Abstract: The high-resolution proton spectrum of 1,5,9-tribromo-*cis,cis,cis*-1,5,9-cyclododecatriene (I) has been analyzed as a five-spin system in terms of chemical shifts and coupling constants. From the magnitudes of the vicinal coupling constants of the ethano hydrogens of I, it is concluded that the methylene groups are in an *s-trans* arrangement. Two possible conformers meet this requirement: the symmetrical and unsymmetrical *s-trans*. Arguments are presented which favor the assignment of the unsymmetrical *s-trans* conformation to I.

The synthesis and characterization of 1,5,9-tribromo-*cis,cis,cis*-1,5,9-cyclododecatriene (I) has been reported previously.[2] Many conformations are possible for the tribromotriene I. Here we consider four

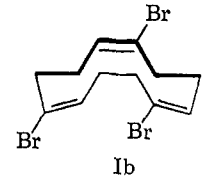


I

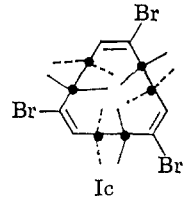
conformers which are representative types: the crown (Ia), the saddle (Ib), the symmetrical *s-trans* (Ic), and the unsymmetrical *s-trans* (Id). The crown conformation is required for maximum σ overlap of the p orbitals.[3] If I were found to be in a crown conformation, then it could be argued that stabilization by π-electron delocalization had out-weighed the nonbonded interactions of the eclipsed hydrogens. The saddle (Ib) has some of the nonbonded interactions, present in the crown, removed. The two *s-trans* conformers (Ic and Id) have the ethano nonbonded interactions minimized, but have present increased intraannular hydrogen repulsions compared to either the crown or the saddle conformations. Molecular models show that the bromine atoms cause no serious steric interactions in these four conformers and, by inspection, that the two *s-trans* conformations possess more favorable arrangements of the carbon–bromine dipoles. Other conceivable conformations for I are intermediate among the four discussed here. Since the relative orientation of the ethano hydrogens differ considerably in the crown, saddle, and *s-trans* conformations, and since a large body of experimental data has established empirical correlations between the magnitudes of the coupling constants of protons with the geometry of the carbon skeleton bearing them, it seemed likely that an analysis of the nmr spectrum of I would furnish the necessary data for the assignment of its conformation.



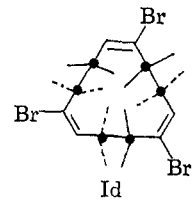
Ia



Ib



Ic



Id

The relative simplicity of the nmr spectrum of I (Figure 1, upper part) suggested that the molecule is rapidly interconverting between equivalent conformers[4] and thereby appears to possess a symmetry higher than C_3. Rapid interconversion between equivalent conformers would allow geminal protons A_1, A'_1 and B_1, B'_1 (Figure 2) to become symmetrically equivalent (but not magnetically) producing an effective C_{3h} symmetry for the whole molecule. However, even with the higher C_{3h} symmetry, one would expect a more complex nmr spectrum unless the 15 protons of I can be partitioned, as shown in Figure 2, into three symmetrically equivalent groups with essentially magnetically equivalent behavior.[5] Accordingly the nmr spectrum of I has been analyzed as an AA'BB'C type using the LAOCOON II program.[6] The nmr parameters obtained from the analysis are summarized in Table I and the calculated spectrum is displayed in the lower part of Figure 1. A resolution factor of 1.0-cps band width at half-peak height was used. This factor has been directly measured from several lines in the experimental spectrum which correspond to calculated single-peak lines.

The broadening of the lines of the experimental spectrum is caused by the long-range couplings between the protons of different groups[7] which have not been included

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(2) K. G. Untch and D. J. Martin, *J. Am. Chem. Soc.*, 87, 3518 (1965).

(3) For a discussion of this kind of π-electron delocalization, see ref 2 and others cited therein.

(4) It was not possible to obtain the nmr spectrum of the "frozen" conformer owing to the insolubility of I in appropriate solvents below 0° at which temperature the nmr spectrum was unchanged from that obtained at 37°.

(5) *I.e.*, that the long-range coupling constants between protons of different groups are sufficiently small to cause only a broadening of the lines of the spectrum in which the over-all pattern is uniquely determined by the coupling among the protons within the *same* group.

(6) S. Castellano and A. A. Bothner-By, *J. Chem. Phys.*, 41, 3863 (1964).

(7) In the temperature range *ca.* 0–70° the line widths are unchanged, indicating that the broadening is not caused by a slow interconversion rate.

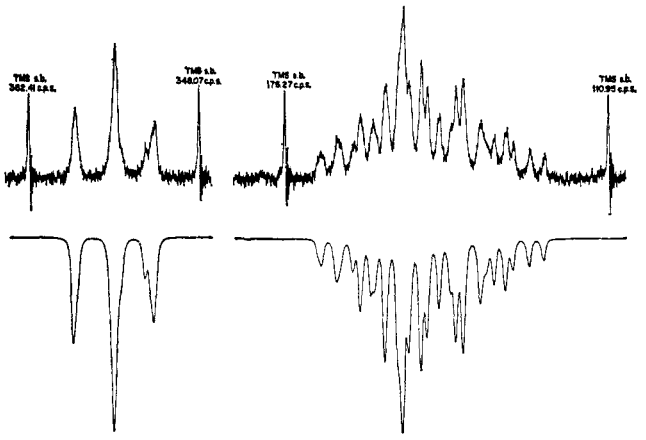

Figure 1. The experimental (upper part) and calculated (lower part) nmr spectrum of 1,5,9-tribromo-*cis,cis,cis*-1,5,9-cyclododecatriene.

in this calculation. Of these, the only ones which may be expected to contribute significantly to the broadening are: $J_{A_2C_1} = J_{A'_2C_1}$ (four-bond coupling constants); $J_{B_2B_1} = J_{A'_2B'_1}$, $J_{A_2B'_1} = J_{A_2'B_1}$ (five-bond coupling constants). A straightforward perturbative calculation demonstrates that these long-range couplings cause a symmetrical[8] line broadening of the order of $J_{A_2C_1} + (J_{A_2B_1} + J_{A_2B_1'})$. Since the line width of TMS in the same solution was 0.3 cps, we conclude that the absolute value of the sum of the above coupling constants is no greater than 0.7 cps.

Table I. Spectral Nmr Parameters of I[a,b]

Chemical shifts	$W_A = W_{A'}$	157.87 ± 0.02
	$W_B = W_{B'}$	138.49 ± 0.02
	W_C	364.70 ± 0.03
Coupling constants	$J_{AA'}$	−13.88 ± 1.09
	$J_{AB} = J_{A'B'}$	10.57 ± 0.03
	$J_{AB'} = J_{A'B}$	5.49 ± 0.03
	$J_{AC} = J_{A'C}$	−0.25 ± 0.03
	$J_{BB'}$	−13.88 ± 1.09
	$J_{BC} = J_{B'C}$	8.46 ± 0.03

[a] All data in cps; ν_0 = 60 Mcps. Chemical shifts referred to TMS as internal standard. [b] The small differences between the present data and those reported in ref 2 are due to a further refinement of the fitting of the spectrum. The large errors associated with $J_{AA'}$ and $J_{BB'}$ are due to the fact that the spectrum is sensitive to the differences of these parameters but not to their sum; see ref 6.

The excellent agreement between the experimental and calculated nmr spectra demonstrates the validity of performing the analysis of the nmr spectrum of I as a five-spin problem. These results clearly show that under particularly favorable circumstances, precise values of many of the parameters of multispin systems can be obtained (15 spins in this case).

The labeling of the protons as shown in Figure 2 is readily made on the basis of the chemical shifts and the magnitudes of the geminal coupling constants ($J_{AA'}$ and $J_{BB'}$) reported in Table I. The magnitude of the vicinal coupling constants ($J_{AB} = J_{A'B'}$, $J_{AB'} = J_{A'B}$) provides the necessary data to make the conformational assignment for I. The values of 10.57 cps for J_{AB} and 5.49 cps for $J_{AB'}$ are in the ranges characteristic for *trans*

(8) Because of the symmetrical broadening of the lines, the errors of the parameters given in Table I, even if smaller than the observed line width, are reliable.

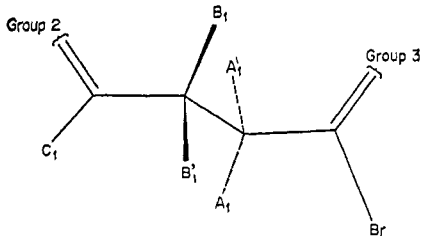

Figure 2.

(10.5–12.4 cps) and *gauche* (2.7–5.4 cps) vicinal coupling constants.[9c] It is only an *s-trans* conformation (Ic or Id) that has the spatial orientation of the ethano hydrogens consistent with the experimental values found for the vicinal coupling constants, namely, pairs of hydrogens *trans* and pairs *gauche*. It is clear that during interconversion between equivalent *s-trans* conformers the *trans* hydrogen pairs remain *trans* and the *gauche*, *gauche*.

In the instance of interconverting crown conformations (Ia), the relative orientation of the hydrogens is preserved in pairs (inner and outer). Here, just as in the case for an *s-trans* conformer, one expects the nmr spectrum to be an AA'BB'C type. However, the values for J_{AB} and $J_{AB'}$ in Ia would fall in the ranges characteristic for *cis* and *gauche* coupling constants which would be *ca.* 7–8 and 3–5 cps, respectively.[9a,b] We therefore conclude that I is not in a crown conformation to any appreciable extent.[10] The last conformation to be considered, the saddle (Ib), can be rigorously excluded on the basis of the following argument: during interconversion among six equivalent saddles (three readily visualized and their enantiomorphs) the geminal hydrogens α to the bromine assume all three possible orientations with respect to the ones β, *i.e.*, *cis* eclipsed, *gauche* (60°), and *gauche* (120°). In this situation the coupling constants J_{AB} and $J_{AB'}$ would necessarily be *equal*. As already shown above, however, J_{AB} and $J_{AB'}$ are *not equal*, and therefore the saddle conformer is also excluded from being present in any significant amount.

We are left then with an *s-trans* conformation for I. A choice between the two possible ones, Ic (two equivalent enantiomorphic conformers) or Id (three equivalent pairs of enantiomorphic conformers), cannot be made with the data provided by the above analysis of the nmr spectrum. However, molecular models show that Ic is more strained that Id. Some of the intraannular hydrogen repulsions are decreased in Id compared to Ic. It would be expected on this ground that the unsymmetrical *s-trans* conformation, Id, is of lower energy and would be the most populated conformation in solution.

Finally, we wish to point out that the experimental values for J_{AB} and $J_{AB'}$ are, respectively, at the lower and upper ends of the ranges for *trans* and *gauche* vicinal coupling constants for six- or larger membered rings. Either one or both of the following considerations would account for these observed magnitudes:

(9) See (a) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959); *J. Am. Chem. Soc.*, 85, 2870 (1963); (b) H. Conroy, *Advan. Org. Chem.*, 2, 265 (1960); (c) A. A. Bothner-By, *Advan. Magnetic Resonance*, 1, 195 (1965).

(10) Independent evidence forces the same conclusion, *i. e.*, the dipole moment measurement, 0.6 D. (benzene).[2]

it is conceivable (a) that small populations of crown and/or saddle conformers are present in equilibrium with Ic and Id; (b) that the *s-trans* conformers, Ic and Id, are somewhat skewed away from a pure *s-trans* arrangement of their ethano hydrogens in order to relieve part of the strain energy due to nonbonded interactions. Of the two explanations, we prefer the latter.

Experimental Section

The 1,5,9-tribromo-*cis,cis,cis*-1,5,9-cyclododecatriene was prepared as previously reported.[2] An analytically pure sample, mp 116–117°, was obtained by recrystallization (three times) from hexane. Approximately 100 mg was dissolved in 0.5 ml of $CDCl_3$ to give *ca.* a 15% solution (w/w) which was degassed on a vacuum line and sealed into a 5-mm nmr tube after the addition of 2% TMS as an internal standard.

The nmr spectra were recorded using a Varian A-60 spectrometer. The spectra were calibrated by the side-band technique using a Hewlett-Packard 201 CR oscillator. The line frequencies used in the calculation were averaged measurements of four spectra, two recorded for each sweep direction of the magnetic field. The computations were performed on a 7090 IBM computer.

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Structural Studies of Ribonuclease. XXIV. The Application of Nuclear Magnetic Resonance Spectroscopy to Distinguish between the Histidine Residues of Ribonuclease[1]

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Contribution from the Department of Chemistry, Cornell University, Ithaca, New York. *Received May 2, 1966*

Abstract: Proton magnetic resonance studies of L-histidine, glycyl-L-histidine, L-histidyl-glycine, and poly-L-histidine showed that deuteration of the imidazole group is accompanied by a downfield chemical shift of about 0.9 ppm of the C-2 proton and about 0.4 ppm of the C-4 proton. The former chemical shift is studied as a function of p*D* in D_2O for DL-histidyl-DL-histidine, and approximate values of the microscopic dissociation constants for the two imidazole groups are determined. With ribonuclease, the C-2 proton resonance falls downfield from the aromatic resonance for all values of p*D*. Using a computer of average transients it is found that the resonance splits into three peaks at values of p*D* between 5.4 and 8. The two groups with average microscopic dissociation constants p*k* of approximately 5.4 and 5.8 have been *tentatively* assigned to the two histidines involved in the active site of the enzyme and the two groups with identical p*k* values of approximately 6.6 to the other two histidine residues. In oxidized ribonuclease the four histidine residues are normalized with $pK' \sim 7.4$.

Ribonuclease was the first protein for which a nuclear magnetic resonance (nmr) spectrum was determined by Saunders, Wishnia, and Kirkwood in 1957.[3] This spectrum was interpreted by Jardetzky and Jardetzky[4] on the basis of measurements of the spectra of the amino acids and peptides in water, D_2O, and concentrated sulfuric acid.[5,6] The spectra of amino acids and some polymers and proteins have also been determined in trifluoroacetic acid.[7,8] More recent studies comprise several on the interpretation of the nmr spectra of various proteins,[9-11] including the sharpening of the spectra and the appearance of new peaks which is produced by opening up the native structure by denaturants and oxidation of disulfide bonds. Other more specialized applications include (i) a study of the slowly exchanging protons attached to nitrogen atoms in ribonuclease,[12] (ii) contact resonances at very high and low field in cytochrome *c*,[9,13] and (iii) measurement of spin lattice relaxation times of water molecules in protein solutions.[14]

From the foregoing, it is apparent that the nmr technique has been of limited use in the solution of problems of protein chemistry. This is largely due to the fact that the spectra, in general, consist of a number of broad bands, owing to a multiplicity of similar protons, and it is usually impossible to separate the contribution from each kind. The exceptions to this pattern occur with protons attached to nitrogen which, however, form very broad bands,[12] the aromatic protons due to tryptophan, tyrosine, phenylalanine, and the C-4 proton of histidine which can be partially separated in favorable cases, and finally the C-2 protons of histidine.[9,10] The last named show promise because they form a fairly sharp resonance on the low-field side of the aromatic resonance, and previous work[15] with L-histidine indicates

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(2) On leave from the Chemistry Department, Australian National University, Canberra, Australia, 1965. The award of a Fulbright Travel Grant to J. H. B. is gratefully acknowledged.

(3) M. Saunders, A. Wishnia, and J. G. Kirkwood, *J. Am. Chem. Soc.*, **79**, 3289 (1957).

(4) O. Jardetzky and C. D. Jardetzky, *ibid.*, **79**, 5322 (1957).

(5) M. Takeda and O. Jardetzky, *J. Chem. Phys.*, **26**, 1346 (1957).

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(7) F. A. Bovey and G. V. D. Tiers, *J. Am. Chem. Soc.*, **81**, 2870 (1959).

(8) F. A. Bovey, G. V. D. Tiers, and G. Filipovich, *J. Polymer Sci.*, **38**, 73 (1959).

(9) A. Kowalsky, *J. Biol. Chem.*, **237**, 1807 (1962).

(10) M. Mandel, *ibid.*, **240**, 1586 (1965).

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(12) A. Wishnia and M. Saunders, *J. Am. Chem. Soc.*, **84**, 4235 (1962).

(13) A. Kowalsky, *Biochemistry*, **4**, 2382 (1965).

(14) D. J. Blears and S. S. Danyluk, *Biopolymers*, **3**, 585 (1965).

(15) C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc.*, **85**, 3736 (1963).