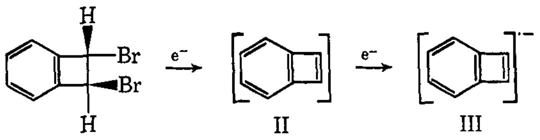
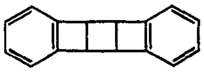
has been prepared previously by Cava[1] by refluxing *trans*-1,2-dibromobenzocyclobutene[2] with zinc in ethanol. The parent compound, being very reactive, was not isolated; instead, its dimer was isolated. In later experiments, Cava[3] was able to trap the parent compound by treating it with dienes.

We have attempted to trap the reactive benzocyclobutadiene as its radical anion. The method involves the addition of *trans*-1,2-dibromobenzocyclobutene (I) to an excess of solvated electrons[4] in a mixture of glyme and diglyme at −60°. The two bromine atoms are reduced off, yielding II; compound II should be reduced in a diffusion-controlled process by the excess solvated electrons before it can dimerize. Following this procedure, we found that treatment of I with excess solvated electrons yielded a paramagnetic species which exhibited a 27-line spectrum.[7] The observed splitting constants were $a_\mathrm{H} = 11.05$ G (two protons), $a_\mathrm{H} = 7.60$ G (two protons), and $a_\mathrm{H} = 1.48$ G (two protons).

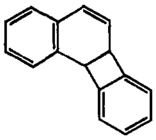


I II III

The possibility existed that the paramagnetic species was merely the radical anion of one of the two known dimerization products of benzocyclobutadiene, namely the head-to-head dimer[8] (IV) or the Diels–Alder dimer[1] (V). These compounds were prepared and reduced under the identical conditions used for the dibromide. They both yielded paramagnetic species, but neither species was similar to that obtained from the dibromide.[9]



IV



V

A second possibility was that we were observing the radical anion of the bromide itself. This is seemingly ruled out by the fact that the diiodo compound upon reduction yielded the same paramagnetic species as the dibromide.[10]

Additional support for the benzocyclobutadiene radical anion comes from the over-all width of the spectrum, 40.3 G, and the hyperfine coupling constants. The observed coupling constants of $a_\mathrm{H} = 1.48$ G and $a_\mathrm{H} = 7.60$ G are comparable with the aromatic proton splitting constants of benzocyclobutene,[12] $a_\mathrm{H} = 1.40$ G and $a_\mathrm{H} = 7.60$ G, and other *o*-dialkylbenzene radical anions. However, the $a_\mathrm{H} = 11.05$ G splitting constant is much larger than the methylene splitting constant of benzocyclobutene,[12] $a_\mathrm{H} = 5.50$ G, or the methyl splitting constants of *o*-xylene,[13] $a_\mathrm{H} = 2.00$ G. Therefore, this large value would seem unlikely for a methylene proton but would be possible for a proton attached to an sp^2 carbon which has a bond angle[14] much smaller than 120°. Nelson and Trost[17] have observed that the *Q* value necessary to explain the unusually large hyperfine splitting constants of the 1,2,5,6 protons of paracyclene must be 30 G. In the benzocyclobutadiene radical anion, the bond angles of the four-membered ring are much smaller than in the five-membered ring of paracyclene, and we might expect a *Q* value for these positions to be even larger. Thus the large splitting constants would be expected even though the spin density in the four-membered ring may be small. In addition, the over-all width of the spectrum would be expected to be much larger than the usual 22–28 G.

All the data we have collected to date indicate that the paramagnetic species is the benzocyclobutadiene radical anion. We are presently carrying out additional experiments which will shed further light on the exact nature of this species.

Acknowledgment. Financial support of this investigation by the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

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(4) The solution of solvated electrons was prepared by allowing a mixture of diglyme and glyme to stand in contact with a potassium mirror at Dry Ice–acetone temperatures for 1–2 hr. A study of the concentration of solvated electrons *vs.* solvent and temperature has been reported.[5,6]

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(7) Reduction of the *trans*-1,2-dibromobenzocyclobutene in a mixture of glyme and diglyme with a potassium mirror did not yield the benzocyclobutadiene radical anion.

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(10) The nmr spectra of *trans*-1,2-diiodobenzocyclobutene[11] and *trans*-1,2-dibromobenzocyclobutene[11] show substantial differences in the chemical shifts of equivalent positions in the two molecules. This difference in electronic environment of equivalent positions would be expected also to show up in the hyperfine coupling constants if one were merely looking at the radical anions of the two dihalo compounds.

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Reuben D. Rieke, Phillip M. Hudnall
Department of Chemistry, University of North Carolina
Chapel Hill, North Carolina 27514

Received April 2, 1969

Origin of the Rotational Strength of Heme Transitions in Myoglobin

Sir:

Recently, Cotton effects associated with the heme electronic transitions have been studied in a number of heme proteins.[1] Several proposals have been made for the origin of these Cotton effects. In principle, the

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sign and magnitude of these effects should yield information about the nature of the heme binding site. Since the three-dimensional structure of myoglobin is known in considerable detail,[2] we have been able to carry out theoretical calculations of the heme–protein interaction in myoglobin.

We have concentrated so far on the Soret band of the heme. The two lowest excited configurations of a D_{4h} porphyrin, $a_{1u}e_g$ and $a_{2u}e_g$, are nearly degenerate and interact strongly,[3,4] yielding a very intense band at about 400 nm (the Soret or B band) and a much weaker system between 500 and 600 nm (the Q bands, or α and β bands). The excited states corresponding to both the B and the Q bands remain doubly degenerate in D_{4h} symmetry, and the α and β bands are assigned to different vibronic components of the Q band.

Myoglobin exhibits a positive Cotton effect in the Soret region.[1b,d,i,j] The exact magnitude and position depend somewhat upon the oxidation state of the iron and the nature of the ligand. From the data of Beychok[1j] and of Willick and Kay,[1i] we have estimated the Soret rotational strength in metmyoglobin to be 0.5 ± 0.05 DBM (1 DBM = 0.9273×10^{-38} cgs unit).

There are three possible mechanisms leading to rotational strength in the Soret band. (1) Nonplanarity of the porphyrin in myoglobin could make the heme an inherently dissymmetric chromophore.[5] It is known that the iron is out of the porphyrin plane in myoglobin[2b] but nonplanarity within the porphyrin has not been reported. Therefore, we ignore this possibility for the present. (2) Mixing of the Soret transition and d–d transitions of the iron could lead to magnetic dipole character in the Soret transition. The absence of large changes in rotational strength upon reduction or on changing the ligand argues against this. This possibility can be tested when CD data become available in the infrared. (3) The Soret Cotton effect may be due to coupling of the Soret transition and excited states of the globin backbone and side chains.[6] We have considered the allowed π–π^* transitions of aromatic side chains, π–π^* and n–π^* transitions in the peptide backbone, and σ–σ^* transitions in alkyl side chains. Our results indicate that this mechanism can account for the observed rotational strengths and that the major factor is the interaction with the allowed π–π^* transitions in aromatic side chains.

The rotational strength for an electrically allowed, magnetically forbidden transition, a, in the ith group of a molecule, is[6]

$$R_{ia} = -\left[\frac{2\pi}{c}\sum_{j\neq i}\sum_{b\neq a}\frac{V_{i0a;j0b}\nu_a\nu_b\mathbf{R}_{ij}\cdot(\boldsymbol{\mu}_{j0b}\times\boldsymbol{\mu}_{i0a})}{h(\nu_b^{\,2}-\nu_a^{\,2})} + 2\sum_{j\neq i}\sum_{b\neq a}\frac{Im V_{i0a;j0b}\nu_a\boldsymbol{\mu}_{i0a}\cdot\mathbf{m}_{jb0}}{h(\nu_b^{\,2}-\nu_a^{\,2})}\right]$$

where $V_{ica;j0b}$ is the interaction potential between transition a in the ith group and transition b in the jth group. ν_a is the transition frequency, $\boldsymbol{\mu}_{k0n}$ and $\mathbf{m}_{k0n}$ are the electric and magnetic transition dipole moments associated with transition from ground state 0 to excited state n in the kth group, respectively, and $\mathbf{R}_{ij}$ is the distance between the ith and jth group in the polymer. The interaction potential is approximated by electrostatic interaction between point monopoles.[6] We used Kendrew and Watson's atomic coordinates for myoglobin.[2c]

We considered 12 out of 23 aromatic side chains which are near the heme in myoglobin. The wave functions and electric dipole transition moments of benzene and phenol were taken from the literature.[7–9] We reproduced the MO calculation of Gouterman, *et al.*,[3] in the four-orbital approximation to calculate transition monopoles for the heme. An analogous set of parameters was used in a PPP calculation for imidazole. The aromatic groups considered, the distances between the heme and the aromatic side chains, and the calculated rotational strength are listed in Table I.

Table I. Calculated Soret Rotational Strength Arising from Interaction with Allowed π–π^* Transitions[a] in Aromatic Side Chains

	Amino acid[c]	Distance,[b] Å	R_x, DBM	R_y, DBM
His	(36, 1C)	14.3	−0.081	0.060
	(64, 7E)	4.7	0.248	−0.427
	(93, 8F)	3.6	0.417	−0.392
	(97, 2FG)	6.5	0.068	−0.168
Phe	(33, 14B)	6.7	0.105	0.160
	(43, 1CD)	5.9	0.261	−0.217
	(46, 4CD)	9.9	−0.093	0.142
	(106, 7G)	14.3	−0.089	0.033
	(138, 15H)	9.6	0.033	0.045
Tyr	(103, 4G)	11.3	0.251	−0.004
	(146, 23H)	11.8	0.136	−0.235
	(151, 2HC)	15.3	0.037	0.014
Total			1.296	−0.989

[a] The four lowest energy transitions of imidazole and phenol and the $A_{1g} \rightarrow E_{1u}$ band of benzene were included in the calculation. [b] Distance between the optical center of the aromatic side chain and the center of the porphyrin. [c] See stereodrawings in H. C. Watson, *Progr. Stereochem.*, in press.

It has been suggested that the Soret Cotton effect in myoglobin and hemoglobin arises from the helical structure of the globin.[1a,j] Because of the high symmetry of porphyrin, it is not likely that the Cotton effect arises from a permanent field provided by the helices.[10] The coupling between the Soret transition and the π–π^* and n–π^* transitions in the polypeptide backbone was treated following Woody and Tinoco.[11] The resulting contribution is quite small, 0.003 DBM.

Interactions with the alkyl side chains lining the heme pocket were also considered, using parameters for σ–σ^* transitions derived from Raymonda and Simpson.[12] The resulting value is 0.08 DBM.

Thus, the Soret Cotton effect in myoglobin arises from a rather complicated coupling mechanism. The aromatic side chains of globin play a major role. Among them histidine (64, 7E), histidine (97, 2FG), phenylalanine (33, 14B), tyrosine (103, 4G), and tyrosine

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(3) C. Weiss, H. Kobayashi, and M. Gouterman, *Mol. Spectry.*, **16**, 415 (1965).

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(146, 23H) give the major contributions. Histidine (64, 7E) is the so-called distal histidine which is near the sixth ligand of the iron. The fifth ligand, histidine (93, 8F), gives only a small effect, because the imidazole sits in a rather symmetrical position with respect to the heme.

The two components of the Soret band, B_x and B_y, have opposite signs for the rotational strength (Table I). This raises the question of why only a single positive band is observed. That the two components are not completely degenerate has been shown by Eaton and Hochstrasser.[13] Their single-crystal polarization measurements indicate a splitting of about 200 cm^{-1}. Such a splitting would lead to two distinct CD bands of opposite sign if the intensities were comparable in magnitude, but even a 2:1 disparity in magnitude can obscure the presence of two bands.[14] The magnitudes of the two individual components depend on the orientation of the Soret transition moments in the heme plane, but the total Soret rotational strength is independent of the direction of polarization. The calculations on which Table I is based assume polarization parallel to the opposing N-N lines. Rotating the transition moments by 45° leads to $R_{B_x} = -0.127$ and $R_{B_y} = 0.434$. Thus the shape of the Soret CD band of heme proteins may be rather sensitive to substituent and conformational changes, leading to variation in the direction of polarization of the Soret components.

Details of these calculations will be published. We are also investigating other heme transitions, and a study of hemoglobin is in progress.

Acknowledgment. The authors wish to thank Dr. J. C. Kendrew and Dr. H. C. Watson for providing their unpublished atomic coordinates of myoglobin, and Dr. G. Willick for communication of his results on the CD of myoglobin. This work was supported by U. S. Public Health Service Grant GM-13910.

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(15) University of Illinois Fellow, 1966–1968.

Ming-Chu Hsu,[15] Robert W. Woody
W. A. Noyes Laboratory of Chemistry
University of Illinois, Urbana, Illinois 61801

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Nitrogen-15 Magnetic Resonance Spectroscopy. A Correlation of the ^{15}N-H Coupling Constants in Aniline Derivatives with Hammett σ Constants

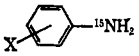
Sir:

We wish to report the observation of a linear correlation between the magnitude of the directly bonded ^{15}N-H coupling constants and the appropriate Hammett σ constants in a series of aniline-^{15}N derivatives. The chemical shifts of the amino protons[1] and the N-H stretching frequencies[2] in a series of anilines have recently been shown to be directly proportional to the Hammett substituent constants, as have the ^{13}C-H coupling constants[3] and chemical shifts of the methyl protons in a series of toluene derivatives.[4]

The magnitude of the ^{13}C-H coupling constant is generally believed to be related to the hybridization and polarity of the C-H bond.[5] An empirical equation, $\% \text{s} = 0.43(J_{^{15}\text{N-H}}) - 6$, relating the hybridization of the nitrogen atom to the directly bonded ^{15}N-H coupling constant, has been proposed[6] based on compounds in which the nitrogen hybridization is reliably known (*e.g.*, NH_4^+, sp^3, $J = 73.2$ Hz; $(C_6H_5)_2C{=}NH_2^+$, sp^2, $J = 92.6$ Hz).[7] This relationship is further supported by the recently reported coupling constants involving the sp-hybridized nitrogen atom in protonated nitriles.[8]

The ^{15}N-H coupling constants measured in $CDCl_3$ and DMSO-d_6 are summarized in Table I. Although the observed trends correspond well with those reported by Bramwell and Randall,[9] their measurements on $CDCl_3$ solutions differ considerably from ours. The source of this discrepancy is not clear.

Table I. ^{15}N-H Coupling Constants in Aniline Derivatives[a]

	$J_{^{15}\text{N-H}}$[b]	
X	DMSO-d_6[c]	$CDCl_3$[c]
4-NO_2	89.4	86.4
3,5-Me_2,4-NO_2	87.0	83.2
3-Br	85.3	80.5
3-Cl	85.1	80.9
3-I	84.4	80.4
4-I	84.0	79.7
4-Br	84.0	79.6
4-Cl	83.7	78.9
3-MeO	83.0	79.4
H	82.6	78.6
3-Me	82.0	78.2
3,5-Me_2	82.1	77.5
4-F	81.6	77.8
4-Me	81.4	76.5
4-MeO	79.4	75.6

[a] All anilines were prepared with a ^{15}N enrichment of at least 95% by Hofmann rearrangement of the corresponding amides. [b] All constants are expressed in hertz and are accurate to ±0.2 Hz. All measurements were made from pmr spectra. [c] All measurements were made with approximately 1 *m* solutions where possible. Otherwise saturated solutions were used. In ^{15}N-aniline, the coupling constant was independent of concentration between 0.4 and 2.2 *m* in $CDCl_3$.

Essentially parallel trends are seen in the two different solvents, although, as has been previously observed,[10] the magnitude of the coupling is larger in DMSO-d_6. The observed ^{15}N-H coupling constant in aniline is intermediate between those observed for typical systems containing sp^3- and sp^2-hybridized nitrogen. This may be attributed to delocalization of the nitrogen lone pair by orbital overlap with the π system of the aromatic ring, resulting in enhanced s character in the N-H bond compared to a normal sp^3-hybridized ammonium ion.

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