Further Studies of the Nuclear Magnetic Resonance Spectra of Unsymmetrical *ortho*-Disubstituted Benzenes

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Abstract: The nmr parameters for a series of unsymmetrical *ortho*-disubstituted benzenes have been determined utilizing data taken at both 60 and 100 Mc. These results are compared with those reported previously for the *o*-dihalobenzenes. Values of the empirical shielding parameter Q were determined for the methoxy, nitro, cyano, and methyl groups. The implications of these results on the theory of proton shielding in aromatic systems are discussed.

For the experimentalist, the present situation regarding the role of various substituents on the chemical shifts of protons in aromatic systems has been aptly summarized by Heller. "*He was pinched perspiringly in the epistemological dilemma of the skeptic, unable to accept solutions to problems he was unable to dismiss as insoluble. He was never without misery and never without hope.*"[1]

Among the various postulates advanced to explain substituent effects may be listed the changes in charge density introduced around the ring, mesomeric and inductive effects, substituent group magnetic anisotropy, localized van der Waals interactions, and field effects introduced by substituent dipole moments.[2]

The many effects are often of equal magnitudes and may operate, several at once, in conflicting ways. Thus, it has not proved possible to separate one from the other or to make any really meaningful quantitative calculations. The situation is even more complex in polysubstituted benzenes where substituent interactions introduce further variables.

While the theory of substituent effects on aromatic proton chemical shifts is still not in a satisfactory state, considerable success has been achieved in establishing empirical substituent additivity rules.[3] These are well exemplified by the rules of Martin and Dailey[2b] which allow one to calculate the chemical shifts of a large number of *para*-disubstituted benzenes with excellent accuracy. Their same substituent constants work well with *meta*-disubstituted benzenes and for the remote protons of *ortho*-disubstituted benzenes.

Martin and Dailey[2b] found that protons adjacent to the substituents in a series of symmetrical *ortho*-disubstituted benzenes gave chemical shifts consistently downfield by substantial amounts from the predicted values. Recently, a series of three unsymmetrical *ortho*-dihalobenzenes was examined in these laboratories,[4] and it was noted that the chemical shifts of protons adjacent to a given halogen, when compared to their symmetrically substituted analogs, were independent of the nature of the nonadjacent halogen. Furthermore, it was found that the chemical shifts of these protons correlated well with the empirical parameter Q described by Hruska, Hutton, and Schaefer.[5] In view of these observations, it seemed important to ascertain if other groups would exhibit the same behavior. Furthermore, it seemed possible that values of Q derived for these other groups might prove to be of predictive (and potentially theoretical) importance.

Experimental Section

Since the principles of the analysis of unsymmetrical four-spin systems of this type have been covered before,[4] the detailed spectral tracings and description of how the parameters were derived have been submitted elsewhere for publication.[6] Suffice it to say, the results given in Table I were taken from dilute solutions in carbon tetrachloride using both 60- and 100-Mc instruments. The analyses were facilitated by the use of the LAOCOON program.[7] Root mean square deviations between calculated and experimental line frequencies were typically 0.03–0.1 cps.

The chemical shifts for the methyl group in a series of substituted toluenes were also determined in dilute solutions (*ca.* 5%) of carbon tetrachloride. These results are given in Table III.

Results and Discussion

The coupling constants and chemical shifts for the compounds in this study are listed in Table I. Chemical shifts were assigned for protons nonadjacent to substituents using the additivity rules of Martin and Dailey.[2b] The remaining protons could usually be assigned from the splitting pattern though in cases of tightly coupled spectra, such as the chlorocyano- and bromocyanobenzenes, this procedure was not completely clear-cut, and shifts were assigned to give results most consistent with the other data in Table I and our earlier work.[4] For convenience of discussion, the data from our earlier work[4] and that of others is summarized in Table II. Table III presents the chemical shift data for the methyl protons in a series of substituted toluenes. These data agree well with those reported by Jackman.[8]

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[1] J. Heller, "Catch 22," Simon and Schuster, New York, N. Y., 1961.

[2] See, for instance: (a) H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 731 (1961); (b) J. S. Martin and B. P. Dailey, *ibid.*, **39**, 1722 (1963); (c) T. Schaefer, W. F. Reynolds, and T. Yonomoto, *Can. J. Chem.*, **41**, 2969 (1963); (d) A. D. Buckingham, *ibid.*, **38**, 300 (1960); (e) M. J. S. Dewar and Y. Takeuchi, *J. Am. Chem. Soc.*, **89**, 390 (1967).

[3] For a summary, see J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High-Resolution Nuclear Magnetic Resonance," Vol. 2, Pergamon Press Inc., New York, N. Y., 1966, p 754 ff.

[4] W. B. Smith and G. M. Cole, *J. Phys. Chem.*, **69**, 4413 (1965).

[5] F. Hruska, H. M. Hutton, and T. Schaefer, *Can. J. Chem.*, **43**, 2392 (1965).

[6] W. B. Smith and J. L. Roark, *J. Chem. Eng. Data*, in press.

[7] S. Castellano and A. A. Bothner-By, *J. Chem. Phys.*, **41**, 3863 (1964). We wish to acknowledge our appreciation to Dr. Bothner-By for providing us with a copy of the program and for carrying out some of our preliminary calculations with the aid of a grant from the National Science Foundation.

[8] L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Inc., New York, N. Y., 1959, p 58.

Table I. Nmr Parameters for Disubstituted Benzenes[a]

Benzenes	J_{34}	J_{45}	J_{56}	J_{35}	J_{46}	J_{36}	ν_3	ν_4	ν_5	ν_6
1-Chloro-2-methoxy	7.99	7.30	7.74	1.19	1.62	0.31	3.19 (3.28)	2.90 (3.00)	3.20 (3.21)	2.74 (2.84)
1-Bromo-2-methoxy	8.31	7.63	7.80	1.54	1.63	0.19	3.23 (3.34)	2.85 (2.91)	3.27 (3.27)	2.56 (2.68)
1-Iodo-2-methoxy	8.05	7.17	7.55	1.38	1.62	0.29	3.29 (3.48)	2.80 (2.91)	3.39 (3.40)	2.31 (2.48)
1,2-Dimethoxy	...	...	...	...	...	...	3.25 (3.32)	3.25 (3.25)	3.25 (3.25)	3.25 (3.32)
1-Chloro-2-nitro	8.23	7.13	7.67	1.17	1.41	0.78	2.18 (1.84)	2.59 (2.63)	2.51 (2.51)	2.46 (2.57)
1-Bromo-2-nitro	7.86	7.53	8.27	1.66	1.22	0.19	2.22 (1.90)	2.56 (2.64)	2.60 (2.57)	2.29 (2.41)
1-Iodo-2-nitro	7.90	7.20	7.71	1.35	1.45	0.28	2.20 (2.04)	2.64 (2.64)	2.71 (2.71)	2.01 (2.21)
1,2-Dinitro	...	...	...	...	...	...	2.03 (1.61)	2.13 (2.28)	2.13 (2.28)	2.03 (1.61)
1-Chloro-2-cyano	7.52	7.58	8.08	1.48	0.95	0.46	2.36 (2.53)	2.62 (2.72)	2.47 (2.47)	2.50 (2.58)
1-Bromo-2-cyano	7.78	7.44	7.84	1.33	1.59	0.43	2.37 (2.59)	2.57 (2.65)	2.55 (2.53)	2.33 (2.42)
1,2-Dicyano	...	...	...	...	...	...	2.19 (2.31)	2.25 (2.25)	2.25 (2.25)	2.19 (2.31)
1-Nitro-2-methoxy	8.54	7.91	7.85	1.21	1.76	0.27	2.94 (3.05)	2.54 (2.55)	3.04 (2.98)	2.30 (1.88)

[a] Coupling constants in cycles per second; chemical shifts in τ. Values in parentheses are predicted from the values of Martin and Dailey.[2b]

Table II. Nmr Parameters from the Literature[a]

Benzenes	ν_3	ν_4	ν_5	ν_6
1,2-Dichloro[b]	2.63 (2.80)	2.88 (2.95)	2.88 (2.95)	2.63 (2.80)
1,2-Dibromo[b]	2.45 (2.70)	2.91 (2.93)	2.91 (2.93)	2.45 (2.70)
1,2-Diiodo[b]	2.19 (2.63)	3.04 (3.07)	3.04 (3.07)	2.19 (2.63)
1-Chloro-2-bromo[b]	2.47 (2.64)	2.99 (3.02)	2.86 (2.87)	2.62 (2.86)
1-Chloro-2-iodo[b]	2.21 (2.43)	3.16 (3.16)	2.79 (2.87)	2.63 (2.99)
1-Bromo-2-iodo[b]	2.22 (2.50)	3.12 (3.07)	2.90 (2.93)	2.45 (2.84)
Chloro[c]	2.76	2.85	2.76	2.71
Bromo[c]	2.82	2.76	2.82	2.51
Iodo[c]	2.98	2.75	2.98	2.33
Methoxy[c]	2.72	3.04	2.72	3.08
Nitro[c]	2.53	2.40	2.53	1.78
Cyano[d]	2.63	2.51	2.63	2.46

[a] Chemical shifts in τ. Values in parentheses are predicted from the value of Martin and Dailey.[2b] [b] Reference 4. [c] Reference 2a. [d] Values not from dilute solutions: R. R. Fraser and R. N. Renaud, *J. Am. Chem. Soc.*, **88**, 4365 (1966).

Table III. Nmr Parameters of Methyl Groups[a]

	τ_{CH_3}		
Toluenes	*ortho*	*meta*	*para*
Chloro	7.64	7.68	7.70
Bromo	7.60	7.67	7.74
Iodo	7.61	7.71	7.71
Cyano	7.49	7.58	7.58
Nitro	7.55	7.53	7.52
Methoxy	7.82	7.70	7.73

[a] The toluene methyl group appeared at τ 7.66 under the conditions employed here.

Martin and Dailey[2b] based their calculations of shielding parameters for the cyano group on the chemical shifts of two *para*-substituted benzonitriles. Coupling their data with ours for 1,2-dicyanobenzene gave the following best set of parameters for the cyano group: d_o, -0.27 ppm; d_m, -0.15 ppm; and d_p, -0.33 ppm. The shifts for the protons in the chlorocyano- and bromocyanobenzenes in Table I were calculated with these values.

As previously noted,[2b,4] the agreement between observed and predicted chemical shifts for protons adjacent to the substituents (H_3 and H_6) is much poorer with the observed values tending to be downfield by 0.1–0.3 ppm. The major exception to this generalization is offered by protons adjacent to the nitro group which uniformly appear 0.2–0.4 ppm upfield from the predicted value. While one is tempted to explain this observation as an *ortho* effect forcing the nitro group out of the plane of the ring and placing the adjacent proton more in a shielding region of the magnetically anisotropic nitro group,[9] one would expect the effect to increase in the series chloro-, bromo-, and iodonitrobenzene which, in fact, it does not. In the *o*-halonitrobenzenes, H_3 appears at τ 2.20 $\pm$ 0.02 with the value for the iodo compound closest to that predicted by the Martin and Dailey rules.

For the *ortho* protons in the various dihalobenzenes (Table II), we previously noted independence of the chemical shifts at H_3 and H_6 on the nonadjacent halogen substituent.[4] This trend is only approximately obeyed when the nonadjacent substituent is a methoxyl, a cyano, or a nitro group. In 1-chloro-, 1-bromo-, and 1-iodo-2-methoxybenzene the chemical shifts (H_6) are 0.11, 0.11, and 0.10 ppm more shielded than the analogous protons in the dihalobenzenes. In the 1-chloro- and 1-bromo-2-cyanobenzenes these differences are -0.13 and -0.13 ppm, respectively; while in the chloronitro-, bromonitro-, and iodonitrobenzenes, H_6 is deshielded by -0.16, -0.16, and -0.20 ppm from its average value in the dihalo compounds. These data support the contention that there is essentially a constant "effect leakage" from methoxy, cyano, or nitro

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[9] I. Yamaguchi, *Mol. Phys.*, 6, 106 (1963).

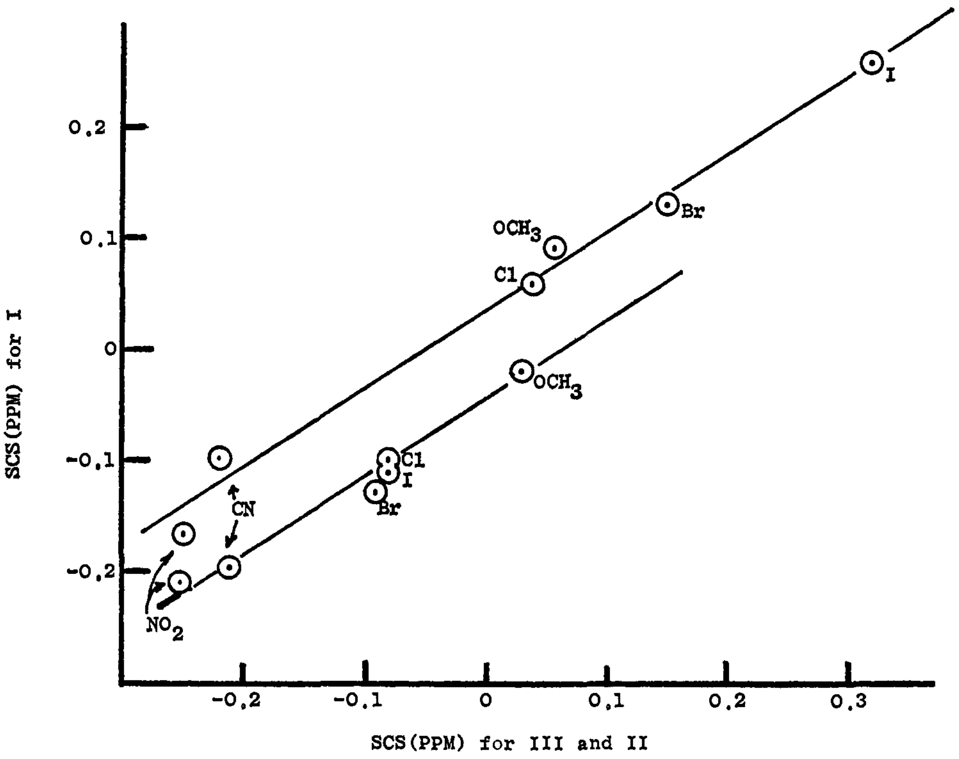


Figure 1. The SCS values of I-H_4 plotted against II (upper) and I-H_6 plotted against III (lower).

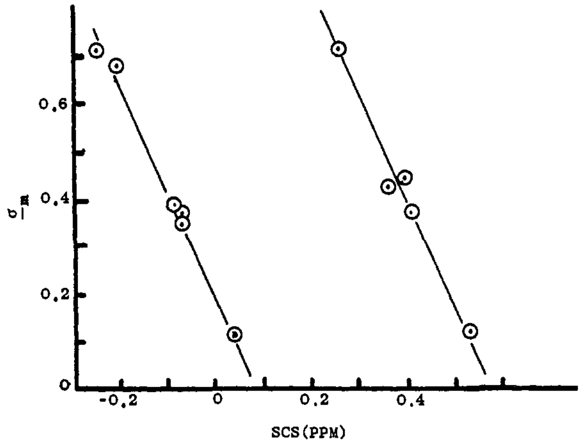
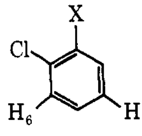


Figure 2. Plots of the SCS values for 1-chloro-2-X-benzene (left) and 1-nitro-2-X-benzene (right) *vs.* σ_m.

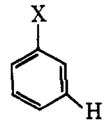
substituents at C_2 on H_6. This point will be supported by further evidence subsequently.

Pertinent to the question of substituent effects in general is the question of whether or not any regular pattern emerges from the chemical shifts around the ring due to the interaction of the two adjacent substituents. The additivity rules of Martin and Dailey[2b] fit well for protons at C_4 and at C_5 in the 1,2-disubstituted benzenes (Tables I and II). Their substituent additivity constants were derived from a series of *para*-disubstituted and monosubstituted benzenes. The chemical shifts at protons 4 and 5 are the sums of one *meta*-substituent constant and one *para* constant. Quite obviously, the interactions of two substituents located *ortho* to each other are not such as to interfere with their additive effects at H_4 and H_5. These results are not consistent with operation of a field effect of the type described by Buckingham.[2d] Attempts to calculate such field effects at positions 4 and 5 treating these fields either as additive entities from each substituent or as resultants from dipole–dipole interactions failed to reproduce the experimental data trends.

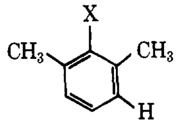
From the data in Tables I and II it is evident that the effects of a substituent introduced at C_2 are different at H_4 and H_6, positions which are both *meta* to C_2. However, the chemical shifts at both positions vary in a regular manner, and the effects reproduce themselves in a fashion independent of the substituent at C_1. This point is emphasized in Figure 1 where a plot is given of the substituent chemical shifts (SCS)[10] for the series I-H_4 *vs.* II and I-H_6 *vs.* III. Similar plots were obtained for series other than the chlorobenzenes. The SCS values for II and III were taken from ref 2e.



I



II



III

It is evident from Figure 1 that the variations of the SCS values at H_4 and H_6 are not only different for a range of substituents at C_2, but, furthermore, *they are independent of the nature of the substituent at C_1.*

The premise now seems generally accepted that the shielding of aromatic protons *meta* to a substituent is due to a combination of "through space" and "through the bonds" mechanisms.[2] If so, then the above observation suggests strongly that the variations in SCS values at H_4 due to the substituents at C_2 are the result

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(10) As defined by M. J. S. Dewar and A. P. Marchand, *J. Am. Chem. Soc.*, **88**, 3318 (1966): the difference in chemical shift for a proton in a substituted benzene and the appropriate proton shift when the substituent is hydrogen.

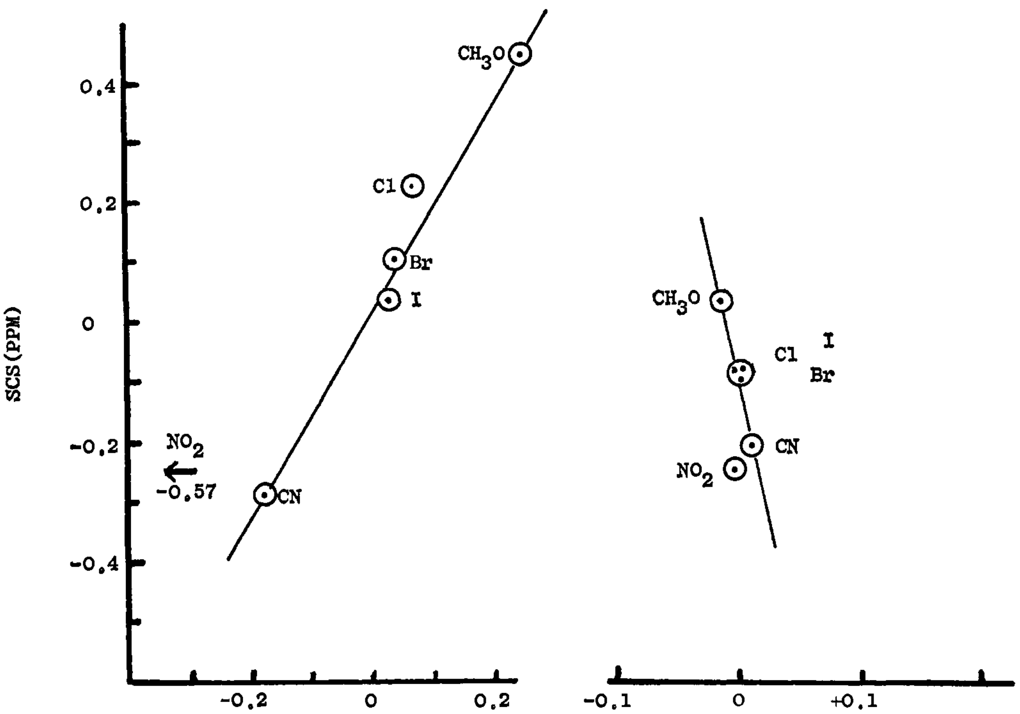

Figure 3. Plots of SCS values for H_5 (left) and H_6 (right) in 1-chloro-2-X-benzenes *vs.* chemical shift calculated by Wu and Dailey.[12]

of the operation of both types of mechanisms, while the change in shielding at H_6 is due to the "through the bond" mechanism only, the intervening substituent at C_1 effectively insulating H_6 from a field effect generated by a C_2 substituent.

Recently, Dewar and Takeuchi[2e] have postulated a "steric inhibition of diamagnetism" to explain the differences in SCS in system *III* as compared to *II*, the presumption being that the methyl groups on III hinder the induced circulation of the unshared electrons about the substituent X. The plot in Figure 1 clearly rules against this hypothesis since such an effect would have to apply equally at H_4 and H_6 in I, a result contrary to fact.

Protons which are *meta* to the substituent in the monosubstituted benzenes do not follow such measures of electronic perturbations as the Hammett σ_m values. The same is true for H_4 in our various series. However, in Figure 2 the SCS values for H_6 are plotted in the series 1-chloro-2-X-benzene and 1-nitro-2-X-benzene, respectively, against σ_m. While not reproduced here, the values of SCS for H_5 (*para* to C_2) give an equally good linear plot against σ_p. The meaning of these observations is not completely clear since no exact theoretical correlation of chemical shifts and σ has been made.[11] However, it is generally assumed that σ_m and σ_p do represent rather closely the electronic perturbations of the substituent on the ground state of the molecule in question even though this cannot be strictly true due to the experimental ways by which σ is measured.

Perhaps a more fundamental relation is that shown in Figure 3 where the SCS values at H_5 and H_6 in the chlorobenzenes are plotted against the chemical shift values calculated for the protons *para* and *meta* to the substituent.[12] Wu and Dailey[12] arrived at their chemical shift values by considering that the effects were due entirely to π-electron density alternations brought about by the substituent, the latter being calculated by the HMO method. As can be seen, the fit for both H_5 and H_6 is good with the exception of the nitro group. This suggests that the parameters used by these workers for the nitro group were in error. Actually, it would be highly desirable to have a complete set of SCF calculations for the monosubstituted benzenes. However, they are not yet available.

The consistency of the σ and the Wu and Dailey values (Figures 2 and 3, respectively) adds further empirical evidence for the contention that σ does measure π-electron density (or a value proportional to it) at the *meta* and *para* positions although it does not rule out the possibility that the effect at H_6 is attenuated by the intervening substituent at C_1. Again, it would appear that the effect of altering the substituent at C_2 while holding the C_1 substituent constant is to change the chemical shift at H_6 by a "through the bond" mechanism.

As mentioned previously, Hruska, Hutton, and Schaefer[5] have described an empirical parameter *Q*, defined as P/Ir^3 where *P* is the C–X bond polarizability, *I* is the first ionization potential taken as the value for the free elements, and *r* is the C–X bond length. Values for *Q* were calculated for bonds to hydrogen and the halogens. They[5] reported linear correlations between *Q* and the *ortho* protons in the aryl halides and with the *cis*-vinyl hydrogens in the vinyl halides. Subsequently, it was shown by Smith and Cole[4] that protons adjacent to the substituents in a series of *ortho* dihalobenzenes were independent of the nonadjacent halogen but rather followed the *Q* relation with the adjacent halogen.

The appropriate data are not available for the *a priori* calculations of *Q* for methoxyl, cyano, and nitro

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[11] See W. Adcock and M. J. S. Dewar, *J. Am. Chem. Soc.*, **89**, 379 (1967), for comment on this issue.

[12] T. K. Wu and B. P. Dailey, *J. Chem. Phys.*, **41**, 2796 (1964).

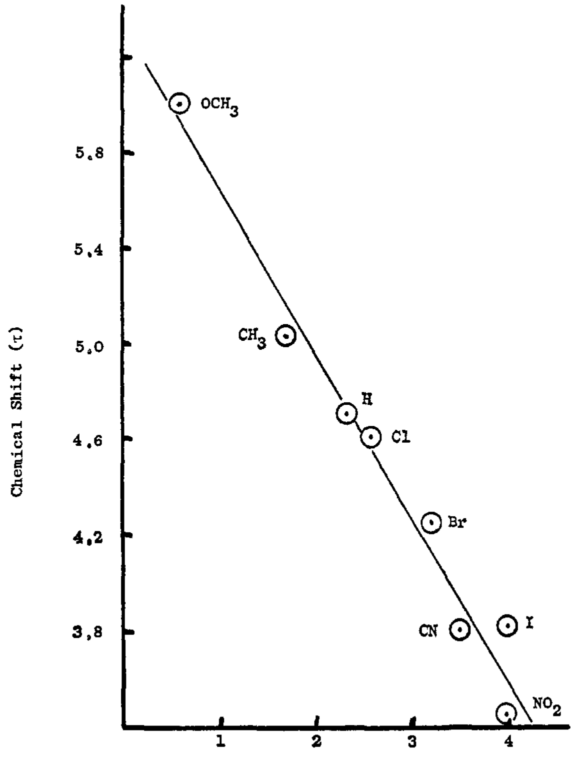

Figure 4. The relation of Q to the chemical shifts of a variety of *cis*-vinyl hydrogens.

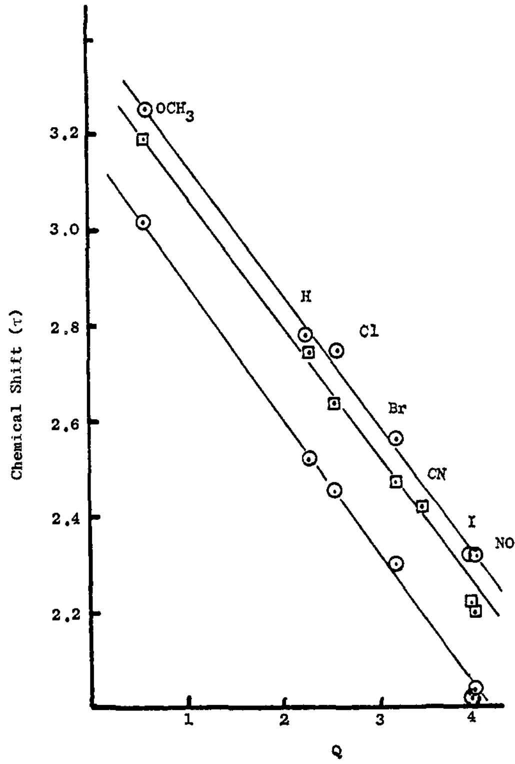

Figure 5. The chemical shift of H_3 in 1-methoxy-2-X-benzene (upper) and 1-chloro-2-X-benzene and 1-nitro-2-X-benzene (lower) graphed against Q.

groups. However, using the previously published plot for the *o*-dihalobenzenes[4] and the average values for the H_6 chemical shift in the 1-halo-2-methoxy (cyano or nitro) benzenes allowed us to derive experimental values of Q as follows: methoxyl 0.6, cyano 3.5, and nitro 4.0. Using the published values[13] for *o*-xylene provided a value of Q of 1.7 for the methyl group. Probably these derived values are good to ±0.1.

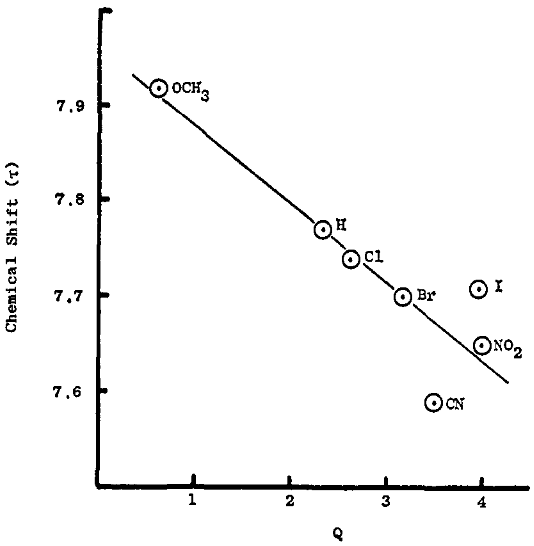

Figure 6. The methyl group chemical shift in a series of *o*-toluenes plotted against Q.

To test these new Q values, a plot of Q *vs.* the chemical shift of the *cis*-vinyl hydrogen was prepared (Figure 4).[14] As can be seen, the new Q values fit the relation previously established.[5] Presumably, the predictive merit of these new values is now established.

A further test of the new Q values is offered in Figure 5 where Q is plotted against the chemical shifts for H_3 in a series of disubstituted benzenes in which the substituent at C_1 is fixed while that at C_2 is varied. Similar plots were obtained with the other families. These plots suggest further predictive utility for the Q–chemical shift relation. Furthermore, the parallel nature of the plots in Figure 4 supports the earlier conclusion that the leakage of effect from the C_2 substituent on H_6 or from the C_1 substituent on H_3 is constant, *i.e.*, independent of the nature of the intervening substituent.

Ideally, one would like to calculate proton chemical shifts from first principles starting from the Ramsey formulation.[15] However, the exact calculation for proton spectra is complicated due to our lack of knowledge of the exact wave functions for the ground and all excited states. In discussing substituent effects it has become the custom to follow the dissection of the Ramsey equation provided by Saika and Slichter[16] who considered the chemical shift to be made up of diamagnetic and paramagnetic contributions both from the atom (in this case a proton) in question and from neighboring atoms. For protons in C–H bonds it has usually been assumed that the local paramagnetic contribution was nil.[17]

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[13] Varian Catalog of Nmr Spectra, Varian Associates, Palo Alto, Calif., 1962.

[14] Chemical shifts for ethylene and the vinyl halides were taken as quoted in ref 5. The value for nitroethylene was taken from R. R. Frazer, *Can. J. Chem.*, **38**, 2226 (1960), and methyl vinyl ether from J. Feeney, A. Ledwith, and L. H. Sutcliffe, *J. Chem. Soc.*, 2021 (1962).

[15] N. F. Ramsey, *Phys. Rev.*, **78**, 699 (1950).

[16] A. Saika and C. P. Slichter, *J. Chem. Phys.*, 22, 26 (1954).

[17] J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p 176.

The local diamagnetic terms are usually discussed in terms of factors which will affect the electron density about the proton, *i.e.*, electronegativity of adjacent substituents, Hammett σ functions, and resonance and inductive effects of substituents. Nonlocal paramagnetic effects may be field effects,[2d,10] van der Waals effects,[2c] or various magnetic anisotropy effects.

The shifts of the protons H_3 in the series of 1-chloro-2-X-benzenes give, at best, a very rough correlation with σ_p or with group dipole moments. With the other families no relation is evident with either of these parameters. We conclude that resonance, inductive, and field effects as discussed by Buckingham[2d] are not the dominant factors in determining chemical shifts *ortho* to the substituent.

The consistency of the Q–chemical shift plot (Figure 5) can be accounted for by the operation of a single dominant mechanism or the accidental collocation of a variety of effects. The latter seems highly unlikely.

In Figure 6 is given a plot of the chemical shifts of a series of *ortho*-substituted toluenes against Q. The linear behavior of the relation suggests that the same mechanism determines the shielding for *o*-methyl as for an *o*-hydrogen. Exceptions are noted for the iodo and cyano group (both large and axially symmetrical) where other effects are apparently operative. This relation offers further evidence against the operation of inductive and resonance effects since these would be greatly attenuated at the methyl protons.

In conclusion, the dominant *ortho* effect is seen as a shielding perturbation introduced into the paramagnetic term of the Ramsey equation. Such effects are well accepted for neighboring fluorines[16,18] and carbon-13[19] but have been considered unimportant in hydrogen since presumably the energy gap to the available 2p orbitals is quite large. The question of the perturbing effects of a neighboring dipole or charge do not seem to have been quantitatively considered. However, Hruska, Hutton, and Schaefer[5] have given expression to some thoughts on a qualitative relation between Q and the paramagnetic term. Our results lead us to believe the source of the *ortho* shielding enigma lies here waiting examination by the theorists.

Acknowledgment. We wish to express our sincere appreciation to the Robert A. Welch Foundation for the support of this work.

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(18) M. Karplus and T. P. Das, *J. Chem. Phys.*, **34**, 1683 (1961).

(19) M. Karplus and J. A. Pople, *ibid.*, **38**, 2803 (1963).

Proteins as Random Coils. III. Optical Rotatory Dispersion in 6 *M* Guanidine Hydrochloride[1]

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Abstract: Measurements have been made of the optical rotatory dispersion curves of protein polypeptide chains in 6 *M* guanidine hydrochloride solutions. All of the curves have the characteristics of randomly coiled polypeptides, but individual proteins differ appreciably in the magnitude of the rotation at any given wavelength. A literature search indicates that the intrinsic rotations of the various amino acids in a randomly coiled polypeptide chain must differ widely, from large positive values for tyrosine to large negative values for proline. The mean residue rotation of a protein polypeptide chain must therefore depend on amino acid composition, and calculations based on this, using such intrinsic residue rotation as can be determined from existing data, in fact account semiquantitatively for the observed variations. For proteins which contain disulfide bonds, measurements were made both with disulfide bonds intact and with these bonds ruptured by reduction. The observed differences in rotation were found to be ascribable to the difference in intrinsic residue rotation between oxidized and reduced half-cystine residues multiplied by the number of such residues per mole of protein. The over-all conclusion is that optical rotatory dispersion indicates that no noncovalent structure exists in polypeptide chains in 6 *M* guanidine hydrochloride, either in the presence or in the absence of disulfide bonds.

It has been shown in the previous papers of this series[3,4] that proteins dissolved in 6 *M* guanidine hydrochloride (GuHCl), in the presence of a reducing agent (RSH), have intrinsic viscosities and sedimentation coefficients characteristic of randomly coiled polypeptide chains. In the same solvent, but in the absence of RSH, some of the proteins studied possess disulfide bonds. The intrinsic viscosities were found to be diminished thereby, and the sedimentation coefficients increased, but the extent of change was found to be such that it could be reasonably ascribed to the purely physical restrictions which disulfide cross-links necessarily impose on an otherwise random coil. It was postulated therefore that no noncovalent structure exists in pro-

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