Nmr Chemical Shifts in Carcinogenic Polynuclear Hydrocarbons

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For polycyclic aromatic hydrocarbons displaying a range of carcinogenic activities, the relation between molecular orbital calculations and recently measured chemical shifts, derived from analysis of high-field nmr spectra, is examined. The modified and unmodified McWeeny methods of evaluating the dominant ring-current contributions are compared with experimental shieldings (relative to benzene) for naphthalene and benz[a]-anthracene. For dibenz[a,h]anthracene, benzo[c]phenanthrene, and dibenzo[c,g]phenanthrene, shieldings calculated by the McWeeny method also correlate with experimental shieldings; large downfield shifts cause bay protons to lie on subsidiary lines (the phenanthrenic proton effect). This agreement enables nmr shifts to provide a test of LCAO calculations underlying theories of carcinogenesis. The significance of small deviations from otherwise satisfactory correlations with LCAO-based ring-current theory for the K and L regions of carcinogens is discussed in terms of molecular geometry.

The primary agents responsible for the carcinogenic activity of coal tars,[1,2] tobacco smoke,[3] and air pollutants[4] are thought to be polycyclic aromatic hydrocarbons and their alkyl derivatives. One approach toward discerning their mode of action at the cellular and molecular levels involves associating this biological property[5,6] with physicochemical measurements.

Two sets of parameters, the chemical shifts, ν, and spin–spin coupling constants, J, result from the analysis of a high-resolution nmr spectrum; values of both can vary somewhat with concentration, solvent, temperature, and, in particular, with the influence of a neighboring, biologically important, molecule. However, knowledge of ν and J, even if only to moderate accuracy, allows the application of molecular orbital (MO) theory to polycyclic hydrocarbons[7] to be checked and provides a way of determining the electron distribution within the molecule. For example, π-electron ring currents, which may be calculated by MO methods,[8-11] make large contributions to ν in polybenzenoid hydrocarbons; anisotropies of susceptibility within bonds in the σ framework can also be important.[12] Moreover, in sterically hindered molecules, ν can be indicative of deviations from planarity;[12] the detailed geometrical compatibility of hydrocarbon[13] and receptor will be important in the binding associated with carcinogenic activity. In this paper we discuss the relation between recent chemical shift[14,15] measurements and MO calculations on polynuclear hydrocarbons of varying carcinogenic activities as[16-18] listed in Table I. Empirical correlations[11,19,20] can also be made between *ortho* H–H coupling constants and MO calculations; the Pullman[17,21,22] theory of carcinogenesis defines threshold values of localization energy (electron density) in the active K (*ortho*) and L (*para*) regions.

Ring-Current Contributions to Chemical Shifts in Carcinogenic Polynuclears.

Despite controversy about the origin of shifts[23-25] and magnetic susceptibilities[26] in polynuclears, it is generally accepted that the dominant contributors[27] to chemical shielding in polynuclears are the π-electron ring currents first invoked to account for magnetic susceptibility anisotropies.[28] The magnetic field promotes ring fluxes, defined in terms of the modified π-binding energy,[29] which may be regarded as either specific to a given ring[8,30] or as line currents in the component bonds.[31]

In the magnetic-field modified LCAO theory, a wave equation is set up for π electrons only; shift contributions from σ anisotropies must be considered separately. Further, LCAO ring currents and chemical shifts calculated for alternant polynuclear hydrocarbons are relatively insensitive to the refinements of a many-electron (SCF) Hamiltonian, variation of resonance integrals (β), or choice of atomic orbitals (AO) giving independence from β. Although inclusion of overlap makes little difference to either π-electron energies[32,33] or LCAO-calculated anisotropic magnetic susceptibilities[34,35] of polynuclears, recent calculations with this

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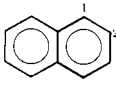
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TABLE I

CARCINOGENIC POTENCY OF HYDROCARBONS STUDIED IN THIS INVESTIGATION

| Compd | Carcinogenic potency (or activity)[a] | | | Nmr shifts reported in ref |
	Ref 16	Ref 17	Ref 18	
Benz[a]anthracene (I)	Inactive	Uncertain	+	14
7-Methylbenz[a]anthracene (II)	+++	+++	NR	14
7,12-Dimethylbenz[a]anthracene (III)	++++	++++	NR	14
9-Methyl-7,8-ethanobenz[a]anthracene (20-methylcholanthrene) (IV)	++++	++++	NR	14
Dibenz[a,h]anthracene (V)	++	++	+++	14
Benz[a]pyrene (VI)	++++	++++	+++	14, 15
Benzo[c]phenanthrene (VII)	+	+	NR	b
Dibenzo[c,g]phenanthrene (VIII)	Inactive	Inactive	NR	c

[a] Activity: ++++, very marked; +, very weak; NR means not reported. [b] K. D. Bartle and D. W. Jones, *J. Chem. Soc., A*, 437 (1969). [c] K. D. Bartle, D. W. Jones, and R. S. Matthews, *ibid.*, 876 (1969).

TABLE II

NAPHTHALENE. COMPARISON OF EXPERIMENTAL SHIELDING CONSTANTS, $\sigma_\pi/\sigma_{\pi benzene}$ (WITH RESPECT TO BENZENE), WITH VALUES CALCULATED BY SEVERAL METHODS

| | Dipole approxmtn (Pauling model) Ref 38 | Hückel LCAO | | | | | Refined LCAO | | | |
| | | Pople method Ref 11 | McWeeny method | | | SCF Ref 39 | Variable β Ref 40 | β independence Ref 41 | Inclusion of overlap Ref 36 | Exp(t Ref 11 |
			Ref 37	Ref 39	Ref 36					
H(1)	1.43	1.44	1.31	1.31	1.28	1.28	1.24	1.39	1.20	1.28
H(2)	1.16	1.22	1.17	1.17	1.15	1.15	1.09	1.21	1.11	1.10

refinement[36] suggest that ring-current shifts for some protons deviate significantly from "simple" LCAO values. Comparison of calculated[11,36-41] and experimental proton-shielding constants, $\sigma_\pi/\sigma_{\pi benzene}$ (with respect to benzene), for naphthalene (Table II) shows that LCAO-calculated shifts correlate well with experimental values. It is concluded that a comparison of calculated and experimental shifts in polynuclear carcinogens should afford a measure of the applicability to these compounds of LCAO theory on which the Pullman theory[17,21,22] of carcinogenesis depends.

Low symmetry and/or poor solubility render the measurement of chemical shifts in many polynuclear carcinogens difficult from nmr spectra recorded at moderate frequencies; recently, spectra for a number of benz[a]anthracenes (of carcinogenic potency given in Table I) have been analyzed[14,15] at 220 MHz. The fair correlations (Figures 1–3) between calculated[36,37,42] shielding parameters, $\sigma_\pi/\sigma_{\pi benzene}$, and values derived from the experimental shifts confirm earlier valuations[42] of ring-current calculations in the approximate solution of parent hydrocarbon spectra in terms of relative chemical shifts. As with analogous polynuclear hydrocarbons, bond anisotropies for peripheral protons are small enough to be neglected,[11,12,37,43] but bay protons experience here such anomalously large downfield

shifts that they lie on a subsidiary (broken) line (Figures 1–3) displaced −0.35 shielding unit (0.54 ppm) from the main line.
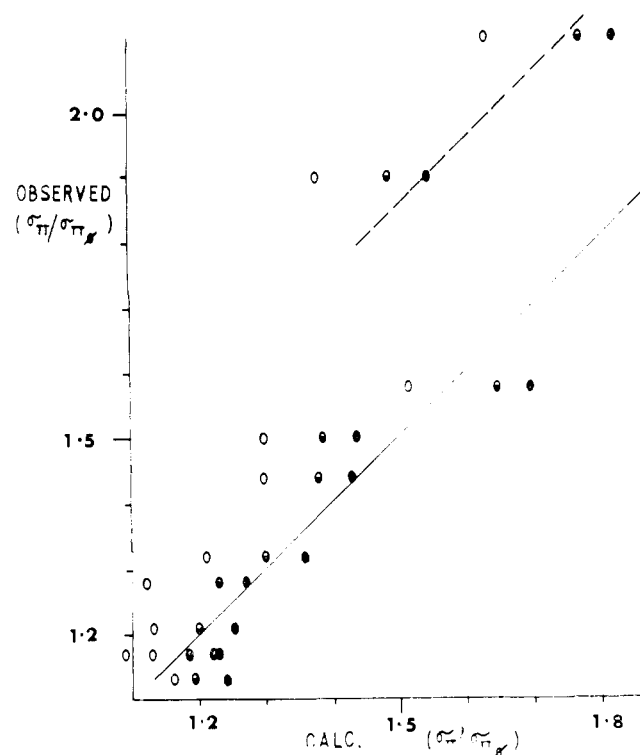
Several explanations of this phenanthrenic proton

Figure 1.—Graph of observed and calculated values of shielding parameter, $\sigma_\pi/\sigma_{\pi benzene}$, relative to benzene, for hydrogens in benz[a]anthracene. Full line is for equality of observed and calculated parameters; bay protons lie on dashed line. Closed circles[37] and half circles[36] correspond to values of $\sigma_\pi/\sigma_{\pi benzene}$ calculated by the McWeeny method; open circles correspond to values of $\sigma_\pi/\sigma_{\pi benzene}$ calculated with inclusion of overlap.[36]

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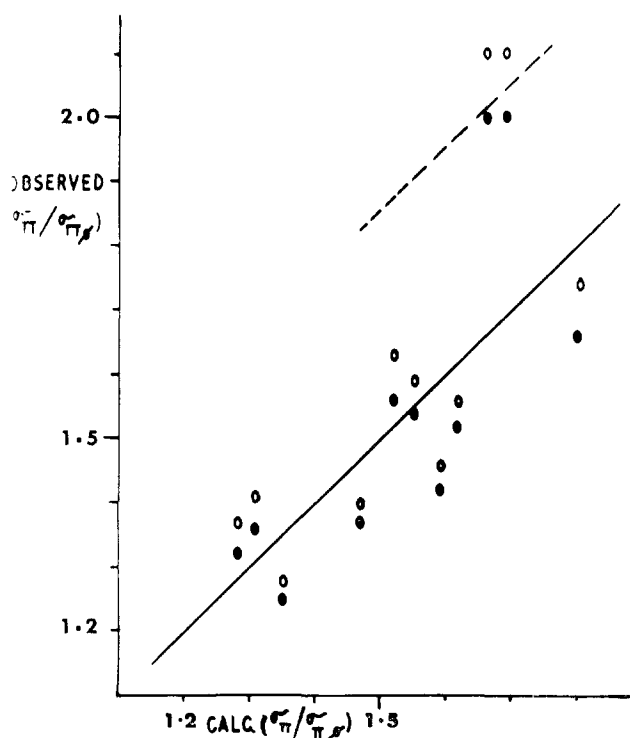(43) K. G. Kidd, G. Gutowsky, and T. Schaefer, *Can. J. Chem.*, **45**, 2155 (1967).

Figure 2.—Observed and calculated (McWeeny) shieldings, σ_π $\sigma_{\pi\text{localized}}$ for benzo[a]pyrene; lines as in Figure 1. Open circles are for observed values[15] at infinite dilution in CCl_4; closed circles for observed values[14] for solution in CS_2.
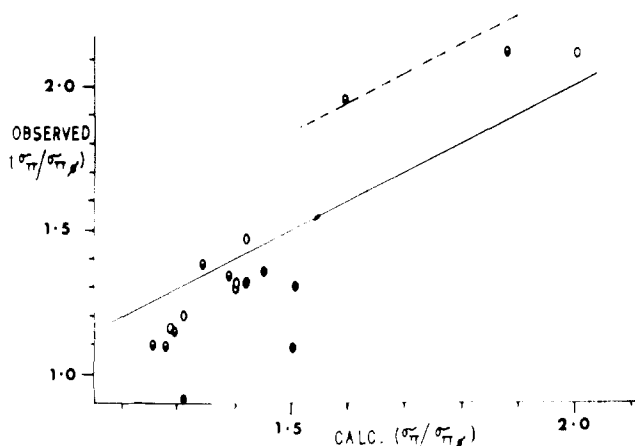

Figure 3.—Observed and calculated (McWeeny) shielding parameters, $\sigma_\pi/\sigma_{\pi\text{localized}}$ for dibenz[a,h]anthracene (half circles), dibenzo[c,g]phenanthrene (closed circles), and benzo[c]phenanthrene (open circles), all in CS_2 solution.

effect have been proposed. First, attribution to local anisotropies of susceptibility ($\Delta\chi$) appears plausible; this calls for downfield shifts of 0.26 ppm, with respect to benzene, in phenanthrene[44] and between 0.27 and 0.45 ppm in triphenylene,[12] depending on the values taken for $\Delta\chi_{C-C}$ and $\Delta\chi_{C-H}$ and whether the modification[45] of the McConnell equation[46] is used. Secondly, recent work by Cheney[47] suggests that the steric compression[48] to which bay protons are subject induces

charge polarization in the H–C bond with consequent deshielding. This is estimated at 0.76 ppm (by an approximate a priori calculation) or 0.51 via empirical correlation based on "experimental steric" shifts deduced by substracting calculated ring-current shifts, δ_{RC}, from the observed shift; for certain calibration molecules, however, such as benzo[c]phenanthrene, δ_{RC} is based on an inappropriate[49] planar model. There may be a parallel[42,47] between this sterically induced charge polarization and the third mechanism proposed, an electric-field effect which is difficult to evaluate and arises from repulsion between hydrogen orbitals.[50,51]

Over-all agreement for I (Figure 1) is better between experiment and McWeeny-method σ_π, $\sigma_{\pi\text{localized}}$ calculated by Caralp and Hoarau[36] than for either the shielding parameters calculated ostensibly by the same method by Memory, et al.,[37] or those with overlap inclusion[38] which in general are about 0.1 shielding unit too low. Agreement between experimental and LCAO σ_π, $\sigma_{\pi\text{localized}}$ values[42] is also good for V, but our measurements[14] or σ_π, $\sigma_{\pi\text{localized}}$ for VI at low concentration in
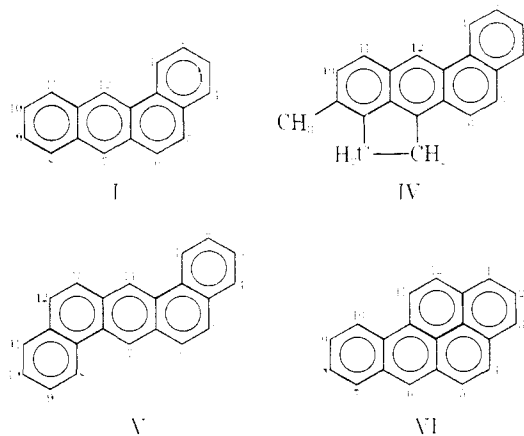
CS_2 are shifted to high field of both McWeeny values[42] and infinite-dilution (CCl_4) experimental results.[15] This discrepancy arises because dilution shifts[52] for VI are much larger[15] than those found for phenanthrene[44] and other alternant polycyclics,[1] and for fluoranthenes;[53] in fact, differences between our δ values[14] and those of Haigh and Mallion[15] correlate rather well with the downfield shifts observed[15] when VI is diluted. LCAO-based ring-current theory thus appears to account well for proton chemical shifts in I, V, and VI, and to endorse the applicability of such simple MO methods to these polycyclic carcinogens;[54] small deviations from theory may be significant, however, as is discussed below.

A Possible Geometric Effect in Carcinogenesis by Benz[a]anthracenes. The reason why H-14 in V suffers (Figure 3) about 0.1 shielding unit extra upfield shift (not experienced by the opposed H-1) may be that it is slightly displaced from the plane of the rings; in

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the crystal,[55] C-1 lies very nearly in, and C-14 slightly (0.01 Å) out, of the mean molecular plane. There is reduced deshielding of H-12 in I (Figure 1), for which the refinement of the noncentrosymmetric crystal structure[56] is not available. Moreover, in crystals of the potent 7.12-dimethyl derivative III, some C atoms lie appreciably out of the molecular plane[57] (0.56 Å for 12-CH₃) in contrast with the earlier view[58] of planarity as a prerequisite of carcinogenicity. Our observation[12,14] that the bay proton H-1 is shielded rather than deshielded[12] when Me is introduced suggests that this nonplanarity persists in solution. Since the corresponding C-11 atom in the equally active carcinogen VI also projects slightly from the mean ring plane,[59] there is evident a gradual increase in potency of some benz[a]anthracenes, from the almost planar I, through V, to VI and III, as increasingly bulky groups at the 12 position protrude from the molecular plane. While molecular geometry, and especially planarity, of carcinogens is involved in theories of interaction with DNA (mutagenesis[91]) and with proteins (genetic regulation theories),[61] its role is less clear for the benz[a]anthracene series than for the acridine series.[62]

With the recent availability of full assignments for the spectra of VII (also designated 3,4-benzophenan-threne[49]) and VIII (also designated 3,4,5,6-dibenzo-phenanthrene[63]), it has been possible to make improved correlations with theoretical shielding parameters.[37] Apart from the hindered 1 position, calculated values of $\sigma_\pi/\sigma_{\pi\text{Lenzene}}$ in VII tend to be slightly downfield of experimental values, but nonhindered positions in VIII show a much bigger difference.[42] This may be a consequence of nonplanarity in that orbital overlap, ring currents, and deshielding are all reduced.

Chemical Shifts in the K and L Regions. Alkyl-Substituted Polynuclear Carcinogens.—Agreement between observed and calculated chemical shifts is good for the K regions of I and V (Figures 1 and 3); although the experimental shielding parameters are greater than those calculated for both the K-region protons H-4 and H-5 in VI (Figure 2), this correlation is probably fortuitous, since similar discrepancies are observed for the

"pyrenic" protons H-1 and H-12. In both I and VI, Haigh and Mallion have noted[15] that the K region protons exhibit anomalous dilution shifts.

The high-field shifts induced in the para H-12, when one H of the L region is substituted by Me (in II) or CH₂ (in IV), are smaller than in compounds of lower molecular weight. Thus ν_{12} is at 0.06 ppm higher field in II than in I, but the p-CH₃ shifts in toluene,[64] 1,8-dimethylnaphthalene,[65] and 4-methylphenanthrene[44] are +0.16, +0.23, and +0.15 ppm, respectively; the difference of 0.20 ppm between values in I and IV compares with a high-field shift of 0.31 ppm induced by p-CH₂ in acenaphthene.[65] The shielding of H-7 by p-CH₃ in 12-methylbenz[a]anthracene is also small.[66] If p-CH₃ shifts occur because inductive or hyper-conjugative interaction with the π system increases electronic change at the para C,[64,67] then our results[14] on the carcinogens II and IV suggest that the charge density at C-12 in the benz[a]anthracene system changes only slightly when a CH₃ or CH₂ is substituted at C-7.

On the other hand, while the K region proton H-6 in both II and III is subject to strong deshielding (0.33 and 0.16 ppm, respectively) by Me-7, via the spatial peri effect,[44,65] H-6 in IV is scarcely affected by the peri-methylene. Association of the carcinogenic activity of these molecules with a geometric factor in the K region cannot therefore be ruled out.

Conclusions.—Experimental nmr chemical shifts in polycyclic carcinogens correlated quite well with values calculated from MO theory and, for this purpose, LCAO theory is adequate. Consequently, pmr shifts can be used to assess the validity of LCAO calculations on which some theories of carcinogens depend. Those cases showing small deviations are consistent with the association of carcinogenic activity in benz[a]anthra-cene derivatives with the stereochemistry of the C-12 substituent in the L region; geometric effects are also possible in the K region.

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