that the Fe–CN bond strength is unchanged upon putidaredoxin binding, while the iron-bound C–N bond is weakened under the situation. The weakened iron-bound C–N bond likely makes a less efficient electron spin delocalization from the iron to the ^{15}N atom. Here, we note that the binding of putidaredoxin to the enzyme regulates the electron spin transfer from the iron to the ligand. If the oxy form (Fe^{3+}–O–O$^-$) of P-450$_{cam}$ is assumed to be similar to the cyano form (Fe^{3+}–C–N$^-$), this electronic configuration may be favorable to a heterolytic scission of the O–O bond when one more electron is given from the electron donor to the heme in the oxy form.[17]

Concerning the electronic and structural changes in the heme vicinity upon putidaredoxin binding, three possibilities were considered: (1) an increase in the Fe–S$^-$(Cys) bond strength, (2) a decrease of the steric restriction to the Fe–C–N bond, and (3) a formation of hydrogen bonding of cyanide N atom with nearby amino acid residues. As stated in the preceding sections, any of these effects serve to change the cyanide ^{15}N signal position to upfield region. Recent X-ray studies[1] revealed that the heme of P-450$_{cam}$ is deeply buried in the peptide, and the closest approach to the protein surface occurs at its proximal face, at a distance of about 8 Å. So, if putidaredoxin binds to the protein surface of P-450$_{cam}$ at the proximal side of the heme, it is likely that its binding alters the Fe–S bond strength, supporting the first possibility. Further, when the conformational change at the heme distal side would be induced upon the putidaredoxin binding, the second and/or third possibilities are also likely. Further studies will be required to reveal this problem.

(17) (a) Poulos, T. L.; Freer, S. T.; Alden, R. A.; Edwards, S. L.; Skoglund, U.; Takio, K.; Eriksson, B.; Xuong, N.; Yonetani, T.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 575–580. (b) Poulos, T. L.; Kraut, J. *Ibid.* **1980**, *255*, 8199–8205.

Distortion in Six-Membered Saturated Rings by Natural Abundance Deuterium NMR

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Abstract: Distortions in six-membered aliphatic ring systems were examined using deuterium chemical shifts and multiple linear regression analysis. A new set of regression parameters has been developed that successfully interprets not only spectra for those methylcyclohexanes with normal chair conformations but also spectra for *cis*- and *trans*-decalin and for three highly distorted trimethylcyclohexanes with skew-boat type distortions to their equilibrium conformations. The origin of deformations in the cyclohexane ring and the effect of distortions on ring hydrogen/deuterium chemical shifts are discussed in terms of gauche interactions between vicinal C–C and C–D bonds. The fits for 115 deuterium shifts in these cyclic alkanes exhibited a multiple $R = 0.9816$ and $\sigma = 0.0623$ ppm for an overall shift range of about 1.5 ppm. As the 14 structural parameters obtained in this study are very sensitive to distortions and conformational features, the results indicate that ^2H NMR methods should be very powerful in those cyclic paraffins in which highly second-order banding is observed in the corresponding ^1H NMR spectrum.

I. Introduction

The use of very high field spectrometers for ^2H NMR has been shown to be valuable in extracting structural information not readily available from complex ^1H spectra.[1] Similar to ^{13}C spectra, proton-decoupled, natural abundance ^2H spectra exhibit single resonances for chemically distinct sites. The absence of scalar couplings in these ^2H spectra produces a simple spectrum, which is extremely helpful in determining chemical shifts when the ^1H spectrum is either banded or exhibits overlapping multiplets. Compounds containing six-membered saturated rings usually suffer severe banding in their ^1H spectra and hence are particularly suitable candidates for study by ^2H NMR.

It is well-known[2,3] that the chair conformation of cyclohexane is energetically more stable than the boat conformation and that cyclohexane interconverts rapidly between degenerate chair conformations at room temperature.[4] Methylcyclohexane preferentially assumes the chair conformation in which the methyl group is equatorial,[5–7] since the strain energy associated with an axial methyl is ~1.8 kcal/mol higher than that for an equatorial methyl. In multiple-substituted methylcyclohexanes there are four different conformational cases: case 1, two equal energy con-

formations exist and the compound interconverts between them (e.g., 1,1-dimethylcyclohexane); case 2, one chair conformation is energetically favored (>99%) over the other (e.g., 1,*cis*-3-dimethylcyclohexane); case 3, one chair conformation is only slightly favored over the other (e.g., 1,*trans*-2,*cis*-4-trimethylcyclohexane); case 4, substantial distortion to the ring occurs due to extensive steric crowding of substituents (e.g., 1,*cis*-2,*trans*-3-trimethylcyclohexane).

In the first three cases it has been possible to make $^2H/^1H$ NMR spectral assignments very successfully using substitution parameters established by multiple linear regression.[1] In the last case, where some contribution from skew-boat type conformations has been proposed, it has not proved possible to predict or to interpret spectra satisfactorily using the parameters developed from compounds exhibiting "pure" chair conformations. This work develops a parameter set that successfully characterizes the

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(1) Curtis, J.; Dalling, D. K.; Grant, D. M. *J. Org. Chem.* **1986**, *51*, 136.
(2) Pitzer, K. S. *Chem. Rev.* **1940**, *27*, 39.
(3) Hendrickson, J. B. *J. Am. Chem. Soc.* **1961**, *83*, 4537.
(4) Dalling, D. K.; Grant, D. M. *J. Am. Chem. Soc.* **1967**, *89*, 6612.
(5) Brownstein, S.; Muller, R. *J. Org. Chem.* **1959**, *24*, 1886.
(6) Anet, F. A. L.; Ahmad, M.; Hall, L. D. *Proc. Chem. Soc.* **1964**, 145.
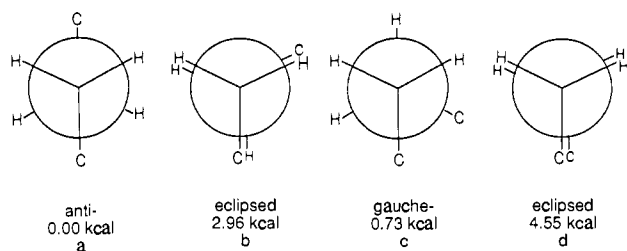(7) Muller, N.; Tosch, W. C. *J. Chem. Phys.* **1962**, *37*, 1167.

Figure 1. Energy of rotational conformations of butane. Lowest energy anti form (a) is set arbitrarily to zero. Highest energies are associated with eclipsed conformations (b and d). Conformation of cyclohexane methylene groups is similar to that for *gauche*-butane.

conformational structure in distorted methylcyclohexanes as well as those with simpler conformations.

A close examination of the parameters for vicinal substitution indicates that the primary factor in differentiating hydrogen chemical shifts in deformed rings is the interaction of a specific C_α–H bond with either the gauche C_β–C_γ or C_β–H bonds. The magnitude of these interactions is spatially dependent upon the dihedral angle, assuming a constant C_α–C_β bond length in the ring. Modulating the interaction by changes in the dihedral angle between the vicinal bonds successfully accounts for the variation in vicinal parameters at a first level of approximation.[1]

This dependence of chemical shift on dihedral angle offers an opportunity for studying the methylcyclohexanes with intermediate energy differences especially those having conformations with distortions in the six-membered rings. In addition to the 17 cyclohexane compounds previously reported,[1] *cis-* and *trans*-decalin are now examined with this approach. The effect of ring junctures in the decalins is found to be sufficiently similar to those of axial or equatorial methyl substitution that a common parameter set is found to be appropriate.

II. Functional Dependence on Dihedral Angle

In studies of the relative energetics of *gauche*-butane, *anti*-butane, and eclipsed butane[8] it is apparent that increased energy is required as butane rotates to one of the two eclipsed conformations (see Figure 1). The energy of a conformation depends on the dihedral angles between vicinal bonds and on the type of vicinal bonds involved in these interactions. The functional form of this energy is proposed to depend on cos α, where α is the dihedral angle, since the interaction energy for any particular pair of bonds is a maximum at $\alpha = 0°$ and a minimum at $\alpha = 180°$. The interaction energy of two C–H bonds is given by Allinger[8] as +0.42 kcal/mol, that of a C–H and C–C bond as −0.05 kcal/mol, and that of two C–C bonds as −0.13 kcal/mol at the dihedral angle of 60° found in *anti-* and *gauche*-butane. The lowest energy form of butane (*anti*-butane) has two C–H:C–H interactions and four C–H:C–C interactions while *gauche*-butane has three C–H:C–H, two C–H:C–C, and one C–C:C–C interactions.

Closure of a ring to form cyclohexane locks each methylene group into a conformation analogous to Figure 1c for *gauche*-butane. The dihedral angles for gauche interactions with the ring hydrogens are thereby restricted to small variations around a constant angle. These small variations imposed by the ring cause substitution of C–C for C–H bonds to now appear to be the largest factor in differentiating chemical shift environments among the ring protons in cyclohexane ring systems.

In order to have a compound with an axial methyl, since the strain energy of an equatorial methyl group is much lower than that for an axial methyl, it is necessary to have multiple equatorial methyls as well. One such compound, 1,*cis*-2,*trans*-3-trimethylcyclohexane, is the most distorted from a simple chair conformation. Not only is there strain from an axial methyl but also there is the strain due to vicinal methyl groups. Figure 2 demonstrates the distortions from a dihedral angle of 60° caused

(8) Allinger, N. L.; Tribble, M. T.; Miller, M. A.; Wertz, D. H. *J. Am. Chem. Soc.* **1970**, *93*, 1637.
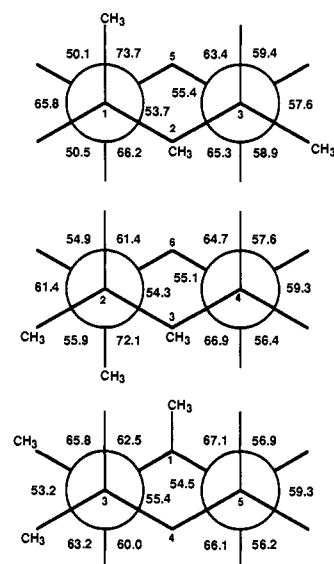
1,cis-2,trans-3-trimethylcyclohexane

Figure 2. Distortions from the ideal angle of 60° in 1,*cis*-2,*trans*-3-trimethylcyclohexane.

by these steric forces. The MM2 force field program[9] was used to calculate the dihedral angles shown. Even for the most distorted methylcyclohexanes the chemical shift appears to be approximately linear for small variations (±12°) in this dihedral angle.

Expanding the gauche dihedral angle about a standard angle (60°) allows one to express the gauche effect in the angular deviations. Let α be the dihedral angle of interest, which can be written as $\theta + \phi$, where ϕ is the angular deviation from the standard angle, θ. Normalizing the cos α angular dependence by dividing with cos θ gives:

$$\frac{\cos \alpha}{\cos \theta} = \frac{\cos (\theta + \phi)}{\cos \theta} = \frac{\cos \theta \cos \phi - \sin \theta \sin \phi}{\cos \theta} =$$
$$\cos \phi - \tan \theta \sin \phi \quad (1)$$

Since this normalized interaction must be unity when $\alpha = \theta$, the angular variation (v) in the shift is referenced to the default condition by the expression

$$v = \delta_{G1}\left(\frac{\cos \alpha}{\cos \theta} - 1\right) = \delta_{G1}(\cos \phi - \tan \theta \sin \phi - 1) \quad (2)$$

where δ_{G1} is a regressional parameter for the effect of a gauche interaction on chemical shift and tan (60°) = 1.732. For $\phi \sim 0$ and cos $\phi \sim 1$, the functional dependence of v on ϕ depends on sin ϕ. For small angles sin $\phi \sim \phi$ and the v dependence becomes linear in the angle of variation.

$$v \sim -\delta_{G1}(1.732\phi) \quad (3)$$

Note that eq 2, and thus eq 3, has the desired characteristics. It is zero when $\alpha = \theta$, greater than zero when $\alpha < \theta$, and less than zero when $\alpha > \theta$. This linear approximation in ϕ simplifies the dependence of chemical shifts upon angular distortion.

Two other possible distortions and their effect on the gauche interaction were considered using force field calculations. They are distortions arising from vicinal bonds in the tetrahedral angle of the C_α–D bond relative to the C_α–C_β bond and in the lengths of the C_α–C_β bond connecting the two interacting vicinal bonds. Even in the most distorted molecule (1,*cis*-2,*trans*-3-trimethylcyclohexane) the deviations from the tetrahedral angle were not more than 3°, giving rise to less than a 2% variation (0.002–0.004 ppm) in the predicted chemical shift using the shift coefficient for a gauche interaction. Lengths of the C–C bonds in the ring in the force field calculations varied between 1.533–1.538 Å and of C–H bonds for ring hydrogens between 1.114–1.120 Å over

(9) Allinger, N. L.; Yuh, Y. H. *QCPE* **1980**, 395.

Table I. Chemical Shift Assignments by Regression with Distortion of Dihedral Angles

item	cyclohexane	site	exptl[a]	predicted	item	cyclohexane	site	exptl[a]	predicted
1	methyl-	2,6e	1.64�	1.65	27	1,1,4-	3,5e	1.34⎤	1.41
2		3,5e	1.66⎦	1.66	28		2,6e	1.46⎦	1.43
3	1,*trans*-2-	3,6a	0.92⎤	0.95	29	1,*cis*-2,*trans*-3-	5a	1.32⎤	1.38
4		1,2a	0.93⎦	1.08	30		5e	1.32⎬	1.42
5	1,*cis*-3-[b]	2a	0.55	0.52	31		6e	1.32⎦	1.38
6		4,6a	0.77	0.80	32		6a	1.46⎤	1.49
7		5a	1.27	1.32	33		4e	1.48⎦	1.58
8		1,3a	1.36	1.34	34	1,*trans*-2,*cis*-4-	2a	1.20⎤	1.18
9		2e	1.65	1.59	35		3a	1.20⎬	1.12
10		5e	1.72	1.63	36		4a	1.26⎦	1.22
11		4,6e	1.65	1.62	37		3e	1.37⎤	1.36
12	1,*trans*-2,*trans*-4-	6a	0.97⎤	0.99	38		5e	1.39⎬	1.39
13		2a	0.98⎦	1.02	39		6e	1.43⎦	1.40
14		3e	1.59⎤	1.55	40		5a	1.48	1.55
15		6e	1.62⎬	1.58	41	*trans*-decalin[c]	9,10	0.80	0.84
16		ke	1.63⎦	1.60	42		1,4,5,8a	0.92	0.92
17	1,1,3-	4a	0.71⎤	0.75	43		2,3,6,7a	1.24	1.20
18		2a	0.75⎦	0.77	44		1,4,5,8e	1.52	1.61
19		5e	1.33	1.41	45		2,3,6,7e	1.67	1.65
20		6e	1.42	1.44	46	*cis*-decalin[c]	1,5a 4,8e	1.30⎤	1.26
21		5a	1.50	1.52	47		2,6a 3,7e	1.33⎦	1.34
22	1,1,2-	4a	1.11	1.17	48		1,5e 4,8a	1.54	1.39
23		3e	1.35⎤	1.36	49		2,6e 3,7a	1.56	1.48
24		6e	1.39⎦	1.41	50		9,10	1.62	1.56
25		5e	1.45	1.42					
26		4e	1.63	1.62					

[a] Shifts connected by brackets were too close to assign unambiguously. Reassigned by ordering in regression. [b] 1,*cis*-3-Dimethylcyclohexane was found to have erroneous shifts reported in the previous work. The shifts for this compound have been corrected in this table. [c] Compound not previously reported or assigned. Assignments for all other sites can be found in ref 1.

the entire set of compounds. These effects, like the tetrahedral distortions, appear to make negligible contributions to the ^{2}H shifts and the much larger relative distortions in the dihedral angle are left to account for the measurable variations in the shift.

III. Experimental Section

cis- and *trans*-decalins (99% pure) were obtained from Aldrich Chemical Co. The methylcyclohexanes were obtained from either standard commercial sources or from the American Petroleum Institute (all in excess of 99% purity) as described in ref 1. Deuterium spectra on these compounds were obtained on a Varian XL-400 spectrometer equipped with proton-decoupling capability and an internal fluorine lock. Samples were prepared in 5-mm-o.d. tubes with 40% hexafluorobenzene (C_6F_6) as lock solvent and 5% TMS as a shift reference. Shimming was performed on ^{19}F. A spectrum of methylcyclohexane in the fluorinated solvent was also obtained to determine the effect of solvent on the methylcyclohexane data. No differences in chemical shifts were detected within experimental error.

IV. Results and Discussion

A. Spectral Assignments. Good spectral assignments for many of the methylcyclohexanes exist from our previous work,[1] but chemical shift assignments in four distorted trimethylcyclohexanes (1,1,2-; 1,1,4; 1,*cis*-2,*trans*-3-; and 1,*trans*-2,*cis*-4-) have only been tentative because the earlier parameter set was developed for relatively undistorted methylcyclohexanes and failed for seriously distorted molecules. In addition, some of the initial assignments were also tentative because the experimental shifts were too close to be assigned unequivocally. These tentative assignments have been reconsidered along with the decalin data in a new regressional analysis of the data.

The closely spaced resonances in *trans-* and *cis*-decalin render shift assignments by normal techniques in these rather complex cases difficult except at the 9,10-positions where site populations may be used to make some unequivocal assignments (see Figure 3). Initial assignments were made using the previous parameter set with a final assignment given by the ordering of experimental and predicted shifts in regressional analyses, which included angular distortion explicitly. Table I contains the shift assignments for the decalins. The shifts in the methylcyclohexanes, which were too close to be assigned unambiguously and which were tentatively assigned differently than previously reported, are also included in the table. While some assignments continue to remain ambiguous, many are now considered to be better than those from

the previous regression because structural distortion of individual sites is now better understood and correlated for these data.

B. Regression Analysis. Inclusion of both the decalins and the distorted trimethylcyclohexanes in a regression analysis using the structural and distortional parameters from ref 1 resulted in a multiple correlation coefficient of 0.970 and a standard error of the estimate of 0.0810 ppm compared with a multiple $R = 0.9959$ and $S = 0.0348$ for the regression on the pure chair compounds. Table II demonstrates the effect made by different refinements in the regression parameters in predicting chemical shifts on representative compounds for both pure chair and distorted methylcyclohexanes. It is clear that the regression, which includes distortional parameters explicitly, is superior in predicting the entire class of compounds.

The development of a parameter set to include distortion was found to depend principally on gauche interactions. The parent equatorial site is taken as the default case since this shift is not perturbed by vicinal interaction with any gauche C–C bonds. A deuteron at an equatorial site in the parent has four interactions with C–H bonds with dihedral angles of nearly 60° and no such interactions with C–C bonds. In principle, distortions affecting both interactions with gauche C–H and C–C bonds should affect chemical shifts. However, as a practical matter, only distortions associated with the dihedral angle between gauche C–D and C–C bonds were found to be significant, due to the dominating effect of substituting a C–C bond for a C–H bond. The contribution from angular distortion is thus summed into the parameter for gauche C–C bond substitution (see Figure 4). All other substituent effects were summed into a single parameter for each substitution site as also contained in Figure 4. It was found necessary to discriminate further between axial and equatorial parameters and to include a parameter to account for the interaction between axial 1,3 proton sites explicitly.

The chemical shift dependence of gauche interactions requires good estimates for the dihedral angles to produce coefficients to be used in the regression. Experimental data for the compounds in this study generally is unavailable, and estimates, therefore, were taken from force field calculations using the MM2 program,[9] the theory of which has been discussed previously.[10] Figure 5 contains the angles used to calculate the coefficients for angular

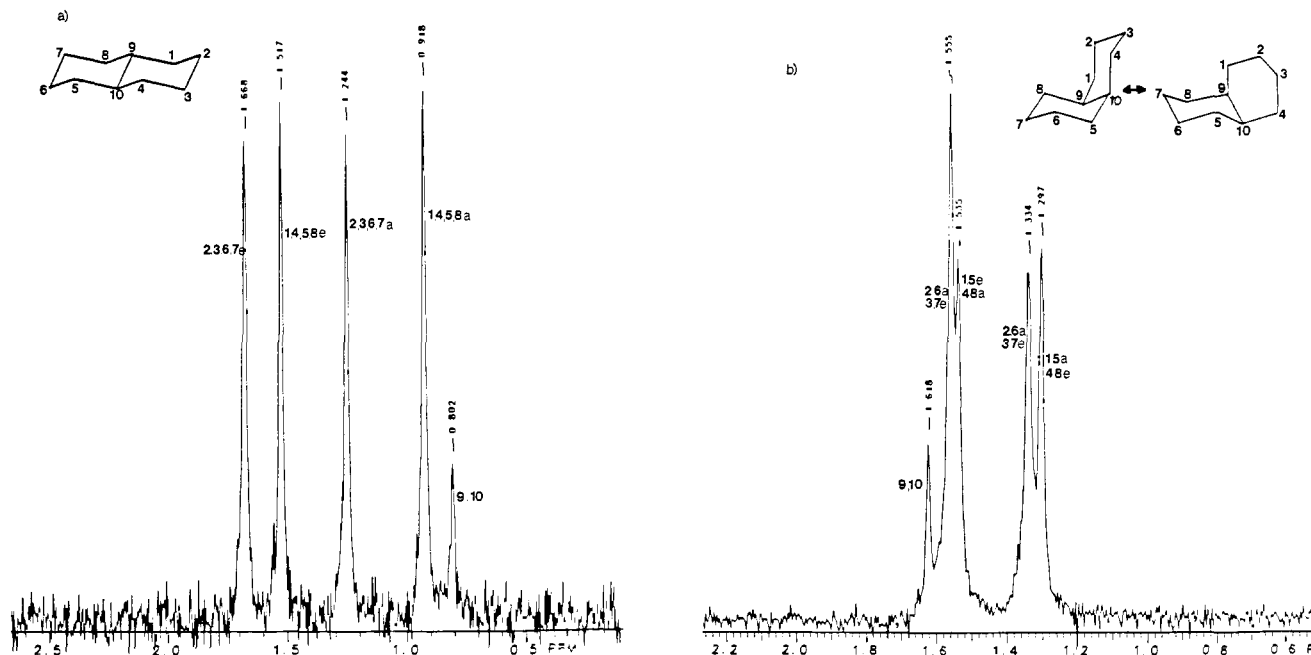(10) Allinger, N. L. *Adv. Phys. Org. Chem.* **1976**, *13*, 1.

Figure 3. Natural abundance deuterium spectra of (a) *trans*-decalin and (b) *cis*-decalin. The peaks in *trans*-decalin are well-resolved. Assignments are from the parameters established in the regression. *cis*-Decalin interconverts rapidly between two equal energy conformations at room temperature. There would be no resolution in a proton spectrum due to second-order coupling effects. Assignments of close resonances are by ordering in the regression.

Table II. Prediction of Chemical Shifts of Methylcyclohexane and 1,*cis*-2,*trans*-3-Trimethylcyclohexane

site	exptl	chair compds[a]	all compds[b]	distortion[c]
		Methylcyclohexane		
1a	1.30	1.32	1.34	1.30
2,6a	0.87	0.88	0.95	0.91
3,5a	1.22	1.24	1.35	1.29
4a	1.12	1.13	1.23	1.18
2,6e	1.64	1.67	1.55	1.64
3,5e	1.66	1.66	1.54	1.67
4e	1.61	1.64	1.51	1.67
estimate of error for this compd	0.017	0.096	0.038
		1,*cis*-2,*trans*-3-Trimethylcyclohexane		
2a	1.20	1.11	1.15	1.24
3a	1.11	1.07	1.17	1.16
4a	0.86	0.86	0.91	0.88
5a	1.32	1.34	1.41	1.37
6a	1.46	1.32	1.42	1.48
1e	1.65	1.95	1.85	1.77
4e	1.48	1.62	1.57	1.54
5e	1.32	1.49	1.47	1.34
6e	1.32	1.38	1.35	1.42
estimate of error for this compd	0.109	0.084	0.053
estimate of error for all compds	0.084	0.081	0.063

[a] Predicted chemical shifts from substitution and average distortion parameters developed by a regression using only pure chair compounds. Assignment is good for pure chair compounds but poor for distorted ones. [b] Predicted shifts from the same parameter set but with all compounds included in the regression. There is improvement in the distorted compounds but loss of accuracy of fit for nondistorted ones. [c] Predicted chemical shifts from parameter set including distortion explicitly. Regression included all compounds. The fit for the entire set of compounds is best with this set.

effect on chemical shifts for 1,*cis*-3,*trans*-5-trimethylcyclohexane and Table III contains the coefficients for the parameters as used in the regression.[11] The results of a stepwise multiple linear regression analysis using all of the shifts produced a parameter set with a multiple correlation coefficient of 0.9816 and a standard error in the estimate of 0.0629 (see Table IV).

(11) Similar data for the other compounds is given as supplementary material.
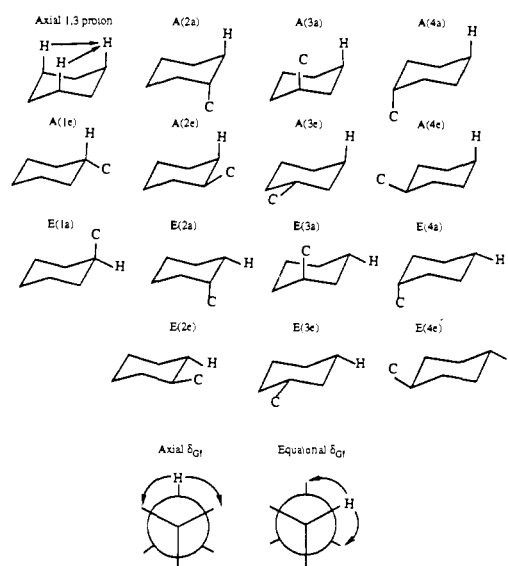

Figure 4. Parameters for a regression including explicit distortion showing important structural features.

Table III. Coefficients for Parameters for 1,*cis*-3,*trans*-5-Trimethylcyclohexane

site	C–H	1e	2a	2e	3a	3e	4a	4e
1,3a	1.7846	1	0	0	1	1	0	0
2a	3.7930	0	0	2	0	0	1	0
4,6a	2.7005	0	1	1	0	0	0	1

site	C–H	1a	2a	2e	3a	3e	4a	4e
2e	2.1256	0	0	2	0	0	1	0
4,6e	2.1197	0	1	1	0	0	0	1
5e	0.0000	1	0	0	0	2	0	0

An examination of the parameters in Table IV clearly demonstrates that the largest effect on the chemical shift of axial sites is vicinal substitution. The three parameters (δ_{G1}, A(2a), and A(2e)) are the largest in magnitude of the axial set. For equatorial sites, remote substitution has a negligible effect on chemical shift as may be observed in Table IV. The utility of the set of parameters in predicting chemical shifts even for highly distorted

Table IV. Parameters for Chemical Shifts of Cyclohexane Ring Systems[a]

axial			equatorial		
param	no. of cases	value	param	no. of cases	value
A(1e)(trans)	23	0.086 ± 0.020	E(1a)(trans)	7	0.206 ± 0.040
A(2e)(cis)	36	−0.122 ± 0.038	E(2e)(trans)	30	0.192 ± 0.034
A(2a)(trans)	18	0.258 ± 0.025	E(2a)(cis)	12	0.055 ± 0.041
A(3e)(trans)	31	0.049 ± 0.017	E(3a)(cis)	33	b
A(3a)(cis)	15	0.092 ± 0.030	E(3a)(trans)	14	−0.199 ± 0.028
A(4e)(cis)	27	−0.057 ± 0.023	E(4e)(trans)	23	b
A(4a)(trans)	11	−0.082 ± 0.030	E(4a)(cis)	9	b
axial 1,3 proton	68	−0.035 ± 0.024	E(no methyls)	1	1.671 ± 0.038
gauche C–H interaction	36	−0.205 ± 0.031			

Statistical Data

multiple R	0.9816
standard error of the estimate	0.0629
size of data set	115
number of fitting param	14

[a] A or E indicates whether the ring site is axial or equatorial. The number and letter in parentheses indicates the relative carbon to which the substituent is attached and whether it is axial (a) or equatorial (e). Parameters using 17 methylcyclohexanes and 2 decalins and including explicit angular information. [b] Failed to enter in the stepwise regressional analysis. Value was therefore zero within statistical error limits.
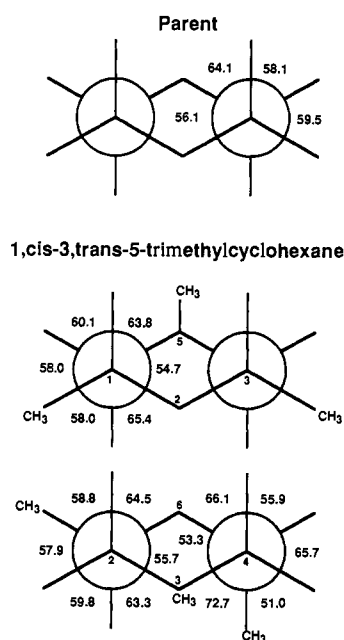

Figure 5. Distortion of dihedral angles by equatorial and axial substitution: (a) parent cyclohexane, (b) 1,*cis*-3,*trans*-5-trimethylcyclohexane. Sites are indicated by number; direction of bond is indicated by atomic labels. Angles are taken from force field calculations.

Figure 6. Plot of 115 experimental chemical shifts versus shifts predicted by a regression including distortion to the ring explicitly. The fit was produced with multiple $R = 0.9816$ and $S = 0.0629$.

compounds can be clearly seen in Figure 6, which is a plot of experimental chemical shifts versus predicted shifts for the entire set of compounds.

Of the 11 largest residuals in the fit, 8 were in sites near to a 1,2 diequatorial substitution (site 1,2 in 1,*trans*-2-; site 4,6a in 1,*trans*-2,*cis*-3-) or a 1,2 axial/equatorial substitution (sites 1,2 and 3,6 in 1,*cis*-2-; sites 1,4e,5,8a and 2,6a,3,7e in *cis*-decalin; sites 1e and 4e in 1,*cis*-2,*trans*-3-) and 2 were related to the presence of an axial *gem*-methyl (site 5e in 1,*cis*-3,*trans*-5- and site 1,3 in 1,*trans*-3-). These 10 large residuals could be explained by simply invoking a slightly larger distortion for deuteriums near sterically crowded centers in these molecules and may reflect the need for slightly modified force field parameters.

The final large residual (site 5e in 1,*trans*-2,*cis*-4-) was in a compound that contained sterically crowded substitutions but was for a deuterium at an equatorial site more remote from the substitution. However, the site is adjacent to an axial methyl. This effect on chemical shift of a ring site remote from the substitution site indicates that while the major distortion in the ring is local, an overall flattening elsewhere in the ring could cause steric shifts throughout the ring. There were no large residuals
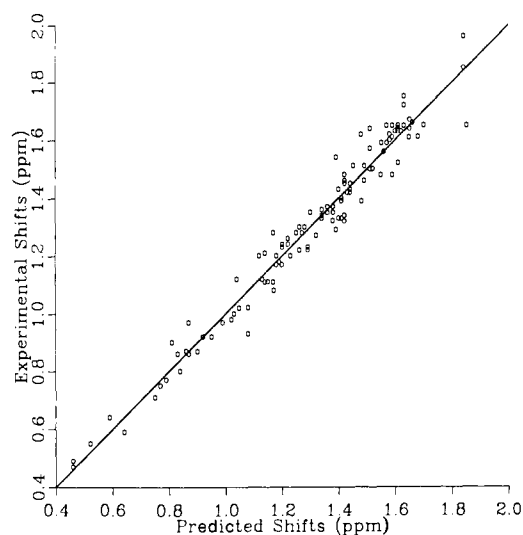
in any compound that did not include some combination of an axial methyl, 1,2 diequatorial carbons, or 1,2 axial/equatorial carbons.

An examination of the force field structures indicated that 1,3 diequatorial substitutions, because they represent only modest perturbations, can be treated essentially linearly as each methyl is considered as an isolated substitution. Such ring distortions from isolated equatorial substitutions are minor. This conclusion is confirmed by the success of assignments in compounds with either 1,3 or 1,4 diequatorial methyls. Large distortions in angles from the force field calculations were associated with axial substitutions or 1,2 diequatorial substitutions. These effects are confirmed by the shift data, although somewhat larger distortions than those resulting from the force field calculations would be preferable to account for the chemical shifts in close spatial proximity to the distorting methyl groups.

Of the methylcyclohexanes with intermediate energy differences between conformations, the shifts in methylcyclohexane and 1,1,4-trimethylcyclohexane are satisfactorily predicted without reference to distortion providing an appropriate ΔE is used. These two compounds had an energy difference of ~1.8 kcal/mol.[4] The other three compounds with intermediate differences (1,1,2-; 1,*trans*-2,*cis*-4-, and 1,*cis*-2,*trans*-3-trimethylcyclohexane) required the distortional parameter set for satisfactory prediction. These three compounds have energy differences of ~0.9 kcal/mol and all three contain 1,2 diequatorial methyls in conjunction with an

axial methyl. The 1,2 diequatorial substitution thus seems to be a factor leading to skew-boat features in the conformation.

V. Conclusion

The development of a new parameter set including gauche interactions, which reflect molecular distortions from idealized geometry, is an improvement over the previous set, which only involved positional methyl substitutions. The new approach is used to predict chemical shifts for the same methylcyclohexanes with chair conformations along with the decalins and methylcyclohexanes with significant steric distortions to the chair conformation. In addition to a wider applicability, the new parameter set uses only 14 parameters, instead of the 18 used in the previous set.

Supplementary Material Available: Tables of coefficients for multiple stepwise linear regression with explicit distortion and associated structures of compounds (24 pages). Ordering information is given on any current masthead page.

15N Chemical Shifts of Backbone Amides in Bovine Pancreatic Trypsin Inhibitor and Apamin

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Abstract: 15N chemical shifts of backbone amides were measured at natural abundance for apamin and bovine pancreatic trypsin inhibitor using heteronuclear multiquantum proton-detected correlated spectroscopy (HMP-COSY). Chemical shift differences from values expected for random-coil peptides were calculated and examined with respect to structural features, including torsion angles and intramolecular hydrogen bonds. A correlation with the torsion angle Ψ_{i-1} was observed for the β-sheet residues. No other dominant factor was found to account for the large (up to ±15 ppm) variation from the model peptides. The results suggest that these 15N shifts are sensitive to nonbonded interactions, though there is no systematic variation with respect to a single structural parameter.

Empirical correlations between molecular structure and 15N chemical shifts continue to pose a challenge.[2,3] An important question is whether 15N chemical shift data can provide information about the environments of backbone amides or side chains in a protein that would contribute to understanding its three-dimensional structure. Although current methods for calculating solution structures of proteins and nucleic acids rely primarily on distances from nuclear Overhauser effects, additional geometric constraints derived from chemical shifts could aid the computation or increase the confidence in the final structures obtained. In addition, the study of molecular interactions will be aided by the rationalization of chemical shift changes that are observed.

Analyses of gramicidin,[4] oxytocin,[5] aluminochrome,[6] vancomycin,[7] and actinomycin[8] for example, have shown that changes in solvent and hydrogen bonding have large effects on 15N chemical shifts. The importance of the latter effect has been also discussed theoretically.[9] A correlation between van der Waals interactions

and chemical shifts of second-row elements is consistent with many β and γ effects.[10] Work on homopolypeptides in the solid state has revealed a small but significant downfield shift of amide signals in β sheets relative to α-helix structures.[11]

The recent application of proton-detected heteronuclear correlated spectroscopy and isotope labeling to proteins[12] has provided a growing list of chemical shift data for backbone amides and side-chain nitrogens. To date, spectra that give at least some residue-type assignments have been obtained from proteins such as bovine pancreatic trypsin inhibitor (BPTI),[13] *Staphylococcus* nuclease,[14] thioredoxin,[15] T4 lysozyme,[16,17] *Salmonella* phage P22 c2 repressor,[18] f1 coat protein,[19] *Anabena* flavodoxin,[20] turkey ovomucoid third domain,[21] λ *cro* repressor, and others.[22] Residues

(1) This paper is dedicated to the memory of the late Emil Thomas Kaiser (1938-1988).

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