Communications to the Editor

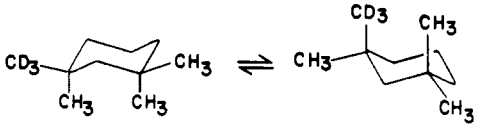
Isotopic Perturbation of Degenerate Conformational Equilibria

Sir:

Deuterium substitution in a compound equilibrating rapidly on the NMR time scale between a number of equivalent structures can cause a significant deviation in their relative populations, and this can lead to a splitting of the averaged resonances which are observed in the unlabelled compound. The splittings provide an accurate method for determining small equilibrium isotope effects. In previous communications, the deuterium isotope effects in some rapidly equilibrating carbonium ion systems were observed using this method.[1-4] In those cases relatively large splittings occur owing to the large chemical shift differences between exchanging nuclei.[5]

Baldry and Robinson[6] have reported a "conformational equilibrium isotope effect" in the *N*-(trideuteriomethyl)-*N*,3,3-trimethylpiperidinium ion, the effect being detected as a barely resolved splitting in the 25-MHz *C*-methyl carbon signal. We have now examined the use of deuterium substitution to lift the degeneracy in the ^{13}C chemical shifts of the methyl groups in 1,1,3,3-tetramethylcyclohexane (I). This molecule is more symmetrical than the *N*,*N*,3,3-tetramethylpiperidinium ion and this created some complications which were resolved by the use of high-field ^{13}C NMR.

The trideuterated compound I-d_3 was made by the 1,4 addition of CD_3MgI to 3,5,5-trimethylcyclohex-2-en-1-one (isophorone) in the presence of Cu_2Cl_2, followed by Wolf-Kishner reduction of the resulting 3,3,5,5-tetramethylcyclohexan-1-one-d_3. This molecule can exist in two different conformations, A and B, which are in rapid equilibrium at room temperature, since $\Delta G^{\ddagger}$ for ring inversion in I is known to be only ~9 kcal/mol.[7,8]



A B

The methyl region of the 63.1-MHz ^{13}C NMR spectrum (proton decoupled) of I-d_3 at 17 °C is shown in Figure 1. The two sharp outer peaks show a chemical shift separation of 0.184 ± 0.006 ppm (11.6 ± 0.4 Hz at 63.1 MHz), and can be assigned to the two methyl groups on C-3. Since the axial methyl groups in I resonate at higher field than do the equatorial methyl groups,[8] the low-field line in Figure 1 must represent the resonance of the C-3 CH_3 group which spends more time in the equatorial than in the axial position. The converse is true for the high-field line. The central broad resonance between the two sharp peaks can be assigned to the methyl group on C-1, the broadening being due to spin coupling with the deuterons of the geminally situated CD_3 group. The *intrinsic* deuterium isotope effect[9,10a] on the chemical shift of this CH_3 carbon is expected to result in an upfield shift. Since the broad resonance is upfield of only one of the two C-3 methyl peaks, its axial–equatorial population ratio must correspond to that of the low-field C-3 methyl group; i.e., the position of the C-1 CH_3 carbon in the absence of an intrinsic deuterium chemical shift isotope effect would be superposed on the low-field C-3 methyl group. Thus, the C-1 CH_3 group spends more time

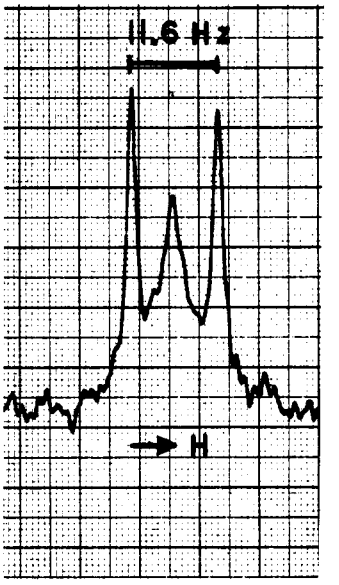


Figure 1. The methyl region of the 63.1-MHz ^{13}C NMR spectrum (protons noise decoupled) of I-d_3 in CS_2 solution at 17 °C.

equatorial than axial, and the converse must be true for the CD_3 group, which is geminally related to the CH_3 group. This proves that B is the favored conformation. The ^{13}C chemical shift of the CD_3 group should be ~0.5 ppm upfield from that of the CH_3 groups, but the CD_3 carbon would not be visible in the spectrum because its signals are expected to be very weak.[11]

The splitting observed for the C-3 methyl resonances is not the result of an intrinsic deuterium isotope effect of the axial CD_3 group on the axial CH_3 group. Even in molecules where the steric compression is much larger than in B, no intrinsic deuterium isotope effect on ^{13}C chemical shifts can be observed as long as there are at least four bonds separating the deuteron(s) from the ^{13}C nucleus.[10]

Unlike the doublet observed for the two *C*-methyl groups in the piperidinium ion,[6] the CH_3 groups in I-d_3 give rise to three signals (Figure 1). The presence of the central methyl peak hinders the identification of the outer peaks at low spectrometer frequencies (e.g., 20 MHz).

The ratio of B:A can be obtained from the NMR spectrum by using the relation[4]

$$K = [B]/[A] = (\Delta + \delta)/(\Delta - \delta)$$

where Δ is the chemical shift difference in the *frozen* equilibrium of B and A and δ is the observed chemical shift difference between the two methyl carbons. In the present case Δ is 9.03 ppm (measured at −100 °C in CS_2) and δ is 0.184 ppm giving an equilibrium constant of 1.042 ± 0.001.[12] The free-energy difference between conformers A and B is thus 24 ± 1 cal/mol.

The fact that the molecule prefers to have the deuterated methyl group in the axial position might be expressed in simple terms by saying that the CD_3 group acts as if it were "smaller"[13-15] than the CH_3 group. This type of effect has been referred to as a "steric isotope effect".[16] A more fundamental description is that the potential surface for hydrogen vibration

0002-7863/80/1502-3945$01.00/0

in the methyl group is perturbed by the 1,3 diaxial interaction, thus increasing the vibrational frequency and hence the zero point energy of methyl groups in the axial position. The CD_3 group has a lower zero point energy and thus is raised *less* in energy than the CH_3 group. If methyl groups in the equatorial position are unperturbed, as is likely, the energy difference between the conformations is simply due to this factor.[17-18]

It is important to be aware of the possible presence of conformational equilibrium isotope effects when using intrinsic chemical shift isotope effects for assignment of ^{13}C resonances. While rigid molecules pose no problem, systems in which deuterium substitution either breaks the conformational degeneracy, or perturbs a nondegenerate equilibrium, can potentially show a conformational equilibrium isotope effect, as well as an intrinsic chemical shift isotope effect.[19]

Acknowledgements. We thank the National Science Foundation for the support of this work.

References and Notes

(1) Saunders, M.; Jaffe, M. H.; Vogel, P. *J. Am. Chem. Soc.* **1971**, *93*, 2558.
(2) Saunders, M.; Vogel, P. *J. Am. Chem. Soc.* **1971**, *93*, 2561.
(3) Saunders, M.; Vogel, P. *J. Am. Chem. Soc.* **1971**, *93*, 2559.
(4) Saunders, M.; Telkowski, L.; Kates, M. R. *J. Am. Chem. Soc.* **1977**, *99*, 8070.
(5) Saunders, M.; Hagen, C. L.; Rosenfeld, J. *J. Am. Chem. Soc.* **1968**, *90*, 6882. The shift is estimated from the *tert*-amyl cation to be 2.1 ppm in the ^1H NMR.
(6) Baldry, K. W.; Robinson, M. J. T. *J. Chem. Res. (S)* **1977**, 86; *J. Chem. Res. (M)* **1977**, 1001.
(7) Friebolin, H.; Schmid, H. G.; Kabuss, S.; Faisst, W. *Org. Magn. Reson.* **1969**, *1*, 147.
(8) Doddrell, D.; Charrier, C.; Hawkins, B. L.; Crain, W. O.; Harris, L.; Roberts, J. D. *Natl. Acad. Sci. U.S.A.* **1970**, *67*, 1588.
(9) Saunders, M.; Kates, M. R. *J. Am. Chem. Soc.* **1977**, *99*, 8071.
(10) (a) Anet, F. A. L.; Dekmezian, A. *J. Am. Chem. Soc.* **1979**, *101*, 5449. (b) The ^{13}C NMR spectra of I and I-d_3 were also measured under conditions[8] so that ring inversion is slow on the NMR time scale (−110 °C). The spectrum of I-d_3 at −110 °C gave the expected spectrum. The resonances of the axial and equatorial CH_3 groups on C-1 were not resolved from the corresponding resonances of the CH_3 groups on C-3. The intensities of the axial and equatorial CH_3 groups on C-1 should differ by 8%. To measure such an intensity difference reliably, a very high signal-to-noise ratio is required, and the small amount (50 mg) of I-d_3 available for study precluded this. Furthermore, if the C-1 and C-3 methyl resonances are not resolved at low temperatures, as actually happens, the predicted intensity differences between the axial and equatorial CH_3 resonances falls to only 4%. This shows that the detection of the equilibrium isotope effect in I-d_3 by direct intensity measurements at low temperatures is very much more difficult than by chemical shift measurements, even though the latter are obtained at much higher temperatures, where the equilibrium constant becomes very close to 1.
(11) The CD_3 carbons should have a very long T_1 relaxation time. Since the interval between pulses (tip angle of ~40°) was 2 s, these carbons should show the effects of saturation. In addition, the nuclear Overhauser effect may be small and coupling to the deuterons will result in the resonance being a multiplet. All of these factors greatly reduce the signal-to-noise ratio of the signal.
(12) The error in the equilibrium constant includes a possible temperature dependence of Δ between −100 and 17 °C of ±0.1 ppm.
(13) Brown, H. C.; McDonald, G. J. *J. Am. Chem. Soc.* **1966**, *88*, 2514.
(14) Bartell, L. S. *J. Am. Chem. Soc.* **1961**, *83*, 3567.
(15) Bartell, L. S. *Tetrahedron Lett.* **1960**, *6*, 13.
(16) Carter, R. E.; Melander, L. *Adv. Phys. Org. Chem.* **1973**, *10*, 1.
(17) Wolfsberg, M. *Annu. Rev. Phys. Chem.* **1969**, *20*, 449-478.
(18) Van Hook, W. A. *ACS Monogr.* **1970**, No. 167, 1-84.
(19) Some apparently puzzling effects of deuterium substitution on ^{13}C chemical shifts undoubtedly have their origin in conformational equilibrium isotope effects: 2-methylcyclohexanone-*2,6,6*-d_3 (Wehrli, F. W.; Wirthin, T. "Interpretation of Carbon-13 NMR Spectra"; Heydon: New York, 1976; p 108); cyclodecanone-*2-d* (Wehrli, F. W.; Jeremić, D. J.; Mihailović, M. L.; Milosavljević, S. *J. Chem. Soc., Chem. Commun.* **1978**, 302). An explanation for the shifts in the latter compound in terms of a conformational equilibrium isotope effect has been given: Andrews, G. C.; Chmurny, G. N.; Whipple, E. B., personal communication. See also ref 10a.

Frank A. L. Anet,* V. J. Basus
Department of Chemistry, University of California
Los Angeles, California 90024

A. P. W. Hewett, Martin Saunders*
Department of Chemistry, Yale University
New Haven, Connecticut 06520
Received December 26, 1979

Interaction of an Iron Macrocyclic Complex with Apohemoglobin and Apomyoglobin

Sir:

For many years, attempts have been made to prepare iron complexes which could react reversibly with dioxygen. One of the main objectives of these efforts was to establish the mode of binding of the oxygen molecule to the iron atom. The realization that Fe(II) complexes undergo irreversible oxidation via a dioxygen bridged dimer, Fe^{II}-O-O-Fe^{II}, have led Baldwin[1] and Collman[2] independently to a successful solution to this problem. They reasoned that the dimerization can be impeded by a sterically hindered ligand and, thus, in Baldwin's case, that the steric hindrance was built into the periphery of a macrocyclic ligand, while Collman prepared the now famous "picket fence" porphyrin. It was later realized that the steric hindrance is not obligatory for the reversible uptake of dioxygen and that simple iron porphyrins, such as Fe^{II}-TPP, can also bind dioxygen reversibly at low temperature.[3]

We thought that it would be of interest to use globin as the sterically hindered environment and investigate its interaction with relatively simple macrocyclic iron complexes. We reasoned that, if such complexes could enter the heme cavity, then, with the right choice of iron macrocycle, reversible oxygenation would take place. In this communication, we report the interaction of human apohemoglobin and horse heart apomyoglobin with the iron complex of the macrocyclic ligand, 5.14-dihydrodibenzo[*b,i*][5.9.14.18]tetraaza[14]annulen (L)[4,5] (Figure 1).

Refluxing stoichiometric amounts of L and $Fe(CH_3COO)_2$ in DMF led to the isolation of the red brown complex Fe(L)-(CH_3COO).[6] Apohemoglobin and apomyoglobin were prepared from human hemoglobin and horses heart myoglobin respectively, by the acid-butanone method.[7] The protein preparations were checked by reconstitution and reaction with dioxygen.

The electronic spectra of Fe(L)(CH_3COO) and its imidazole adduct are shown in Figure 2. Addition of the complex dissolved in a minimum amount of DMF to an aqueous solution of globin gave rise to the electronic spectra depicated in

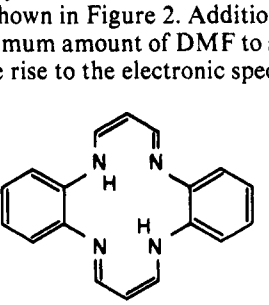


Figure 1. The macrocyclic ligand used in the study.

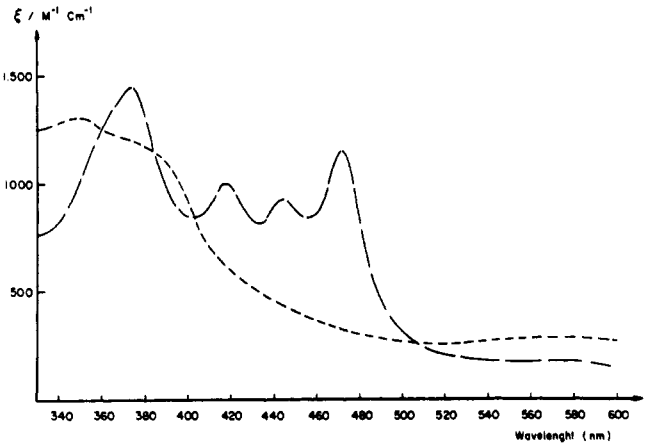


Figure 2. Visible spectra of FeL^+ (- - -) of its imidazole adduct (— —) in DMF.

0002-7863/80/1502-3946$01.00/0