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(11) These studies would have taken several man-years by manual methods (literature searching, punching coordinates, and checking errors) but required only ~10 min computer time altogether on an IBM 370/165.
(12) Geometrical correlations have been linked to the potential energy surface of molecules (ref 13). A similar argument has been used by Brown[14] who plotted O—H---O interactions and suggested that the high frequency of occurence of linear arrangements corresponds to a potential minimum.
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(16) In several cases in Figure 1a the iodine makes contact with two oxygen atoms of a neighboring molecule (e.g., an ester).

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Small Errors in C-H Bond Lengths May Cause Large Errors in Rotational Correlation Times Determined from Carbon-13 Spin-Lattice Relaxation Measurements

Sir:

Measurements of spin-lattice relaxation times (T_1) of resonances of proton-bearing carbons in proton-decoupled ^{13}C NMR spectra have been used extensively to study rotational motions of large molecules in solution.[1,2] Because ^{13}C-^1H dipole-dipole interactions with directly bonded hydrogens provide an overwhelmingly dominant relaxation mechanism[2] (even at high magnetic field strengths[3]), it is necessary to know the values of the pertinent carbon-hydrogen bond lengths (r_{CH}) in order to extract values of rotational correlation times (τ_R) from the measured T_1 values.[1,2] In this report we show that the widespread practice of setting r_{CH} = 1.09 Å (a typical value obtained from rotational spectroscopy) can cause very large errors in the value of τ_R. We show that small errors (2-3%) in the assumed value of r_{CH} can result in values of τ_R in error by as much as a factor of 2. This sensitivity of τ_R to the choice of r_{CH} is caused by the *combined* effects of the dependence of $1/T_1$ on r_{CH}[6] and the nonlinear relationship between $1/T_1$ and τ_R (when dealing with large molecules at typical magnetic field strengths; see Figure 1).[4] We show that the temperature dependence (at 14.2 kG) of the T_1 values and nuclear Overhauser enhancements (NOE) of the α-carbon resonances of aqueous hen egg-white lysozyme can be used to determine τ_R *and* r_{CH}. We discuss the choice of magnetic field strength for maximizing the accuracy of τ_R when r_{CH} is not known accurately. We show that the reported discrepancies between τ_R values measured at 14.2 and 63.4 kG[5] are eliminated (without invoking anisotropic rotations[5] or internal librational motions[6]) when the corrected value of r_{CH} is used.

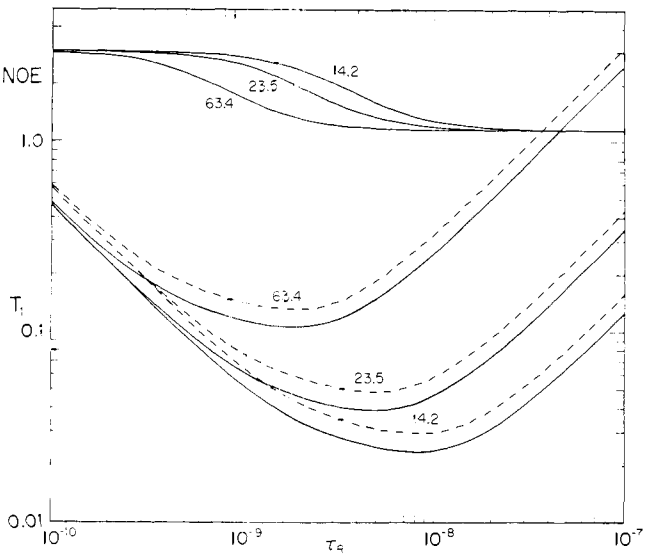

Figure 1. Semilog plots of theoretical T_1 (in seconds) and NOE (ratio of intensities with and without proton decoupling) vs. τ_R (in seconds) for a ^{13}C spin undergoing ^{13}C-^1H dipole-dipole relaxation, in the case of isotropic rotational reorientation and under conditions of proton decoupling.[4] Plots are given for various magnetic field strengths, indicated in kilogauss. The NOE values are independent of the choice of the carbon-hydrogen distance.[4] The T_1 plots were computed for a C-H group with r_{CH} = 1.09 (solid curves) or 1.13 Å (dashed curves).

The appropriate choice of r_{CH} for the interpretation of ^{13}C relaxation data is the average $\langle r^{-3}\rangle^{-1/3}$ for the vibrational ground state.[7] Only a few determinations of rotational motions from ^{13}C T_1 measurements have incorporated vibrational corrections for r_{CH}.[7,8] These estimates indicate that $\langle r^{-3}\rangle^{-1/3}$ may be 1-2% greater than r_{CH} values based on rotational spectra or electron diffraction data.[7,8] In addition, results from NMR spectroscopy in liquid crystal solvents (which yields distance parameters related to $\langle r^{-3}\rangle^{-1/3}$) suggest that, at least in some cases, the vibrational correction may be >2%.[9] Furthermore, the reported frequency dependence of some ^{13}C T_1 values has been used to suggest a value as large as 1.15 Å for the C^α-H bond length in some peptides.[10]

Consider a carbon (with a single directly bonded hydrogen) which is part of a molecule undergoing isotropic rotational reorientation with a correlation time τ_R. Figure 1 shows theoretical plots of T_1 and NOE vs. τ_R (in a range of τ_R values expected for molecular weights between 10^3 and 10^6), at 14.2, 23.5, and 63.4 kG.[4] The NOE values are independent of the choice of r_{CH}. For the T_1 computations, r_{CH} values of 1.09 (solid lines) and 1.13 Å (dashed lines) were used. Let us assume that the true values of r_{CH} and τ_R are 1.13 Å and 8 ns, respectively. Then the measured T_1 at 14.2 and 63.4 kG will be 29.6 and 270 ms, respectively. If we use r_{CH} = 1.09 Å in the interpretation of the data, Figure 1 will yield τ_R values of 18 ns (14.2 kG) and 10 ns (63.4 kG).[11] Clearly, if the true $\tau_R \approx$ 8 ns, an erroneous choice of r_{CH} is much more serious for T_1 data at 14.2 kG than for data at 63.4 kG. In general, measured T_1 values which are near the minimum of the T_1 vs. τ_R curve may yield very erroneous values of τ_R, unless r_{CH} is known with great accuracy. The minimum in the T_1 curves occurs at τ_R values of ~2, 5, and 8 ns at 63.4, 23.5, and 14.2 kG, respectively. Values of τ_R in the range 1-10 ns are expected for many substances (in "nonviscious" solvents at room temperature) which have molecular weights in the range 2000 to 20 000.[4,10,12] Clearly, when ^{13}C spin-lattice relaxation times are used for determining the τ_R values of such molecules, it is desirable to choose a magnetic field strength which does not place T_1 near the minimum in the T_1 vs. τ_R plot.

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Table I. α-Carbon Spin-Lattice Relaxation Times and Nuclear Overhauser Enhancements at 14.2 kG for Aqueous Hen Egg-White Lysozyme[a]

temp, °C	T_1, s[b]	NOE[c]	temp, °C	T_1, s[b]	NOE[c]
22	27.9	1.43	44	26.3	1.68
25	27.4	1.45	46	28.3	1.87
29	29.0	1.42	49	26.2	1.60
35	30.9	1.62	50	28.8	1.74
37	28.7	1.51	57	28.7	1.72
40	28.9	1.55	62	32.5	1.96

[a] Hen egg-white lysozyme (six times crystallized) was obtained from Miles Laboratories, Elkhart, Ind. The sample was 7 mM protein in H_2O (0.1 M NaCl). At temperatures up to 50 °C, the pH was adjusted to ~3.0, a value low enough to prevent appreciable self-association (R. S. Norton and A. Allerhand, *J. Biol. Chem.*, **252**, 1795 (1977), and references cited therein), but high enough to avoid unfolding of the protein. The pH was raised to 5.0 for measurements above 50 °C. Carbon-13 NMR spectra were obtained essentially as described by E. Oldfield, R. S. Norton, and A. Allerhand, *ibid.*, **250**, 6381 (1975). [b] T_1 values were measured from partially relaxed Fourier transform (PRFT) NMR spectra.[2] Typically, eleven PRFT spectra were used at each temperature, and the observed intensities of the α-carbon envelope were analyzed with the use of an exponential fit (see M. Sass and D. Ziessow, *J. Magn. Reson.*, **25**, 263 (1977)). Estimated random error is ±10%. [c] We define the NOE as the ratio of intensities with and without proton decoupling. The NOE values were measured with the use of gated proton decoupling (see R. Freeman, H. D. W. Hill, and R. Kaptein, *ibid.*, **7**, 327 (1972)). Estimated random error is ±10%.

We now show that measurements of T_1 values at or near the minimum of the T_1 vs. τ_R curve can be used for estimating the proper value of r_{CH} (for use in the analysis of T_1 data at any magnetic field strength), if the corresponding NOE values are also measured. In Table I we show the temperature dependence of the T_1 and NOE values (at 14.2 kG) of the α carbons of 7 mM hen egg-white lysozyme in H_2O (with 0.1 M NaCl).[13] The NOE value gradually increases from ~1.4 at 22 °C to ~2.0 at 62 °C. On the basis of the NOE curve for 14.2 kG (Figure 1) we conclude that τ_R changes from about 7 ns at 22 °C to ~3 ns at 62 °C. For this range of τ_R values, T_1 (at 14.2 kG) should be nearly invariant and very close to the minimum T_1 (see Figure 1). Indeed, the experimental T_1 values are essentially independent of temperature (Table I). We estimate that the minimum in the measured T_1 is ~28 ms (see Table I), which is considerably larger than the expected minimum T_1 of 23.8 ms if r_{CH} = 1.09 Å, and slightly lower than the expected minimum T_1 of 29.6 ms if r_{CH} = 1.13 Å (Figure 1). We shall now show that our data (Table I) are most consistent with an r_{CH} value of ~1.11 Å (for the α carbons of a protein).

Figure 2 shows a semilogarithmic plot of the measured NOE at 14.2 kG vs. the reciprocal of the absolute temperature (closed circles). From these measured values, the NOE curve for 14.2 kG in Figure 1 yielded values of τ_R (shown as open circles in Figure 2). In order to improve the precision of the temperature dependence of τ_R, these τ_R values were fitted to an Arrhenius equation, which yielded the dashed straight line in Figure 2 (with an activation energy of 3.4 kcal/mol). The τ_R values given by the Arrhenius equation were then used to compute T_1 and NOE values (shown as solid lines in Figure 2). The spin-lattice relaxation times were computed for several values of r_{CH}. The experimental T_1 values (closed triangles in Figure 2) are in much better agreement with the computed curve for r_{CH} = 1.11 Å than with the one for 1.09 Å. The best-fit value of r_{CH} is 1.111 Å (with an estimated precision of 0.005 Å).

The α-carbon T_1 values of aqueous lysozyme at 63.4 kG are 208 ms at 29 °C and 149 ms at 48 °C.[14] These T_1 values yield correlation times of 6.6 and 4.1 ns, respectively, if we set r_{CH}

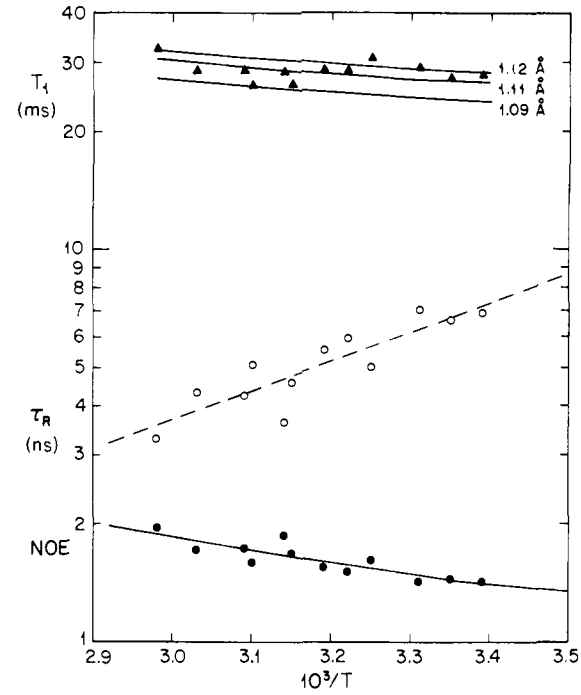

Figure 2. Semilog plots of T_1 (in milliseconds), τ_R (in nanoseconds), and NOE vs. the reciprocal of the absolute temperature, for aqueous hen egg-white lysozyme (see footnote *a* of Table I). The experimental T_1 (closed triangles) and NOE values (closed circles) are those of the α-carbon envelope at 14.2 kG (see Table I). The open circles are τ_R values determined from the experimental NOE values. The dashed line is the Arrhenius fit for the τ_R values. The NOE and T_1 curves were calculated (at 14.2 kG) with the use of the τ_R values given by the dashed line. The T_1 curves were computed for a C-H group, with r_{CH} values of 1.09, 1.11, and 1.12 Å.

= 1.11 Å (7.6 and 4.9 ns if r_{CH} = 1.09 Å). These values are in excellent agreement with those determined from our low-field NOE data (6.3 and 4.4 ns, respectively, from the dashed line in Figure 2). Furthermore, although the T_1 data at 14.2 kG (Table I) cannot be used for accurate determinations of τ_R, the T_1 value of ~27–29 ms in the temperature range 20–50 °C yields a τ_R of ~4–14 ns if we take r_{CH} = 1.11 Å, but a τ_R of ~15–18 ns if we choose r_{CH} = 1.09 Å. Clearly, the choice of r_{CH} = 1.11 Å removes the discrepancy between the low-field and high-field results for hen egg-white lysozyme.[5] Furthermore, this choice of r_{CH} yields τ_R values which are in fairly good agreement with the reported value of 10.0 ± 0.5 ns at 20 °C based on depolarized light-scattering experiments.[15] (Figure 2 yields a τ_R of 7.5 ns at 20 °C). Note that we have assumed that the isotropic rigid rotor model is applicable to the C^{α}–H vectors of native lysozyme. Under this assumption, the use of r_{CH} = 1.11 Å (but not 1.09 Å) gives us correlation times which are internally consistent (at 14.2 and 63.4 kG) and also consistent with results based on depolarized light-scattering experiments. Although we cannot rule out the possibility that the presence of fast internal motions and/or anisotropic overall rotation would bring r_{CH} = 1.09 Å into consistency with the experimental data, our belief that the isotropic rigid rotor model is valid (for the analysis of the relaxation behavior of the α-carbon resonances of native lysozyme) is reinforced by published evidence that $\langle r^{-3}\rangle^{-1/3}$ should be $\gtrsim$1.10 Å.[7-9] In any case, our conclusion that a very small error in the choice of r_{CH} greatly affects the accuracy of correlation times obtained from T_1 values which are near the minimum of the T_1 vs. τ_R curve is based on theoretical considerations (Figure 1) and is not affected by any departure from isotropic behavior of the lysozyme backbone.

The use of r_{CH} = 1.09 Å has been so widely accepted that many authors have not even bothered to indicate what value

of r_{CH} was used to interpret their ^{13}C T_1 data.[1,2] We hope that the results presented in this report will lead to a general re-evaluation of the choice of r_{CH} in ^{13}C relaxation studies.

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(13) See footnotes of Table I for details about the protein sample and the NMR measurements at 14.2 kG.
(14) Sample was 6.4 mM protein in H_2O (with 0.1 M NaCl) at pH 3.1. ^{13}C NMR measurements at 63.4 kG were carried out essentially as described in ref 5, except that a 15-mm probe (instead of the previous 10-mm probe) and quadrature detection were now used, and the number of accumulations per spectrum was increased from 8192 to 16 384 (and 32 768 for some spectra).
(15) See footnote 8 of D. R. Bauer, S. J. Opella, D. J. Nelson, and R. Pecora, *J. Am. Chem. Soc.*, **97**, 2580 (1975).

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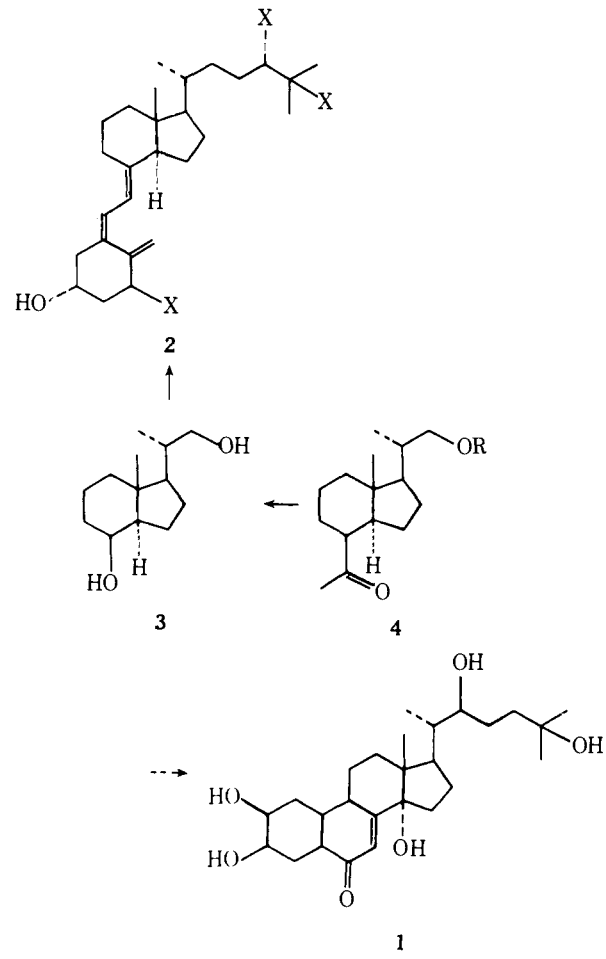
A Stereocontrolled Approach toward Vitamin D Metabolites. A Synthesis of the Inhoffen–Lythgoe Diol

Sir:

Steroids and their transformation products possessing modified side chains such as the molting hormones (e.g., ecdysones **1**)[1] and the metabolites of vitamin D_3 (e.g., **2**)[2] have spurred much research concerned with the synthesis of systems incorporating a functionalized side chain.[3,4] The vitamin D_3 problem is further complicated by conversion of the steroid nucleus into the seco system. Of the synthetic approaches, only Lythgoe's[5] and more recently Okamura's[6] directly construct the conjugated triene system. Thus, a strategy which produces a C,D unit incorporating the asymmetric center at C(20) (steroidal numbering) and which is suitably functionalized for elaboration into these biologically important systems as well as various analogues appears to represent a useful synthetic goal. We describe a synthesis of such a unit, e.g., **4**, which, by conversion into the Inhoffen–Lythgoe diol **3**, effects a formal total synthesis of both vitamin D_3 and some of its metabolites.[5]



As outlined in Scheme I, the starting material for the sequence is the hydroxy ketal **5**, which is readily available in 45% overall yield from 3-carboxytricyclo[2.2.1.$0^{2,6}$]heptan-5-one.[7] The sulfone **6**[8] is prepared from the iodide by displacement with sodium benzenesulfinate.[9] Conversion into its anion in a THF–HMPA mixture and then addition of a 10% (w/v) solution of ethylene oxide in ether led to the hydroxyethylated product **7**.[8] Desulfonylation with $Li/C_2H_5NH_2$ was plagued by double-bond reduction and with unbuffered Na(Hg)[10] by formation of undefined byproducts, whereas desulfonylation by 6% Na(Hg) buffered with Na_2HPO_4 proceeded quantitatively.[11] The desulfonylated product was directly converted into the THP derivative of the bishomologated hydroxy ketone **8**.[8] Methylation proceeds highly stereoselectively from the less hindered endo face to give **9**[8] (>30:1). NMR shows a single doublet, δ 1.01 for **9**, whereas the exo methyl isomer, obtained by partial equilibration of **9**, shows this methyl group at δ 1.20. At this point, all four critical centers corresponding to C(13), C(14), C(17), and C(20) in steroid numbering have been created with the correct relative configuration.

Baeyer–Villiger oxidation using basic hydrogen peroxide gives initially the unrearranged hydroxy acid which upon direct subjection to TsOH in benzene is converted into the rearranged lactone **10**:[8] IR 1780, 1655 cm^{-1}; NMR δ 5.43 (d, J = 7 Hz, 1 H), 4.55 (br S, 1 H), 1.28 (d, J = 7 Hz, 3 H), 1.11 (s, 3 H). After reduction to the diol, reaction with 1 equiv of *tert*-butyldimethylsilyl chloride allowed selective protection of the primary alcohol. Transposition of the chirality of the allyl alcohol unit to an allylic inverted carbon unit envisioned the use of allylic alkylation via organopalladium chemistry[12,13] or [3.3]-sigmatropic rearrangement. Attempts to effect a modified Claisen rearrangement (Claisen–Johnson[14] ortho ester or Claisen–Ireland–Arnold[15] enolate) failed presumably because of steric crowding. On the other hand, the much less

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