$$\Delta O = \frac{1}{\pi T_{2,obsd}} = \frac{1}{\pi}\left(\frac{1}{T_{2f}} + \frac{P}{T_{2b} + \tau}\right) \quad (2)$$

Depending on the relative values of the lifetime of the bound nucleotide (τ) and the transverse relaxation times of free and bound nucleotides (T_{2f} and T_{2b}, respectively), plots of ΔO vs. P may allow calculation of T_{2b} or τ.

Figure 1 shows the ^{17}O NMR signal of [β-$^{17}O_2$]ATP titrated with small percentages of AK purified from porcine muscle.[11] The successive line broadening is consistent with eq 2, whereas the Lorentzian line shapes suggest that the broadening is not due to chemical exchange and that the condition $\omega^2\tau_c^2 << 1$ is met.[8] Figure 2 shows the plots of ΔO vs. P for [γ-$^{17}O_3$]ATP, [β-$^{17}O_2$]ATP, and [α-$^{17}O_2$]ATP. In all cases the ΔO increases upon the first addition of AK due to an increased viscosity. With further addition of the enzyme solution, the change in viscosity is less significant,[12] yet the signals of all three samples of ATP (without Mg^{2+}) show an approximately linear increase in ΔO. The result of [γ-$^{17}O_3$]ATP has been confirmed by titrating an enzyme solution with a negligible volume of ATP solutions, which rules out the viscosity change as the predominant factor in causing the line broadening. At the end of each experiment, neither hydrolysis of ATP nor deactivation of AK was detectable.

The slopes of the plots of the ΔO of ATP represent $1/\pi(T_{2b} + \tau)$. The fact that the slopes are *different* for α-, β-, and γ-^{17}O of ATP suggests that T_{2b} instead of τ is the predominant factor in causing the line broadening; i.e., $\tau << T_{2b}$. The slopes are 2500, 1950, and 900 Hz for α-, β-, and γ-^{17}O of ATP, respectively. The ΔO_f, obtained from the signals of free ATP after correcting for 100 Hz of line broadening and 20 Hz of inhomogeneity, is 690, 570, and 355 Hz for α-, β-, and γ-^{17}O of ATP, respectively. Although the slopes may be considered as ΔO_b, it is more accurate to obtain ΔO_b by extrapolating the linear curves in Figure 2 to 100% AK, particularly when ΔO_f is not negligible compared to ΔO_b. The ΔO_b values thus obtained (after correction) are 3200, 2520, and 1250 Hz for α-, β- and γ-^{17}O of ATP, respectively.

If it is assumed that the differences between ΔO_f and ΔO_b are mainly due to changes in τ_c, the results suggest the τ_c of the ^{17}O of ATP increases by only a factor of 3–5 upon binding to AK. The values could be as high as 10–20 if there is a decrease in e^2qQ/h due to H-bonding or other reasons.[13,14] Since the increase in τ_c should be in the order of 10^2 if binding is rigid,[15] the triphosphate moiety of ATP most likely has appreciable internal rotational freedom in the AK–ATP binary complex. This is consistent with the previous finding that the ^{31}P NMR properties of ATP without (Mg^{2+}) change only slightly upon binding with AK[6a] and suggests that the phosphate moiety of bound ATP may interact only weakly with the enzyme in the absence of Mg^{2+}.[16]

A major concern in the use of ^{17}O NMR in macromolecular systems is that the simple eq 1 is no longer valid when $\omega\tau_c \geq 1$.[8,10b,17] This seems to be less of a problem for small enzymes such as AK. The upper limit of $\omega\tau_c$ is ca. 1 at 40 MHz if the substrate assumes the same τ_c as the enzyme molecule (15–20 ns).[18] In many cases,[3] such as the one described in this paper, the bound substrate can assume a certain degree of rotational freedom (relative to the enzyme) and justifies the use of eq 1. The requirement that is hard to meet seems to be the "fast exchange limit" ($\tau << T_{2b}$). Although it is the case for ATP, we have found that the ΔO of [γ-$^{17}O_3$]MgATP and [$^{17}O_3$]AMP are less sensitive to additions of AK, which suggests that the latter two cases are probably in the "slow exchange limit" due to a long τ, a short T_{2b}, a large chemical shift change,[8,9] or a combination of these.

(9) As pointed out in ref 8, another requirement for eq 2 to be valid is that the difference in chemical shift between the free and the bound state ($\Delta\delta$) is less than $1/T_{2b}$. The ^{17}O chemical shifts of nucleotides rarely change by >50 ppm.[10]

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(12) A small volume of ATP solution was used in order to minimze changes in viscosity. According to the sample conditions described in Figure 1, the concentrations of AK at 2% and 10% (relative to ATP) were 0.42 and 0.82 mM, respectively. Thus, the viscosity change should show a "quantum increase" at 2% AK and become negligible later.

(13) The nuclear quadrupolar coupling constant of ^{17}O could decrease for two possible reasons. The first is hydrogen bonding between the phosphate moiety of ATP and the active site residues. A model for the H-bonding effect is that the e^2qQ/h value for $H_2{}^{17}O$ is 10.17 MHz in the gas phase and 6.525 MHz in ice.[14a] The effect of H-bonding on the quadrupolar coupling constant of ^{17}O has been studied in detail.[14b] The second possible reason is that the charges on the phosphate of ATP are localized (to O=P—O^-) due to ionic interactions with positive residues at the active site and that only the signal due to P=^{17}O is being observed (the other, P—$^{17}O^-$, may not be observable due to much shorter relaxation times). A model for this is $(PhO)_3PO$, where the e^2qQ/h values are 3.825 MHz for P=^{17}O and 9.176 MHz for P—^{17}O—Ph (average of three values[14c]). The e^2qQ/h values for KH_2PO_4 are 4.85–5.96 MHz.[14d]

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(16) The dissociation constants of the AK complexes with ATP, GTP, and PPP (without Mg^{2+}) are 35, 1200, and 240 μM, respectively.[6c] This suggests that the adenine moiety is important in binding. The triphosphate also binds to AK, but it is not known whether its binding is limited to the ATP site or it crosses both AMP and ATP sites.

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Axial and Equatorial 4-*tert*-Butyladamantan-2-ones. Synthesis, Circular Dichroism, and Mass Spectra[1]

David A. Lightner* and W. M. Donald Wijekoon

Department of Chemistry, University of Nevada Reno, Nevada 89557-0020

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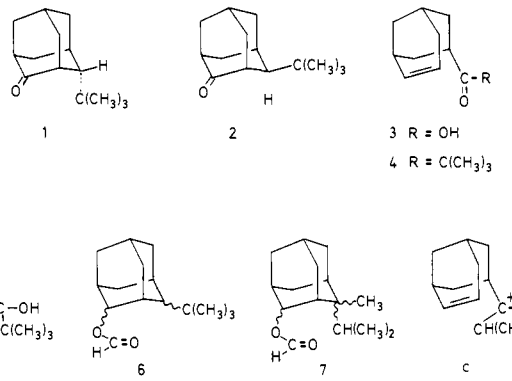
Our interest in unusual (anti-octant[2]) effects in the circular dichroism (CD) spectra of saturated alkyl ketones led us to reexamine[3] the previously reported[4] solvent-dependent anti-octant effects seen with 4(a)-methyl-adamantan-2-one and to extend our investigations to the corresponding *tert*-butyladamantan-2-one. Both 4(a)- and 4(e)-*tert*-butyladamantan-2-one (**1** and **2**) were



unknown at the time of our work, and they are of special interest because (1) no chair cyclohexanones have been prepared with a β-axial *tert*-butyl group and (2) the *tert*-butyl substituent exhibits the same general symmetry as the methyl group but is more

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extended in space (and thus should penetrate farther into front octants[2]). Both 5-*tert*-butyl-[5] and 1-*tert*-butyladamantan-2-one[6] have been reported recently, but their *tert*-butyl groups can assume only the *equatorial configuration with respect to all rings*. 4-*tert*-Butyladamantan-2-one, in marked contrast, exhibits the interesting configurational complementarity of simultaneous axial and equatorial configurations; viz., the substituent is axial in the cyclohexanone ring and equatorial in the cyclohexane ring or equatorial in the cyclohexanone ring and axial in the cyclohexane ring. The only other reported *tert*-butyladamantane exhibiting a similar complementary relationship is 2-*tert*-butyladamantane,[7] in which the *tert*-butyl group has an axial configuration in one cyclohexane ring and an equatorial configuration in the other.

We synthesized **1** and **2** by modifying our previously successful approach to the synthesis of 4-methyladamantan-2-ones and other 4-substituted adamantan-2-ones.[3,8] Thus (+)-*endo*-bicyclo-[3.3.1]non-6-ene-3(*R*)-carboxylic acid (**3**) of known absolute configuration and enantiomeric excess (50%) was reacted with excess *tert*-butyllithium in refluxing pentane for 72 h to afford a 24% yield of (+)-(1*S*,3*R*,5*R*)-*endo3*-bicyclo[3.3.1]non-6-enyl *tert*-butyl ketone (**4**),[9] The major side products of this reaction were the epimeric (exo) ketone of **4** and the alcohol derived from reduction of the carbonyl group in **4**. Lithium aluminum hydride reduction of endo ketone **4** gave a 93% yield of the corresponding diastereomeric alcohols of **5**,[9] which were solvolyzed as before[3] in hot formic acid to effect ring closure to the expected mixture of epimeric 4-*tert*-butyladamantan-2-ol formate (**6**) and the rearranged 4-methyl-4-isopropyladamantan-2-ol formate (**7**). The total mixed formates were cleaved by $LiAlH_4$ then oxidized (Jones oxidation), and the resulting ketones were separated by preparative GC.[10] The ketone products consisted of 80% of a 1:1 mixture of isomeric 4-methyl-4-isopropyladamantan-2-ones[9] (derived from **7**) and only 20% of a 2;1 mixture of the desired 4(a)- and 4-(e)-*tert*-butyladamantan-2-ones (derived from **6**) (**1** and **2**, respectively).[9] Not surprisingly, generation of the carbocation from **5** leads to substantial methyl migration before electrophilic addition to the endocyclic C=C bond and regeneration of the adamantane skeleton. We find no evidence of skeletal rearrangement to homoadamantanes, only a majority of functional group rearranged products (**7**); thus, rearranged carbocation c appears to be the major electrophilic intermediate in the reaction.

The spectroscopic properties of **1** and **2** are in accord with their structural assignments. Thus, **1** exhibits the more shielded CH_3 resonances [^{1}H NMR: **1**, 0.92 ppm (s, 9 H); **2**, 1.04 ppm (s, 9 H)][11] because its *tert*-butyl group lies above and over the C=O. Dreiding models clearly show that in the staggered conformation only one CH_3 lies well over the C=O, but at room temperature apparently rapid, free rotation of the *tert*-butyl group of **1** yields an averaged CH_3 signal. Of special interest is the observation that the major mass spectral fragmentation in **1** and **2** differ strikingly: at 70 eV **1** [$M^{+\cdot}$, 206.1668, $C_{14}H_{22}O$ (13% relative abundance)] shows a base peak of 150.1040 (M − 56) and only an extremely weak 149.0967 (M − 57) peak, whereas **2** [$M^{+\cdot}$ 206.1669, $C_{14}H_{22}O$ (19% relative abundance)] shows a very strong (72% relative abundance) 149.0967 (M − 57) peak and only a weak M − 56 peak. Simple cleavage of the *tert*-butyl group leading to an M − 57 ion is expected to be a major fragmentation pathway for both **1** and **2**, but it is observed only with **2**. In **1** the (unavoidable) proximity of *tert*-butyl and carbonyl groups is favorable

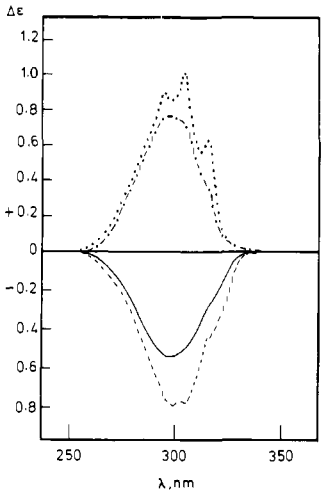

Figure 1. Circular dichroism spectra of 5 × 10^{-3} M (1*S*,3*R*,4*S*)-4(a)-*tert*-butyladamantan-2-one (**1**) at 25 °C (—) and −175 °C (---) and (1*S*,3*R*,4*R*)-4(e)-*tert*-butyladamantan-2-one (**2**) at 25 °C (·-·) and −175 °C (···) run in EPA (ether–isopentane–ethanol, 5:5:2, v/v/v), on a JASCO J-40A instrument equipped with photoelastic modulator and J-DPY data processor and corrected to 100% enantiomeric excess.

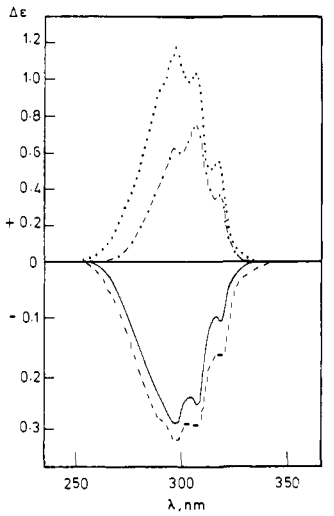

Figure 2. Circular dichroism spectra of 4 × 10^{-5} M (1*S*,3*R*,4*S*)-4(a)-*tert*-butyladamantan-2-one (**1**) at 25 °C (—) and −175 °C (---) and 1*S*,3*R*,4*R*)-4(e)-*tert*-butyladamantan-2-one (**2**) at 25 °C (-·-) and −175 °C (···) run in MI (methylcyclohexane–isopentane, 4:1 v/v), on a JASCO J-40A instrument equipped with photoelastic modulator and J-DPY data processor and corrected to 100% enantiomeric excess.

for the extremely rare ketone homo-McLafferty rearrangement[12] involving transfer of a δ-H (from CH_3) as opposed to the conventional γ-H transfer.

The CD spectra of **1** and **2** are particularly interesting (Figures 1 and 2). In expected agreement with the octant rule,[2,13] the equatorial isomer (**2**) exhibits moderately intense positive n–π* Cotton effects (CEs) at room temperature, $\Delta\epsilon_{304}^{max}$ +0.75 (MI), $\Delta\epsilon_{295}^{max}$ +0.77 (EPA), which change very little down to −175 °C and are quite comparable in magnitude and low-temperature behavior to those exhibited by the corresponding 4(e)-methyladamantan-2-one, $\Delta\epsilon_{305}^{max}$ +0.54 (MI), $\Delta\epsilon_{295}^{max}$ +0.67 (EPA).[3,14] This is somewhat surprising because one would tend to think that the much larger group should make a much larger contribution to the CE; however, in the Kirk and Klyne zigzag model[15] only

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(10) Typically, the axial isomer has a shorter GC retention time.[3,8]
(11) These values are comparable to those of the corresponding 4(a)-methyladamantan-2-one [0.95 ppm (d, 3 H)] and 4(a)-methyladamantan-2-one [1.07 ppm (d, 3 H)].[3]
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one CH_3 of the $C(CH_3)_3$ group can lie on a consignate path; the others must lie on dissignate paths and make anti-octant contributions. The axial *tert*-butyl isomer **1** in marked contrast behaves very differently from the 4(a)-methyladamantan-2-one counterpart in giving surprisingly *strong*, negative n–π^* CEs at room temperature, $\Delta\epsilon_{296}^{max}$ −0.29 (MI), $\Delta\epsilon_{296}^{max}$ −0.54 (EPA), vs. weak, variable CEs, $\Delta\epsilon_{301}^{max}$ +0.023 (MI), $\Delta\epsilon_{306}^{max}$ −0.046, (EPA) of the latter. As with the equatorial *tert*-butyl ketone, the CEs are essentially temperature invariant down to −175 °C, again in marked contrast to the axial CH_3 analogue, for which the CE magnitude increases 10-fold at −175 °C, with sign inversion in MI solvent.[14] Thus, the β-axial *tert*-butyl group behaves as a *strong front octant perturber* that is insensitive to solvent and temperature effects.

Molecular mechanics (MM2)[16] calculations predict a higher energy (1.5 kcal/mol higher) for the sterically more crowded equatorial isomer. Using the adamantanone model, one might therefore assume that although still large the conformational energy difference between chair conformers of 3-*tert*-butylcyclohexanone is less (~3.9. kcal/mol) than the computed *A* value (~5.4 kcal/mol) for *tert*-butylcyclohexane.[17]

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High-Speed Spatially Resolved High-Resolution NMR Spectroscopy

Shigeru Matsui,* Kensuke Sekihara, and Hideki Kohno

Central Research Laboratory, Hitachi Ltd.
Kokubunji, Tokyo 185, Japan

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Several methods have been proposed[1-4] for high-resolution NMR spectroscopic study of spatially inhomogeneous objects, such as biological systems. Among these, the most general and practically useful method appears to be the method employing the multidimensional Fourier transform (FT) NMR techniques,[3] where two- or three-dimensional spatial information is phase-encoded into free induction decays by pulsed applications of field gradients.[5] This type of method, however, is extremely time consuming, because a large number of measurements, which ensure the required resolution in the multidimensions, must be made to obtain full information.

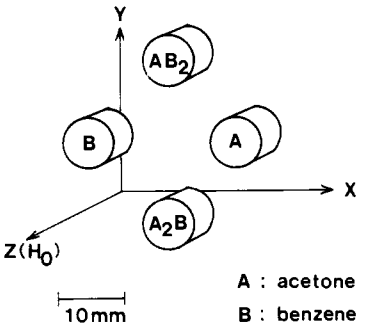

Figure 1. Two-dimensional test sample consisting of acetone (A) and benzene (B). A relaxation reagent, iron(III) acetylacetonate, was added so as to shorten the spin–lattice relaxation times down to about 100 ms for experimental convenience. Each cylinder has a diameter of 8 mm and a length of 10 mm. A, B, AB_2, and A_2B denote the constitutions of the solutions.

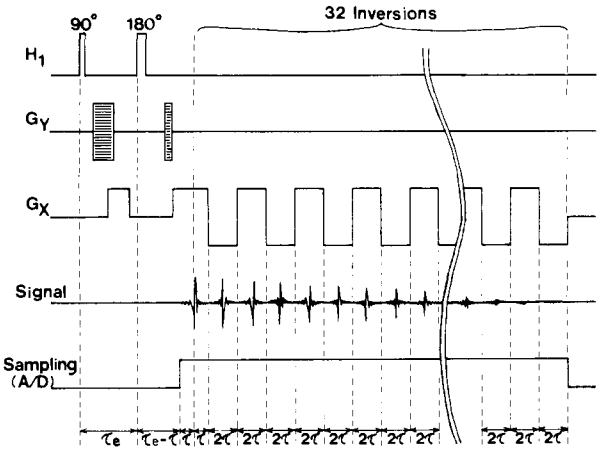

Figure 2. Pulse sequence for high-speed spatially resolved high-resolution NMR spectroscopy. A spatially two-dimensional version is shown. The spin–echo method is employed for compensating the finite-switching-time effects of the field gradient and for observing the initial halves of the first echoes in the echo train signals. The rf pulse interval τ_e = 10 ms. 32 echoes were induced by successive inversions of the field gradient, G_X = 6.8 mT/m, with τ = 1.248 ms and the switching time $\simeq$ 50 μs. Echo trains phase encoded by stepwise applications of the field gradient, G_Y = 6.8 mT/m (the effective phase-encoding time = 1.248 ms), were sampled with an analog-to-digital conversion rate of 78 μs using 1024 points, starting at time $2\tau_e - \tau$.

We wish to describe here our experimental results on a high-speed method of spatially resolved high-resolution NMR spectroscopy, which is closely related to the chemical-shift-resolved NMR imaging methods theoretically described by Mansfield[6a,b] recently. In our method, however, spin–echo trains induced by periodical inversions of a field gradient[6] are further phase modulated (phase encode)[5] by pulsed applications of other field gradients.[7] Such phase-modulated spin–echo trains can be converted to three- or four-dimensional data including both spectroscopic and spatial information by means of suitable data manipulation involving echo rearrangement[6,8] and multidimensional Fourier transformation. Since two-dimensional information (spectroscopic and spatial) can be extracted from each one-dimensional spin–echo train, the dimension of the measurement can

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(7) Our method coincides with Mansfield's methods[6a,b] in the case of one-dimensional objects. For two- or three- dimensional objects, however, his methods necessitate two or three inverting field gradients or rotation of a inverting field gradient. Particularly in view of practical applicability our method possesses important advantages over his methods.
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