

Figure 1. Atom and torsion-angle numbering in acetylcholine.

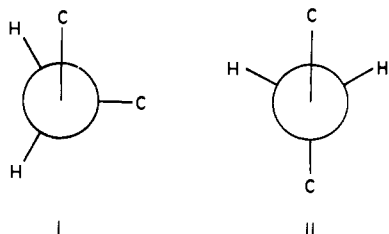


Figure 2. Suggested rotamers for the C(6)-O(1)-C(5)-C(4) fragment. Rotamer I: $\tau_1 \sim 90^\circ$, rotamer II: $\tau_1 \sim 180^\circ$.

Table I. Acetylcholine: ${}^3J_{13\text{C}(6)-\text{O}(1)-\text{C}(5)-\text{H}}$ Values,^a Fractional Rotamer Populations, and ΔG° Values at Different Temperatures for the Fragment C(6)-O(1)-C(5)-C(4)

temp, °C	${}^3J_{13\text{COCH}}$, Hz	fractional rotamer population		ΔG° , cal/mol
		N_I	N_{II}	
10	2.55	0.312	0.688	-443
30	2.66	0.335	0.665	-412
50	2.78	0.360	0.640	-368
70	2.85	0.375	0.625	-347

^a ± 0.05 Hz. Consequently the rotamer populations N and ΔG° values are accurate to ± 0.010 and ± 29 cal/mol, respectively.

occurrence of a conformational equilibrium between different rotamers.

The rotational isomerism of ACh cation in solution can be represented by an equilibrium of the two rotamers shown in Figure 2 on the following grounds. First, torsion angles C(6)-O(1)-C(5)-C(4) found in crystal structure of choline esters cluster round two values: 90 and 180°, except for two carbamoylcholine ions.³ Second, formate and acetate esters of ethyl alcohol show a nearly identical value of ${}^3J_{13\text{COCH}}$ (3.32 and 3.23 Hz, respectively^{10,11}), thus suggesting that their conformational behavior is very similar. There is evidence derived from microwave spectroscopy¹² that ethyl formate exists in solution as a mixture of one trans and two distorted gauche rotamers, the relative C-O-C-C dihedral angles being approximately 85, 180, and 275°. Therefore, it seems reasonable to assume that, on rotation about the C-O axis, the energy of the ACh cation goes through two minima occurring at nearly 90 and 180°. The rotamer with $\tau_1 \sim 275^\circ$ seems highly implausible, keeping the fragment O(1)-C(5)-C(4)-N in its more stable conformation ($\tau_2 \approx 60^\circ$)^{7,8} due to the steric requirements of the C=O and N(CH₃)₃ groups. Since the internal rotation is sufficiently rapid, the observed ${}^3J_{13\text{COCH}}$ value is a weighted average of the corresponding constants for the individual rotamers:

$${}^3J_{13\text{COCH}} = N_I/2[{}^3J_{13\text{COCH}}(30^\circ) + {}^3J_{13\text{COCH}}(150^\circ)] + N_{II}{}^3J_{13\text{COCH}}(60^\circ) \quad (1)$$

where N_I and N_{II} are the fractional populations of rotamer I and II, respectively:

$$N_I + N_{II} = 1 \quad (2)$$

(10) D. E. Dorman, D. Bauer, and J. D. Roberts, *J. Org. Chem.*, **40**, 3729 (1975).

(11) G. J. Karabatsos, N. Hsi, and C. E. Orzech, Jr., *Tetrahedron Lett.*, 4639 (1966).

(12) J. M. Riveras and E. B. Wilson, Jr., *J. Chem. Phys.*, **46**, 4605 (1967).

The coupling constant in rotamer I is given by the mean value $1/2[{}^3J_{13\text{COCH}}(30^\circ) + {}^3J_{13\text{COCH}}(150^\circ)]$, owing to the rapid interconversion of rotamer I with its mirror image.

The ${}^3J_{13\text{COCH}}(30^\circ)$, ${}^3J_{13\text{COCH}}(60^\circ)$, and ${}^3J_{13\text{COCH}}(150^\circ)$ values could be obtained from a Karplus-type relationship:

$${}^3J_{13\text{C,H}} = A \cos^2 \varphi + B \cos \varphi + C \quad (3)$$

Such a relationship has received experimental¹³⁻¹⁷ as well as theoretical¹⁸ evidence. However, reported ${}^3J_{13\text{C,H}}$ values exhibit a considerable scattering which has been attributed to structural parameters^{14,17} (e.g., carbon hybridization, electronegative substituents, nuclei sequence in the coupling pattern). The coefficients A , B , and C for the ${}^3J_{13\text{C,H}}$ coupling transmitted via the specific nuclei sequence ¹³C-O-C-H have been determined as follows. C is considered to be negligible, as a survey of the literature shows that when φ approaches 90°, ${}^3J_{13\text{C,H}}$ becomes undetectable.^{14,15} The A value (7.8 Hz) is derived from averaged ${}^3J_{13\text{COCH}}$ couplings in methyl acetate^{10,11} and formate.¹⁰ Knowledge of A and C enables us to obtain B . A mean B value of -1.8 Hz is computed from the values of vicinal ¹³C,H coupling constants (8.7 and 9.3 Hz) measured in nearly trans and trans arrangements ($\varphi = 158$ and 180°) of the ¹³C(O)-O-C-H fragment in 2,5'-anhydro-2',3'-isopropylideneuridine¹⁶ and vinylene carbonate,¹⁹ respectively. When eq 1-3 and the ${}^3J_{13\text{C}(6)-\text{O}(1)-\text{C}(5)-\text{H}}$ values at different temperatures are employed, one obtains the population fractions reported in Table I.

Although the most stable conformation corresponds to the extended form of the fragment C(6)-O(1)-C(5)-C(4), rotamer I is also significantly populated. The free-energy difference between the two rotamers [$\Delta G^\circ = -RT \ln (N_I/N_{II})$] is only 412 \pm 29 cal/mol at 30 °C. This energy difference is of the order of magnitude of that found by microwave spectroscopy in ethyl formate between the trans and distorted gauche rotamers (200 cal/mol).¹² The slightly greater value agrees with the prediction by Roberts and co-workers¹⁰ that in the acetate and formate esters of primary alcohols an increase of the size of the group attached to the α -carbon should cause an increase in the free-energy difference between the trans and the gauche rotamers.

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(13) A. S. Perlin and B. Casu, *Tetrahedron Lett.*, 2921 (1969).

(14) R. U. Lemieux, T. L. Nagabhusan, and B. Paul, *Can. J. Chem.*, **50**, 773 (1972).

(15) J. A. Schwarcz and A. S. Perlin, *Can. J. Chem.*, **50**, 3667 (1972).

(16) R. U. Lemieux, *Ann. N.Y. Acad. Sci.*, **222**, 915 (1973).

(17) G. K. Hamer, F. Balza, N. Cyr, and A. S. Perlin, *Can. J. Chem.*, **56**, 3109 (1978).

(18) R. Wasylishen and T. Schaefer, *Can. J. Chem.*, **50**, 2710 (1972); A. S. Perlin, N. Cyr, H. J. Koch, and B. Korsch, *Ann. N.Y. Acad. Sci.*, **222**, 935 (1973).

(19) K. A. McLauchlan and T. Schaefer, *Can. J. Chem.*, **44**, 321 (1966).

NMR of Protons Coupled to ¹³C Nuclei Only

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In this communication we describe a simple multinuclear multipulse sequence which enables the cancellation of signals arising from protons bonded to ¹²C nuclei, allowing the observation of just those signals of protons coupled to ¹³C. The sequence will be useful in studying ¹³C-enriched compounds obtained, for instance, in the study of biosynthetic pathways either as an alter-

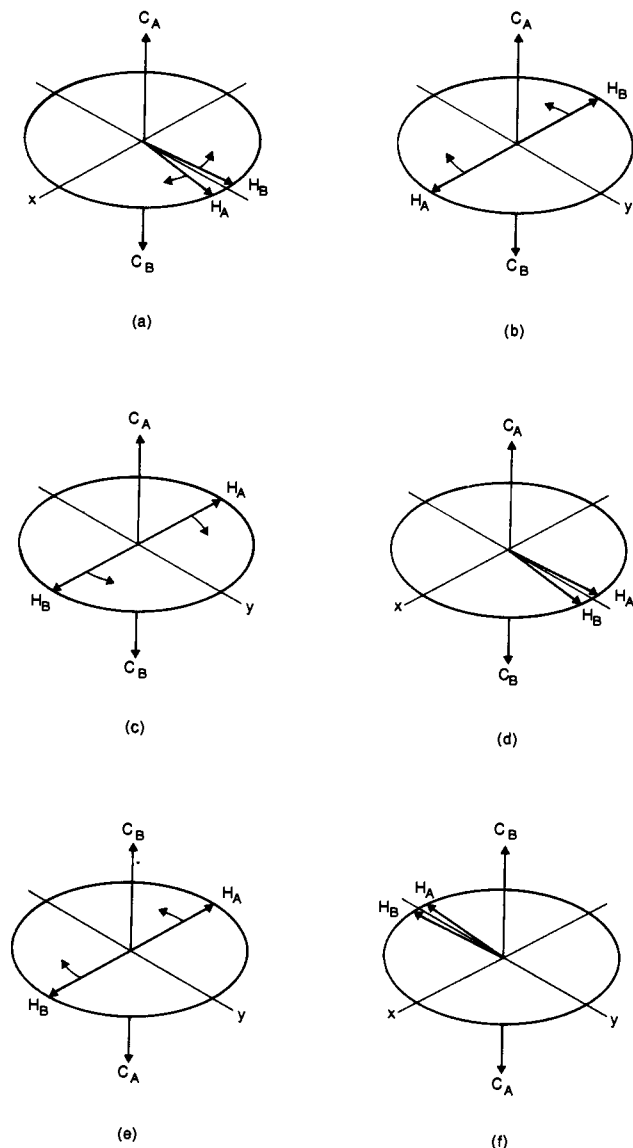
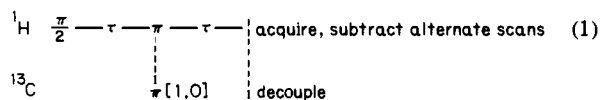


Figure 1. Since precession in the rotating frame due to chemical shift is refocused at the end of the 2τ period by the $\pi[H]$ pulse, precession due only to coupling to ^{13}C is shown. (a) After a $\pi/2[H,x]$ pulse, the magnetization vector of protons coupled to ^{13}C nuclei, which is initially along the y axis of the rotating frame, splits into a clockwise rotating vector (H_A) and an anticlockwise rotating vector (H_B), corresponding to whether the ^{13}C nuclei are in the $+z$ eigenstate (C_A) or $-z$ eigenstate (C_B), respectively. (b) After a period $\tau = (2J)^{-1}$ s, H_A and H_B have precessed 90° in the rotating frame. (c) A $\pi[H,y]$ pulse swaps H_A and H_B . (d) After the second τ period, H_A and H_B are refocused along the y axis. This refocusing is independent of any chemical shift or failure to set τ exactly. (e) If, after the first τ period [Figure 1b], $\pi[H,y], \pi[C]$ pulses are applied, C_A and C_B are also swapped, and H_A and H_B precess in directions opposite to those in Figure 1c during the second τ period. (f) After the second τ period, H_A and H_B are refocused along the $-y$ axis. This refocusing is independent of any chemical shift. Failure to set τ exactly leads to imperfect refocusing along $-y$ axis and a reduced final signal on subtraction of alternate scans.

native to or as an addition to ^{13}C NMR and in studying ^1H - ^{13}C coupling.

The simplest sequence is sequence 1. The key to this sequence



(1) Freeman, R.; Morris, G. A. *Bull. Magn. Reson.* 1979, 1, 5-26.

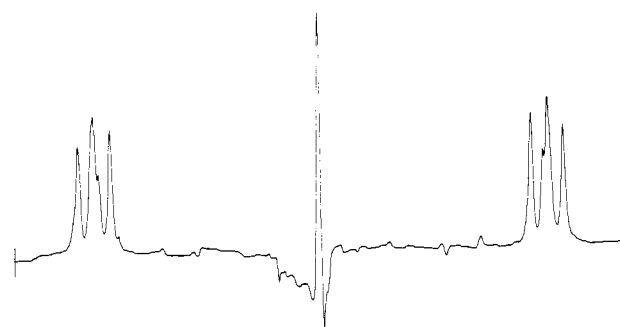
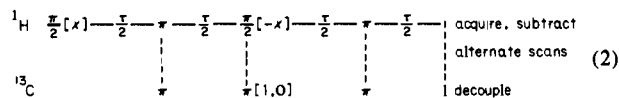


Figure 2. ^1H spectrum³ at 30°C of 50% (v/v) ethylene glycol in D_2O using sequence 2, without ^{13}C decoupling during acquisition, after 500 scans. The sweep width is 200 Hz.

and sequence 2 is the judicious use of refocusing pulses.¹ The $[1,0]$ for the $\pi[C]$ pulse signifies that the pulse is applied for alternate scans only. This $\pi[C]$ pulse has no effect on protons attached to molecules containing ^{12}C , and signals from such protons are canceled by subtraction of alternate scans.

The mechanism of the sequence is illustrated in Figure 1. If, for example, the proton $\pi/2$ and π pulses are on the x and y axes of the rotating frame, respectively, proton magnetization of protons attached to ^{12}C nuclei, which is initially along the y axis, is refocused to the y axis after the second τ period, and the resulting signal is canceled by subtraction of alternate scans. For protons coupled to ^{13}C nuclei we are concerned with the magnetization vectors in Figure 1a: H_A and H_B comprise protons coupled to ^{13}C nuclei in the $+z$ eigenstate (C_A) and $-z$ eigenstate (C_B), respectively. There is no net precession of these proton vectors due to chemical shift during the 2τ period as this is refocused by the $\pi[H]$ pulse as before. Indeed, for scans in which no $\pi[C]$ pulse is applied, precession due to coupling to ^{13}C is also refocused as illustrated in Figure 1a-d. However, the direction of this precession is reversed by the application of a $\pi[C]$ pulse as shown in Figure 1e, and the proton magnetization is refocused along the $-y$ axis at the end of the second τ period, as in Figure 1f, if $\tau = (2J)^{-1}$ where J is the ^1H - ^{13}C coupling constant. Thus alternate application of the $\pi[C]$ pulse and subtraction of alternate scans will cause summation of signals arising from H_A and H_B .

Sequence 1 should be quite successful with modern recent spectrometers employing stable, superconducting magnets. Our system, an iron-core electromagnet, suffers sufficiently from drift in the deuterium lock signal that it is not possible to cancel signals by subtraction of alternate scans to better than 2%. Although this degree of cancellation of signals of protons attached to ^{12}C nuclei is probably sufficient for the study of ^{13}C -enriched samples, it is desirable to improve upon it. To this end sequence 2 was devised. If, for example, the first $\pi[H]$ pulse is on the y axis,



the first $\pi[H], \pi[C]$ pulse ensures that the H_A and H_B vectors are along the $-x$ and x axes at the end of the first τ period [if $\tau = (2J)^{-1}$] but that proton magnetizations of protons attached to ^{12}C are along the y axis. Thus the $\pi/2[-x]$ pulse has no effect on H_A and H_B but returns the unwanted proton magnetization to the z axis. Pulsing about the $-x$ axis reduces the effect of imperfections and inhomogeneities in the initial $\pi/2[x]$ pulse. H_A and H_B are refocused along the y or $-y$ axes after the second τ period, depending on the alternate application of the $\pi[C]$ pulse between the two τ periods. This enables subtraction of alternate scans as in sequence 1 so that residual unwanted signals, resulting from imperfections in the pulses and relaxation during the first τ period, are canceled.

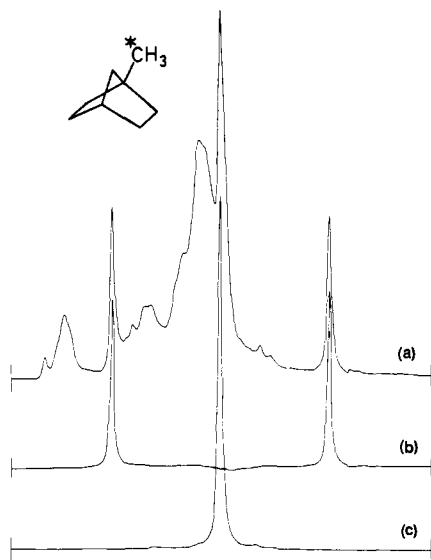


Figure 3. ^1H spectra³ at 30 °C of pure (external D lock) 1-methylnorbornane, approximately 60% ^{13}C -enriched at the methyl group. The sweep width is 250 Hz. Sixty-four scans were collected. (a) Normal spectrum, (b) using sequence 2 without ^{13}C decoupling during acquisition, and (c) using sequence 2 with ^{13}C decoupling during acquisition.

As illustrated in Figure 2 for ethylene glycol, sequence 2 enables cancellation of the unwanted signals to better than 0.2% even for our imperfect magnet. (The ^{13}C satellites are split by homonuclear coupling unlike the central resonance and thus have a normal amplitude of about 0.1% of the central line.) Integration across the center line is considerably less than 0.2% of the uncanceled signal.

The application of the sequence to an enriched sample is shown in Figure 3. Although the ^{13}C satellites are clearly resolved in Figure 3a, the ^{12}CH -canceled spectrum [Figure 3b and the decoupled ^{12}CH -canceled spectrum (Figure 3c)] clearly illustrate what can be achieved for enriched samples where the ^{13}C satellites are normally overlapped by other resonances.

^{13}C -enriched quaternary carbons can be studied by setting τ to approximately correspond to a long-range J value. Indeed, use of larger τ values provides a general method of studying long-range ^{13}C - ^1H coupling in enriched compounds if ^{13}C decoupling is not employed during acquisition. However, the greatest potential use of these sequences is in studying both short- and long-range ^{13}C - ^1H coupling by two-dimensional NMR of unenriched compounds. Incrementation of τ to provide a second J dimension and ^{13}C decoupling during acquisition will produce a resonance in the J dimension for each ^{13}C - ^1H coupling at $J/2$ Hz. Although these peaks will be split by proton homonuclear coupling, there will be a large gain in sensitivity over ^{13}C two-dimensional J spectra,² and the problem of complicated ^{13}C - ^1H coupled multiplets is avoided. Since the proton homonuclear coupling occurs in both dimensions, it will assist in resolution.

Acknowledgment. This research was supported by major equipment grants from the Australian Research Grants Committee. Enriched 1-methylnorbornane was provided by Dr. E. Della, Flinders University.

(2) Thomas, D. M.; Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Field, J. J. *Magn. Reson.*, in press.

(3) Spectra were obtained by using a Bruker HX-90 spectrometer upgraded with an Aspect-2000 computer and associated CXP-series pulse modulation electronics. A 5-mm insert was used which contained a coil doubly tuned to ^{13}C and ^1H . A second coil was used for the D lock.

A New Approach to the Conformational Analysis of Seven-Membered Rings

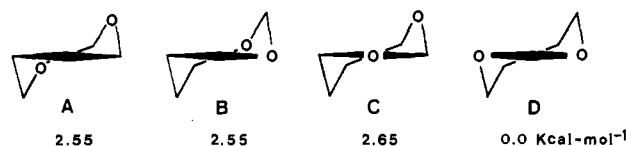
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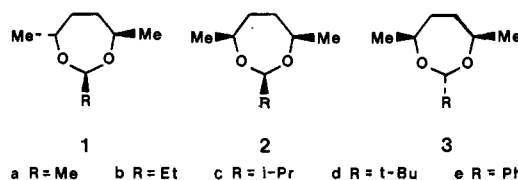
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Progress in the conformational analysis of seven-membered rings has been slow, chiefly because pseudorotation of these rings is facile.^{1,2} Evidence about the nature of the conformational energy surfaces for such systems and about such factors as axial-equatorial energy differences has come mainly from force-field calculations, with experimental support^{1,3} for the nature of the lowest minima. Considerable advances were made in the conformational analysis of six-membered rings through the examination of the properties of derivatives with one or sometimes two groups present to lock the ring in a particular conformation.^{4,5} We show here that the introduction of three groups results in comparable simplification of the conformational situation for seven-membered rings. We illustrate the procedure by using 2,4,7-trisubstituted-1,3-dioxepanes because these compounds are easily synthesized⁶ and equilibrated.

For cycloheptane, there are 14 degenerate lowest energy conformations, the twist-chairs (TC), which pseudorotate into each other through 14 degenerate maxima, the chairs (calculated barrier,¹ 1.3 kcal mol⁻¹). Fourteen boat (B) and twist-boat (TB) conformations (calculated stabilities¹ relative to the TC, 3.42 and 3.39 kcal mol⁻¹, respectively) also pseudorotate into each other and are accessible from the TC conformation via an inversion process (barrier¹ 9.7 kcal mol⁻¹). For 1,3-dioxepane, there are four types of TC conformations, A-D, shown with their calculated stabilities.¹ Chair conformations are again local maxima so they will not be considered further here.



Three stereoisomers (1, 2, and 3) are produced when three



substituents are introduced at positions 2, 4, and 7 of 1,3-dioxepane, if those at 4 and 7 are identical. The relative energies of the TC conformations of the three stereoisomers with all groups methyl (1a, 2a, 3a) were evaluated approximately by making the following assumptions: (1) The four types of 1,3-dioxepane TC conformations have the energies calculated by Bocian and Strauss.¹ (2) The introduction of a methyl group at a particular position in a conformation increases the energy of the conformation by the amount calculated² for a similar introduction for cycloheptane. This A value is decreased by one-quarter of its value whenever

(1) Bocian, D. F.; Strauss, H. L. *J. Am. Chem. Soc.* **1977**, *99*, 2876-2882.
(2) Hendrickson, J. B. *J. Am. Chem. Soc.* **1967**, *89*, 7036-7061.
(3) Groth, P. *Acta Chem. Scand.* **1967**, *21*, 2631-2646. Cameron, T. S.; Cordes, R. E.; Grindley, T. B. *Acta Crystallogr., Sect. B* **1977**, *B33*, 3718-3722. Brookeman, J. R.; Rushworth, F. A. *J. Phys. C*, **1976**, *9*, 1043-1054.

(4) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A.; "Conformational Analysis"; Interscience: New York, 1965.

(5) Eliel, E. L.; *Acc. Chem. Res.* **1970**, *3*, 1-8.

(6) The 2-substituted-4,7-dimethyl-1,3-dioxepane diastereomers were synthesized from a commercial mixture of 2,5-hexanediol isomers and the various aldehydes and were separated by preparative gas-liquid chromatography on a 20-ft column of 30% Carbowax 20M on Chromosorb W.