Pulse Sequence for the Generation of a ¹³C Subspectrum of both Aromatic and Aliphatic Quaternary Carbons

M. Robin Bendall,[*a] David T. Pegg,[a] David M. Doddrell,[a] Stanley R. Johns,[b] and Richard I. Willing[b]

[a] School of Science, Griffith University, Nathan, Queensland, 4111 Australia
[b] Division of Applied Organic Chemistry, C.S.I.R.O., Box 4331 G.P.O., Melbourne, Victoria, 3001 Australia

A simple heteronuclear spin-echo pulse sequence is described which provides an accurate ¹³C subspectrum of just aromatic and aliphatic quaternary carbons, especially when the method is applied in conjunction with the recent distortionless enhancement by polarisation transfer (DEPT) sequence.

Modified spin-echo sequences have been used to separate accurately a ¹³C spectrum into separate methyl/methine (CH_3/CH) and methylene/quaternary (CH_2/q) subspectra[1-3] and the latter have been further separated into separate CH_2 and q subspectra,[4,5] even when the single-bond ¹³C–¹H J value, assumed in setting the free precession periods in the sequences, varies significantly from the actual values. However, accurate separation of CH_3/CH spectra is not possible by spin-echo sequences.[3,6] Recently we have achieved accurate CH_3, CH_2, and CH subspectra using the DEPT sequence,[7,8] a generalisation of the EPT sequence.[3,6] Quaternary resonances do not appear in DEPT spectra. Unfortunately, it is not possible to produce an accurate q subspectrum by subtracting the DEPT subspectra from a normal spectrum because of the different efficiencies of polarization transfer and nuclear Overhauser enhancement. An independent method is required. The randomisation spin-echo method[4,5] is too time-consuming if it is additional to DEPT. The SEBBORD method[9] is accurate only for the aromatic region.[5]

The simple spin-echo technique has been used to generate just a q subspectrum.[1,2] The gated-decoupled sequence (A) is the simplest variation of this method. A simple analysis sequence (A) in terms of the Heisenberg vector model shows that when actual coupling constants differ by ΔJ from the value assumed in the sequence (J), residual error signals of magnitude $\sin^n(\pi/2)(\Delta J/J)$ result for CH_n groups ($n = 1, 2$, or 3).[5] Although the errors are small for CH_2 and CH_3 groups, they are quite significant for CH groups. For a 10% divergence of J, for example, the error is 15.6% of full intensity. These unwanted large residual signals are further illustrated in Figure 1(a)—(c). As illustrated in Figure 1(e)—(h), sequence (B) shows dramatically reduced error signals. For example, compare Figure 1(g) with Figure 1(a) or (b).
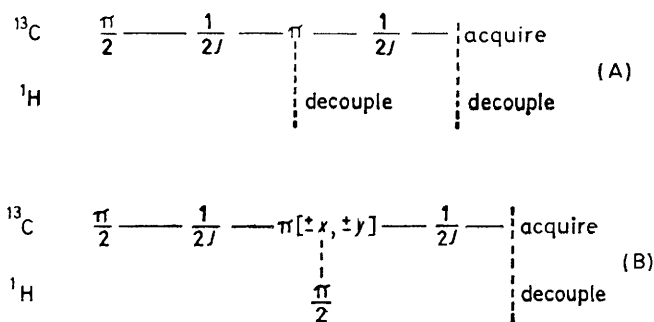
The mechanism of sequence (B) is quite straightforward. As in sequence (A), the only purpose of the π pulse is to refocus chemical shift. The π pulse should be alternated through all four phases, with inversion of receiver phase for 90° shifts, to eliminate errors arising from the pulse.[5,10] After the first $(2J)^{-1}$ s period in both sequences, magnetization

vector components have precessed in the transverse plane because of coupling with the attached protons, so that for any CH_n group they are 180° out-of-phase to each other.[2,4] The decoupling in sequence (A) freezes this precession and the components mutually cancel. In contrast, the $(\pi/2)$[H] pulse in sequence (B) acts so that each vector component splits into new additional components which precess as usual in the second $(2J)^{-1}$ s period, but mutually cancel because for every component there is an equal and opposite one.[11] In both sequences, for divergent J values the various vector components are not exactly 180° out-of-phase after the first $(2J)^{-1}$ s period and cannot exactly cancel each other. For sequence (A) this leads to the residual signals as quantified above, and which are large for CH groups. However, for sequence (B) the residual magnetizations left after the $(\pi/2)$[H] pulse split into vector components which precess almost 180° apart during the second $(2J)^{-1}$ s period [in the same way as during the first $(2J)^{-1}$ s period], thus reducing the errors to $\sin^{2n}(\pi/2)(\Delta J/J)$ for CH_n groups ($n = 1, 2$, or 3). Figure 1(e)—(h) clearly demonstrates the superior cancellation accuracy of sequence (B). For example, a 10% divergence in J for a CH group theoretically gives only a 2.4% error signal.

Residual CH and CH_3 error signals will be positive or negative using sequence (A), when the actual J is smaller or larger than the assumed J respectively[1,2] [e.g. the low-field CH resonance in Figure 1(a)—(c)], but sequence (B) can only give positive errors. Positive or negative signals will, however, result for sequence (B) if the $(\pi/2)$[H] pulse time is smaller or larger than ideal, respectively.[11] In routine use the $(2J)^{-1}$ s periods should be set to a compromise value corresponding to a J value of, say, 135 Hz. The $(\pi/2)$[H] pulse time can then be set to a value slightly larger than the exact 90° time, using a known compound, by ensuring that CH resonances with J close to 135 Hz give slightly negative signals and CH groups with J divergent by, say, 10% give approximately zero signals. This has been done for Figure 1(e)—(h) and in routine use such an adjustment requires only a few minutes work. This method has the effect of significantly reducing the errors expected from the above CH formula for sequence (B).

For CH_2 groups only positive error signals result if J or $(\pi/2)$[H] is mis-set or if the $(\pi/2)$[H] pulse is inhomogeneous.[1,2,4,11] When used in conjunction with the DEPT sequence, a small percentage of a DEPT CH_2 subspectrum may be subtracted from the sequence (B) spectrum as has been done for Figure 1(f)—(h) to reduce the positive CH_2 errors. However, in routine ¹H multiplicity determination we have found this pursuit of perfection unnecessary.

If J 135 Hz is assumed for sequence (B), aromatic and olefinic CH groups will give positive errors of ca. 6%. A perfect aromatic quaternary spectrum can be obtained by repeating the experiment with J ca. 160 Hz assumed. However, if used in conjunction with DEPT, the latter is unnecessary because the ca. 6% errors in the aromatic region can be subtracted using the DEPT CH spectrum. We have found sequence (B) to be an excellent addition to the DEPT method which we believe
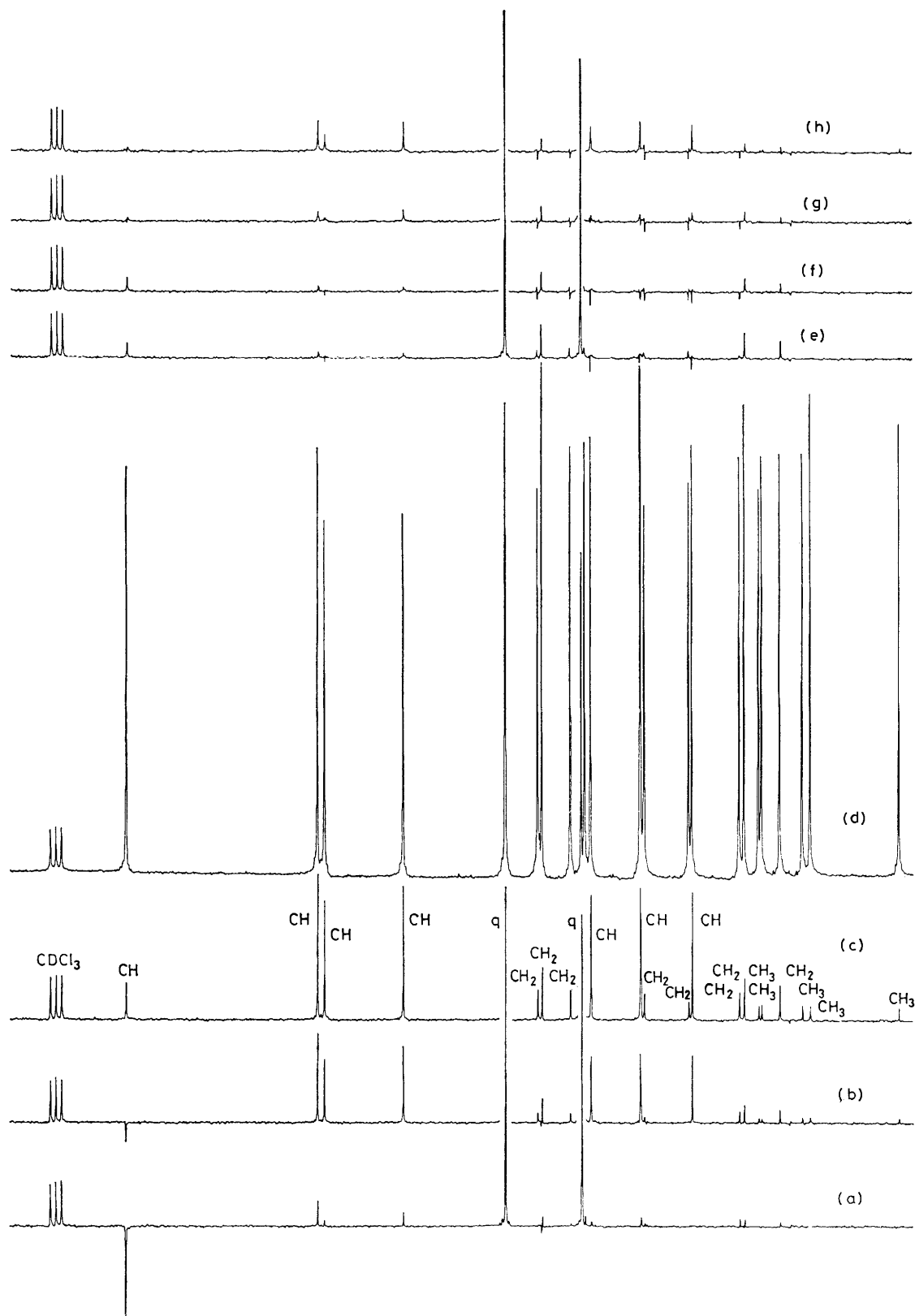
¹³C $\dfrac{\pi}{2}$ ——— $\dfrac{1}{2J}$ ——— π —— $\dfrac{1}{2J}$ —— acquire

¹H   decouple   decouple

(A)

¹³C $\dfrac{\pi}{2}$ ——— $\dfrac{1}{2J}$ —— $\pi[\pm x, \pm y]$ —— $\dfrac{1}{2J}$ —— acquire

¹H $\dfrac{\pi}{2}$ decouple

(B)

Figure 1. Partial ^{13}C spectra of cholesterol at 75.46 MHz. (a) Sequence (A), assumed J 125 Hz; (b) sequence (A), assumed J 137 Hz; (c) sequence (A), assumed J 150 Hz; (d) normal spectrum; (e) sequence (B), assumed J 125 Hz; (f) sequence (B), assumed J 125 Hz, 2% of a DEPT CH_2 subspectrum subtracted; (g) sequence (B), assumed J 137 Hz, 2% of a DEPT CH_2 subspectrum subtracted; (h) sequence (B), assumed J 150 Hz, 2% of a DEPT CH_2 subspectrum subtracted. Each spectrum is the average of the same number of transients.

to be the best method for editing ^{13}C spectra presently available.

This research was conducted on a Bruker CXP-300 spectrometer owned by the Brisbane NMR Centre and sited at Griffith University, and checked using a WM-250 spectrometer (C.S.I.R.O., Melbourne).

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