compares a substituent having a dipole directed along the CX axis, such as F, with one such as COX. Third, even if the point dipole assumption holds, even for a linear substituent, the value of r does not change completely uniformly from one system to another. Thus, the distance from a probe site in the case of a fluorine substituent is to the midpoint of the CF bond while that to a cyano substituent is significantly greater. Thus, if the system is changed, the incremental increase in r is constant, but the relative change will alter from one substituent to another. We can readily illustrate this with system 4 using the F and CN substituents. At $r = 3.44$ Å, the effect of the F is 12% greater than that of the CN, at $r = 4.0$ it is only 5% greater, while at $r = 7$ Å it is 7% *less*. Fourth, as discussed with the cyano group above, substituent electronic effects may change somewhat depending on the substrate.

Conclusions

A simple theoretical method has been devised to calculate inherent σ_F values with a wide variety of substituents. These are in good agreement with values, where known, for nonpolar media and provide reliable values for many other substituents. The method shows the significance of conformational and distance effects on σ_F values that are not generally recognized.

Acknowledgment. We are grateful to the Australian Research Grants Scheme and The Ian Potter Foundation for financial assistance.

Spin-Echo ^{13}C NMR Spectroscopy for the Analysis of Deuterated Carbon Compounds

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Abstract: J modulation of ^{13}C spin-echo signals is used to develop a strategy for the analysis of deuterated carbon compounds, with the aim to distinguish ^{13}C NMR signals of quaternary carbons, CH, CH_2, CH_3, CHD, CH_2D, CHD_2, CD, CD_2, and CD_3 groups. Three different experimental approaches are discussed: (1) spin-echo modulation by $^1J(^{13}C,^1H)$ (^{1}H-SEFT) without ^{2}H decoupling as the simplest method available; (2) spin-echo modulation by either $^1J(^{13}C,^1H)$ or $^1J(^{13}C,^2H)$ with simultaneous ^{1}H and ^{2}H decoupling [these experiments use ^{1}H- and ^{2}H-decoupling facilities and gated decoupling on one decoupler channel]; (3) the combination of two ^{13}C spin-echo sequences with gated ^{1}H as well as ^{2}H decoupling finally leading to TANDEM-SEFT, a pulse sequence that provides the most general approach to the analysis of labeled carbon sites in organic molecules.

Analytical methods that allow a quick and unambiguous characterization of labeled carbon sites are of vital importance for deuterium labeling studies in organic and bioorganic chemistry. It was shown recently that modern pulse techniques in connection with ^{2}H decoupling can be employed successfully for this purpose.[1-3] Rinaldi and Baldwin[1] proposed a ^{13}C {^{2}H}-INEPT pulse sequence for the selective detection of deuterated carbons, while we showed[2,3] that the experimentally less demanding spin-echo sequence allows fully and partially deuterated carbons to be recognized if ^{2}H decoupling is used to control J modulation of transverse ^{13}C magnetization by one-bond ^{13}C,^{2}H spin-spin coupling.[2] It was further demonstrated that the analysis can be considerably improved by difference spectroscopy[3] and that two-dimensional J-resolved ^{13}C spectra can be used to unravel overlapping multiplets.[2]

Since ^{2}H decoupling and ^{19}F field/frequency lock facilities are normally not available with standard FT-NMR equipment, it seemed of interest to investigate an alternative approach that uses the well-known SEFT sequence[4] which is based on ^{1}H broadband decoupling and can be performed with most of the FT-NMR spectrometers presently in use, which are equipped with ^{1}H decoupler and ^{2}H lock channel:

^{13}C: 90°(x)— τ —180°(x)— τ —acquisition (1)

^{1}H: decoupler — on —→|←— off —→|←— on —

At the same time it was desirable to study further the potential of pulse sequences that use ^{1}H as well as ^{2}H decoupling.

Results

The time dependence of transverse ^{13}C magnetization modulated by ^{13}C,^{1}H as well as ^{13}C,^{2}H spin-spin coupling, respectively, has been worked out,[2,4,5] and the following relations for the signal intensity I can be derived if relaxation effects are neglected ($\tau \ll T_2$):

$$\text{CH:}\quad I = I_0 \cos(\pi J_{CH}\tau_1) \tag{1}$$

$$\text{CH}_2\text{:}\quad I = I_0 \cos^2(\pi J_{CH}\tau_1) \tag{2}$$

$$\text{CH}_3\text{:}\quad I = I_0 \cos^3(\pi J_{CH}\tau_1) \tag{3}$$

$$\text{CD:}\quad I = I_0(^1/_3 + {}^2/_3 \cos(2\pi J_{CD}\tau_2)) \tag{4}$$

$$\text{CD}_2\text{:}\quad I = I_0(^1/_3 + {}^2/_3 \cos(2\pi J_{CD}\tau_2))^2 \tag{5}$$

$$\text{CD}_3\text{:}\quad I = I_0(^1/_3 + {}^2/_3 \cos(2\pi J_{CD}\tau_2))^3 \tag{6}$$

If ^{1}H decoupling is used, eq 1-3 apply, and in principle, two experiments should distinguish between the various ^{13}C resonances expected for partially deuterated compounds due to the presence

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[1] Rinaldi, P. L.; Baldwin, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 5791.

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[4] (a) Rabenstein, D. L.; Nakashima, T. T. *Anal. Chem.* **1979**, *51*, 1465A. (b) Brown, D. W.; Nakashima, T. T.; Rabenstein, D. L. *J. Magn. Reson.* **1981**, *45*, 302. (c) For a review, see: Benn, R.; Günther, H. *Angew. Chem.* **1983**, *95*, 381; *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 350. SEFT = spin-echo Fourier transform.

[5] The compact notation for eq 1-3 is due to the following Radeglia, R. *Z. Chem.* **1982**, *22*, 252. Blunt, J. W.; Steel, P. J. *Aust. J. Chem.* **1982**, *35*, 2561.

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Table I. Composition of Test Mixture with Partially Deuterated Alkyl Groups

compound	% w/w	δ
1, $C_6H_5\mathit{CH_3}$	3.5	21.80
2, $C_6H_5\mathit{CH_2D}$	5.5	21.53
3, $C_6H_5\mathit{CHD_2}$	6.5	21.25
4, $C_6H_5\mathit{CD_3}$	24.0	20.97
5, $(C_6H_5)_2\mathit{CH_2}$	11.5	42.62
6, $(C_6H_5)_2\mathit{CHD}$	10.4	42.27
7, $(C_6H_5)_2\mathit{CH}CH_3$	4.5	45.54, 22.43
8, $(C_6H_5)_2\mathit{CD}CH_3$	6.0	45.11, 22.32
9, $(\mathit{CD_2})_6$	10.5	26.40
10, $(CH_3\mathit{CH_2CH_2}CH_2)_2O$	2.0	20.24, 32.82
11, $C_6H_5\mathit{CH_2CH_2}C_6H_5$	0.1	38.52
C_6F_6	17.0	internal lock

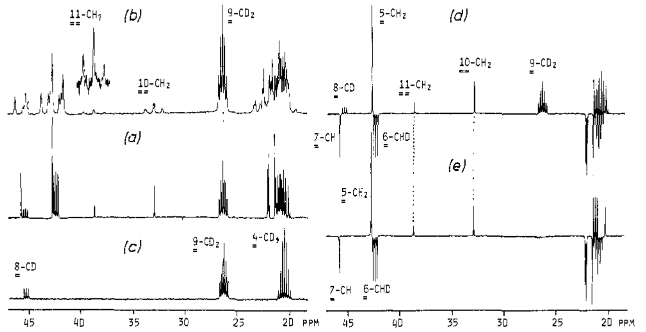

Figure 1. (a) ^1H broadband decoupled 100.61-MHz ^{13}C NMR spectrum of a mixture of compounds **1–11** (cf. Table I); (b) as in a, but ^1H coupled by using the gated ^1H decoupler technique; (c) result of the application of pulse sequence (I) with $\tau = 1/2^1J(^{13}C,^1H)$ (4 ms); (d) as in c, but with $\tau = 1/^1J(^{13}C,^1H)$ (8 ms); (e) difference spectrum obtained by substracting the time domain signal of spectrum c from that of spectrum d.

of nondeuterated sites (C, CH, CH_2, CH_3), partially deuterated sites (CHD, CH_2D, CHD_2), and fully deuterated sites (CD, CD_2, CD_3). It will be shown, however, that signal overlap may complicate the interpretation of the results. In these cases, but also for the analysis of mixed groups CH_nD_m, a new pulse sequence involving ^1H as well as ^2H decoupling is of advantage. It will be described in section III below.

I. SEFT Spectroscopy Using ^{1}H Decoupling (^{1}H-SEFT). The following section develops the strategy for the analysis of carbon sites in partially deuterated compounds on the basis of ^1H-SEFT, i.e., ^{13}C spin-echo spectroscopy with echo modulation by $^{13}C,^1H$ spin–spin coupling J over one bond. It uses the pulse sequence I with gated ^1H decoupling as given above.

In a first experiment, the choice of $\tau = 1/2J$ eliminates all signals from proton bearing carbons.[4] The spectrum thus contains only the singlets of quarternary carbons and the multiplets of the fully deuterated groups that can be recognized by their typical fine structure due to $^{13}C,^2H$ spin–spin coupling (CD, 1:1:1 triplet; CD_2, 1:2:3:2:1 quintet; CD_3, 1:3:6:7:6:3:1 septet).

A second experiment with $\tau = 1/J$ yields positive singlets for quarternary carbons and CH_2 groups and inverted singlets for CH and CH_3 groups.[4] In addition, the signals of fully deuterated groups appear as in the first experiment, and partially deuterated sites give rise to $^{13}C,^2H$ multiplets with positive or negative phase according to the number of protons present: CHD negative, 1:1:1 triplet; CHD_2 negative, 1:2:3:2:1 quintet; and CH_2D positive, 1:1:1 triplet.

A practical demonstration of this approach is now given with the spectra of a mixture of compounds **1–11** (Table I) that contain various groups of interest with their resonances in the aliphatic region of the ^{13}C δ scale where a value of $J = 125$ Hz is appropriate in all cases. From the ^1H broadband decoupled spectrum (Figure 1a) only the signals at δ 26.40, 32.82, and 38.52 can be assigned immediately with the help of a conventional ^1H coupled spectrum (Figure 1b) as belonging to the **9**-CD_2, **10**-CH_2, and **11**-CH_2 groups, respectively. The limitations of ^1H coupled spectra as assignment aids in case of strong signal overlap are obvious, especially in the region between 20 and 25 ppm.

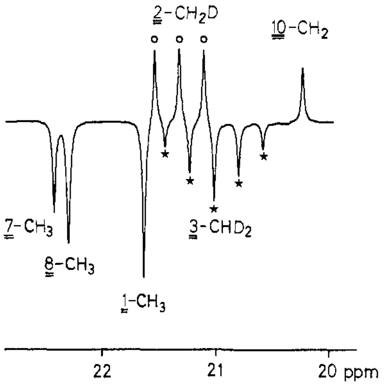

Figure 2. Enlarged upfield region of spectrum e, Figure 1.

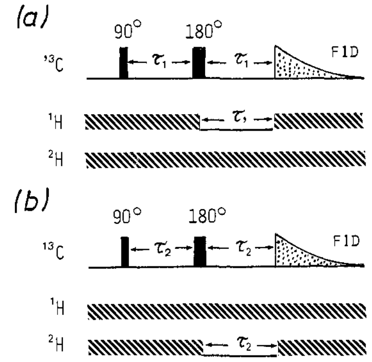

Figure 3. Possible pulse sequences and decoupler timing for ^{13}C spin-echo spectroscopy with simultaneous ^1H and ^2H decoupling and gated decoupling mode limited to one decoupler channel.

The analysis now proceeds with the proposed spin-echo sequence I. Figure 1c shows the result of experiment 1 with the typical multiplets for CD, CD_2, and CD_3 groups. Experiment 2 with $\tau = 1/J$ (Figure 1d) then allows without difficulty the analysis of the region below 25 ppm, since deuterated carbons are recognized by their multiplet structure and the partially deuterated groups can be selected by the different signal phase. For instance, the two 1:1:1 triplets of the CD and CHD groups in **8** and **6**, respectively, are clearly distinguished since only the latter is inverted due to the presence of one proton.

On the other hand, in the region above 25 ppm signal overlap again complicates the analysis. The interpretation of this part of the spectrum can be, however, considerably facilitated in the present case by the use of difference spectroscopy. Spectrum e in Figure 1 shows the result of such an experiment, where spectrum c was substracted from spectrum d (both in Figure 1) in the time domain. The signals around 20 ppm (see Figure 2 for details) are now clearly recognized by their different multiplicity and signal phase. Starting at low field the three CH_3 groups of **7**, **8**, and **1** are seen as inverted singlets, followed by the 1:1:1 triplet of the **2**-CH_2D group with positive phase. The quintet of the **3**-CHD_2 groups is again inverted, whereas the positive singlet at highest field must belong to a CH_2 group because of its positive phase and the absence of $^{13}C,^2H$ coupling. It is identified by its δ value of 20.24 as due to compound **10**.

II. SEFT Spectroscopy Using ^{1}H and ^{2}H Decoupling. As the second experiment described above has demonstrated, the result of the phase-selection step for ^1H-SEFT may suffer from severe line overlap, and sometimes even difference spectroscopy will not allow the situation to be improved. In this respect, ^2H-SEFT offers some advantages. With both methods, however, the analysis of partially deuterated groups (CHD, CHD_2, CH_2D) is still not satisfactory, since the observation of multiplet structures due either to ^1H or ^2H coupling is required. Progress is, however, possible if ^1H as well as ^2H decoupling can be used simultaneously. In its simplest form, one decoupler (^1H or ^2H) may be gated while the other one (^2H or ^1H) is applied continously.

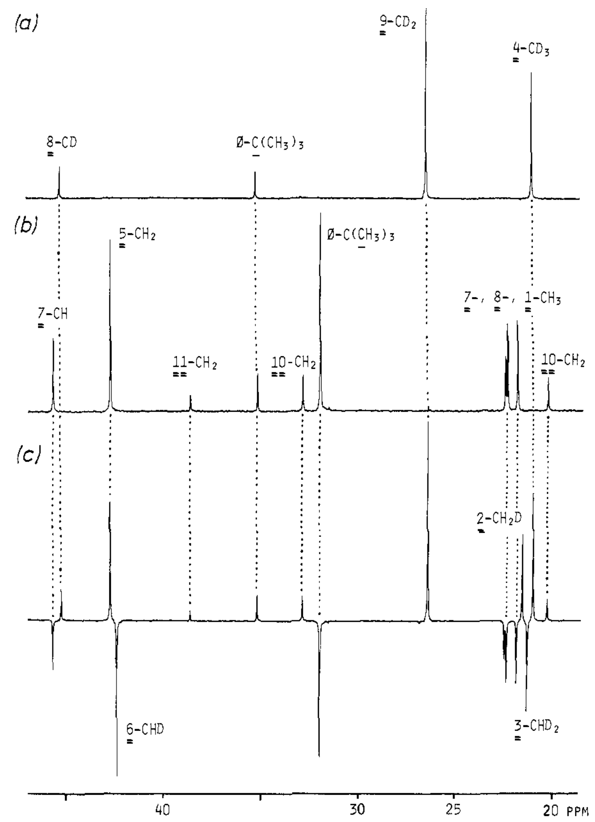

Figure 4. 100.61-MHz ^{13}C NMR spectrum of test mixture 2 (cf. Experimental Section) with the experimental conditions defined in Figure 3: (a) $\tau_1 = 4$ ms; (b) $\tau_2 = 17.4$ ms; (c) $\tau_1 = 8$ ms.

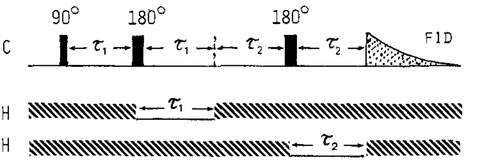

Figure 5. Pulse sequence and decoupler timing for TANDEM SEFT (cf. text).

Three experiments can now be realized for which the pulse sequence and decoupler timing are giving in Figure 3. The spectra edited on the basis of this technique are collected in Figure 4. Here, spectrum a was obtained with gated ^1H decoupling, $\tau_1 = 1/2J(^{13}C,^1H)$, and ^2H decoupling (sequence Figure 3a). It shows the singlet of the quarternary carbon and the lines of fully deuterated groups. All signals belonging to proton-bearing carbons are suppressed. In spectrum b, all signals of deuterated carbons are eliminated by gating the ^2H decoupler with $\tau_2 = 1/3J(^{13}C,^2H)$ in the presence of ^1H decoupling (sequence Figure 3b).[3] Only signals of the quarternary carbon and fully protonated groups are detected. Finally, spectrum c uses ^2H decoupling and phase selection for CH multiplicity by gated ^1H decoupling with $\tau_1 = 1/J(^{13}C,^1H)$. Mixed groups (CHD, CHD_2, CH_2D) only appear in spectrum c and can be recognized by their positive or negative phase, respectively, and their δ value.

III. TANDEM-SEFT. A next step that further increases the versatility of the analysis is the joint application of gated ^1H as well as gated ^2H decoupling. This experiment can be performed using either one spin-echo sequence or two successive spin-echo sequences, a version we call TANDEM-SEFT. The pulse sequence for TANDEM-SEFT is given in Figure 5. It requires the independent timing of both decouplers and is characterized by two delays τ_1 and τ_2, which govern spin-echo modulation by $^{13}C,^1H$ and $^{13}C,^2H$ spin–spin coupling, respectively. It offers four unique experiments which cannot be performed with either gated ^1H or ^2H decoupling alone: (1) selective detection of quarternary carbons ($\tau_1 = 1/2J(^{13}C,^1H)$ and $\tau_2 = 1/3J(^{13}C,^2H)$); (2) phase

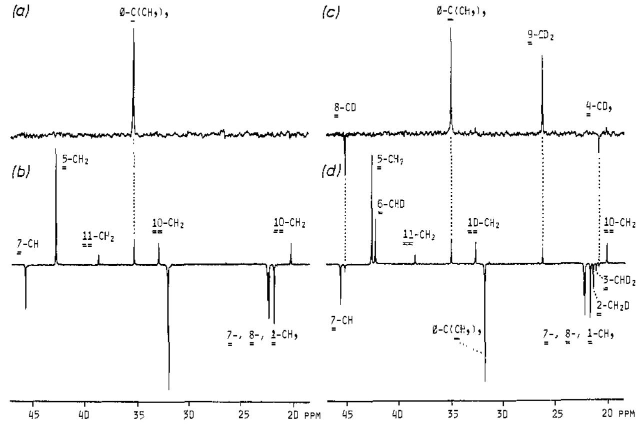

Figure 6. 100.61-MHz ^{13}C NMR TANDEM SEFT spectra of test mixture 2 (cf. Experimental Section) with experiments 1–4 described (cf. text); (a) experiment 1, $\tau_1 = 4$, $\tau_2 = 17.4$ ms; (b) experiment 2, $\tau_1 = 8$, $\tau_2 = 17.4$ ms; (c) experiment 3, $\tau_1 = 4$, $\tau_2 = 26.1$ ms; (d) experiment 4, $\tau_1 = 8$, $\tau_2 = 26.1$ ms.

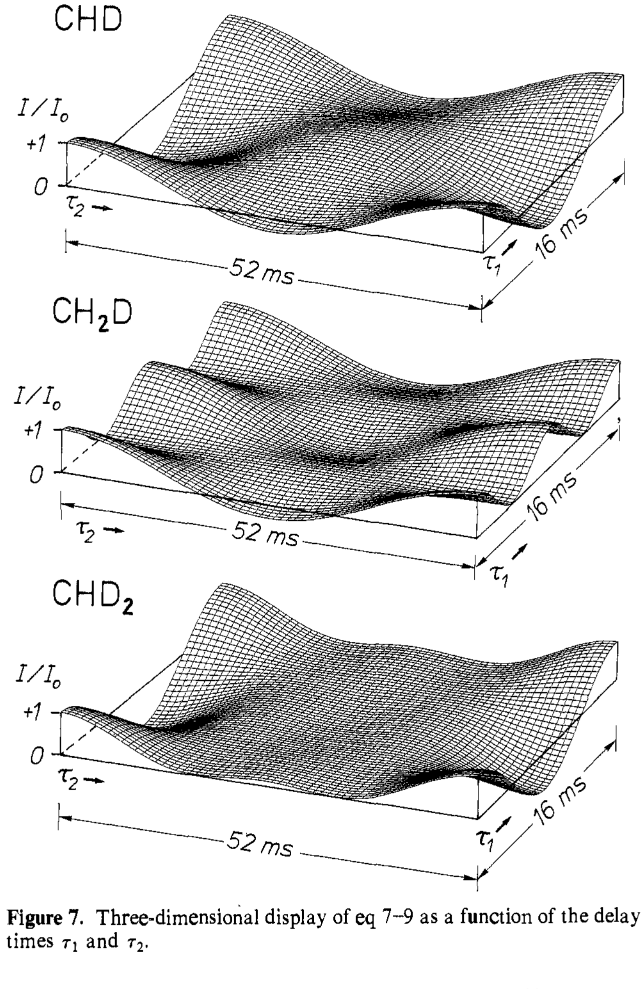

Figure 7. Three-dimensional display of eq 7–9 as a function of the delay times τ_1 and τ_2.

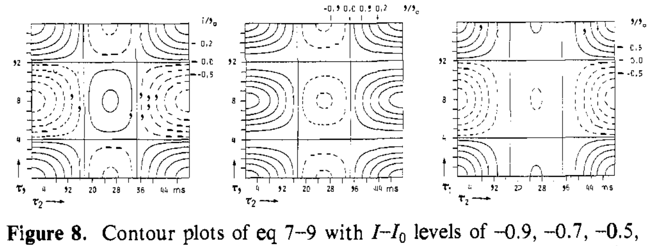

Figure 8. Contour plots of eq 7–9 with I–I_0 levels of –0.9, –0.7, –0.5, –0.3, –0.1, 0, 0.1, 0.3, 0.5, 0.7, and 0.9; dotted lines represent negative values.

Table II. Relative ^{13}C Intensities of CH_nD_m Groups for TANDEM-SEFT Experiments with Different Delay Times τ_1 and τ_2[a]

τ_1	τ_2	C	CH	CH_2	CH_3	CHD	CH_2D	CHD_2	CD	CD_2	CD_3
A		+1	−1	+1	−1	−1	+1	−1	+1	+1	+1
B		+1	0	0	0	0	0	0	+1	+1	+1
	C	+1	+1	+1	+1	−1/3	−1/3	+1/9	−1/3	+1/9	−1/27
	D	+1	+1	+1	+1	0	0	0	0	0	0
A	*D*	+1	−1	+1	−1	0	0	0	0	0	0
B	*D*	+1	0	0	0	0	0	0	0	0	0
A	*C*	+1	−1	+1	−1	+1/3	−1/3	−1/9	−1/3	+1/9	−1/27
B	*C*	+1	0	0	0	0	0	0	−1/3	+1/9	−1/27

[a] $A = 1/J(^{13}C,^1H)$; $B = 1/2J(^{13}C,^1H)$; $C = 1/2J(^{13}C,^2H)$; $D = 1/3J(^{13}C,^2H)$. Typical values for groups with sp^3 carbons are $^1J(^{13}C,^1H) = 125$ Hz, $^1J(^{13}C,^2H) = 19$ Hz, which yields (in ms) $A = 8$, $B = 4$, $C = 26$, $D = 17$; for groups with sp^2 carbons $^1J(^{13}C,^1H) = 160$ Hz and $^1J(^{13}C,^2H) = 25$ Hz and $A = 6$, $B = 3$, $C = 20$, $D = 13$.

selection for CH_n groups with elimination of all signals of CD_m and CH_nD_m groups ($\tau_1 = 1/J(^{13}C,^1H)$, $\tau_2 = 1/3J(^{13}C,^2H)$); (3) phase selection for CD_m groups with elimination of all signals from CH_n and CH_nD_m groups ($\tau_1 = 1/2J(^{13}C,^1H)$, $\tau_2 = 1/2J(^{13}C,^2H)$); and (4) phase selection for all groups due to their ^{1}H and ^{2}H multiplicity ($\tau_1 = 1/J(^{13}C,^1H)$, $\tau_2 = 1/2J(^{13}C,^2H)$). The results of these experiments are collected in Figure 6.

The analytical expression for the signal intensity, *I*, of mixed groups obtained by TANDEM-SEFT, neglecting relaxation effects, is given by the appropriate products of eq 1–6: A graphical display of eq 7–9 as a function of the delay times τ_1 and τ_2 is given in Figure 7 and 8; Table II provides a complete survey of the relative signal intensities for various combinations of τ values—taking into account also different ^{13}C,^{1}H coupling constants—which can be used as a guideline for experimental applications.

$$\text{CHD:} \quad I = I_0 \cos(\pi J_{CH}\tau_1)[\tfrac{1}{3} + \tfrac{2}{3}\cos(2\pi J_{CD}\tau_2)] \qquad (7)$$

$$\text{CH}_2\text{D:} \quad I = I_0 \cos^2(\pi J_{CH}\tau_1)[\tfrac{1}{3} + \tfrac{2}{3}\cos(2\pi J_{CD}\tau_2)] \qquad (8)$$

$$\text{CHD}_2\text{:} \quad I = I_0 \cos(\pi J_{CH}\tau_1)[\tfrac{1}{3} + \tfrac{2}{3}\cos(2\pi J_{CD}\tau_2)]^2 \qquad (9)$$

Conclusions

In its present form, TANDEM-SEFT provides the most general approach to the analysis of labeled carbon sites available on the basis of spin-echo spectroscopy. The use of polarization transfer methods like INEPT[1] and DEPT[6] needs more sophisticated instrumentation with phase shifts for the ^{2}H decoupler channel.[7] TANDEM-SEFT also allows the selective detection of quarternary carbon resonances that are suppressed by polarization transfer methods. A drawback in comparison to techniques using $\{^2H\}^{13}C$ polarization transfer might be seen in the long relaxation times of fully deuterated carbons that lead to relatively long repetition delays. In contrast, the repetition time for polarization transfer experiments of the type indicated is governed by the short ^{2}H relaxation time. It is therefore of importance that with TANDEM-SEFT smaller flip angles[8] for the first ^{13}C pulse can be used without further provision, since two spin-echo sequences allow the inversion of residual *z* magnetization.[9] If the τ_1 and τ_2 evolution periods are incorporated in one spin-echo experiment only, smaller flip angles call for the addition of a second Δ, 180°, Δ cycle (Δ ~ 2 ms). The same is true for the sequences discussed in sections I and II. If a ^{2}H decoupler is not available, the approach described in section I must be used for the analysis of deuterated sites, taking advantage of difference spectroscopy where necessary.

Experimental Section

Compounds. The compounds used in this study are collected in Table I. For the experiments described in sections II and III 3% w/w *tert*-butylbenzene [δ (C) 35.10, δ (CH_3) 31.92] was added (test mixture 2). Labeled systems were prepared by standard methods: **2** was derived from benzylmagnesium chloride with D_2O; **3** was obtained by $LiAlD_4$ reduction of benzaldehyde, reaction of the alcohol with $SOCl_2$, and subsequent treatment of the halide with magnesium and D_2O; **4** was obtained from ethyl benzoate by $LiAlD_4$ reduction and the treatment of the alcohol in the manner mentioned for **3**; **6** was obtained by direct metalation with BuLi followed by reaction with D_2O; whereas **8** was obtained by H/D exchange from $(C_6H_5)_2CHCH_3$ by reaction with NaH in Me_2SO-d_6.

Spectra. ^{13}C NMR spectra were recorded on a Bruker WH-400 spectrometer operating at 100.61 MHz and equipped with ^{1}H (400.13 MHz) and ^{2}H (61.42 MHz) decoupler units and a ^{19}F lock channel (376.5 MHz); C_6F_6 was used as internal lock compound. The ^{2}H decoupler was controlled by the external pulse channel of the Bruker Aspect 2000-pulse programmer, whereas the ^{1}H decoupler was controlled as usual through the FTQ NMR program. The spectral width used was 4000 Hz with 256 transients and 16 K data points. For TANDEM-SEFT 512 transients were used. Digital resolution was 0.488 Hz, and the signal-to-noise ratio was improved by standard exponential multiplication. Measurements were made in nonrotating sample tubes to avoid artefacts through spinning sidebands; the pulse width for the 90° pulse was 22 μs. Difference spectra were obtained by alternatingly adding and substracting groups of 16 scans directly into the same computer memory.

Acknowledgment. We are indebted to P. Rickert for his assistance in the hardware modifications and to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for support.

Registry No. 1, 108-88-3; **2**, 1861-00-3; **3**, 17119-69-6; **4**, 1124-18-1; **5**, 28652-72-4; **6**, 20389-18-8; **7**, 612-00-0; **8**, 87245-79-2; **9**, 1735-17-7; **10**, 142-96-1; **11**, 103-29-7; D_2, 7782-39-0.

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