COMMUNICATIONS

DEPTQ: Distorsionless Enhancement by Polarization Transfer Including the Detection of Quaternary Nuclei

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A pulse sequence DEPTQ yielding the signals and multiplicity information for all carbon types including the signals of quaternary carbons and encompassing all the known advantages of the basic DEPT experiment is proposed. Its behavior has been studied theoretically and experimentally and has been compared critically with alternative methods dedicated for the same purpose. Its marked insensitivity to experimental parameters and its potential for complete and efficient spectral editing makes DEPTQ the ideal experimental platform for a semi- or fully automated analysis of 1D ^{13}C spectra.   © 1998 Academic Press

Key Words: NMR; 1D ^{13}C spectroscopy; ^{13}C multiplicity; spectral editing.

INTRODUCTION

Multiplicity dependent 1D ^{13}C experiments such as DEPT (*1*), SEMUT (*2*), APT (*3*), INEPT (*4*), and PENDANT (*5*) belong to the most common and popular tools for the elucidation and characterization of molecular structures on a routine level, although they are increasingly replaced nowadays by modern ^{1}H-detected heteronuclear 2D shift-correlation experiments including experiments with carbon multiplicity selection (*6–11*). Because of the low J cross-talk dependence on a spread in J couplings, the low dependence on pulse imperfections and the high editing accuracies achieved with DEPT and SEMUT in comparison with APT, INEPT, and PENDANT, and the capability to calculate multiplicity selective subspectra, one of these two experiments is usually applied. The still popular APT experiment is almost exclusively used for partial editing to distinguish CH_3/CH and CH_2/Cq signals from each other, an approach often sufficient to successfully solve simple spectroscopic problems. In contrast to the APT and the SEMUT sequence DEPT yields exclusively the signals of proton-bearing carbons CH_n, whereas the signals of quaternary carbons Cq are suppressed. As a consequence and to complete ^{13}C data DEPT is usually combined with a one-pulse ^{13}C experiment or the "Cq-only" (SEMUT-90) sequence as proposed independently by Doddrell *et al.* (*12, 13*) and Bildsøe *et al.* (*2*).

The second variant is probably superior because there is no possibility of overlap of Cq with CH_n signals but may be hampered by additional relaxation losses with the prolonged pulse sequence compared to the one-pulse experiment. For complete spectral editing, a procedure of increasing interest for fully automated spectral analysis, at least three and four subexperiments with different flip angles of the ^{1}H selection pulse must be performed with DEPT and SEMUT respectively. The behavior of these two experiments for complete spectral editing has been compared, and optimized conditions with respect to the flip angle of the ^{1}H selection pulse and the number of scans for the corresponding subexperiments have been described (*2, 14*). Modifications for both experiments for improving the editing accuracy have been proposed, such as additional phase alternation schemes and composite pulses for DEPT (*12*) and the use of unequal delays and purging pulse sandwiches instead of ^{1}H–180° pulses for molecules with large $^{1}J_{CH}$ variations and to minimize J-cross talk effects for DEPT and SEMUT with (*15, 16*) and without (*17*) ^{1}H broadband decoupling during acquisition. Comparisons showed that SEMUT (*18*) and its modified versions (*14*) provide less S/N in the final subspectra of CH_n and are slightly more sensitive to experimental parameters than DEPT and its correspondingly modified versions.

The aim of our efforts was to develop a pulse sequence based on DEPT, yielding the signals of all carbon multiplicities, including the signals of quaternary carbons, offering the capability for complete and efficient spectral editing, and encompassing all of its advantages such as the low dependence on miscalibrated experimental parameters and simplicity for complete spectral editing. In this Communication a corresponding pulse sequence DEPTQ (Fig. 1) provided with all these attributes is presented and is compared with competitive alternatives for complete spectral editing. Compared to the basic DEPT experiment two further ^{13}C pulses (a third one prior to acquisition is optional) and an extra delay D2 = $1/(2 \, ^{1}J_{CH})$ have been included at the beginning of the pulse sequence.
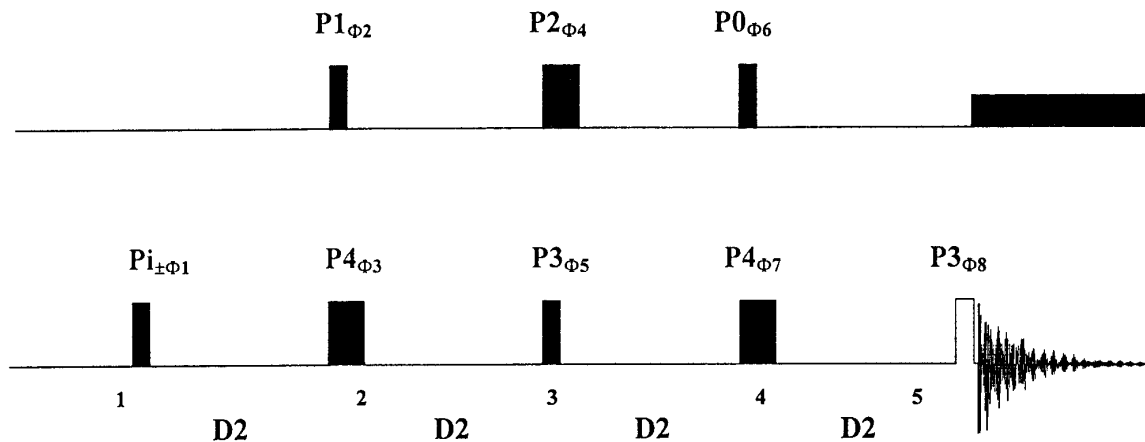
FIG. 1. Schema of the DEPTQ pulse sequence used to record multiplicity-dependent 1D ^{13}C spectra including the signals of quaternary carbons. P1, P2 and P3, P4 denote proton and carbon 90°, 180° pulses respectively. P0 denotes the proton selection pulse, and D2 is set to $1/(2J_{\mathrm{CH}})$. Pi, the initial ^{13}C pulse, may be set to 90° or may be adjusted to the longest T1$_{\mathrm{C}}$. In the latter case and to take full advantage of the additional sensitivity gain for quaternary carbons the last 90° ^{13}C pulse is no longer optional. The phase cycles used in this experiment were $\Phi_1 = 4(-y)$; $\Phi_2 = 4(x)$; $\Phi_3 = x, -x, y, -y$; $\Phi_4 = x, -x, y, -y$; $\Phi_5 = 4(x)$; $\Phi_6 = 4(y)$; $\Phi_7 = x, -x, x, -x$; $\Phi_8 = 4(-x)$; $\Phi_{\mathrm{rec}} = y, y, -y, -y$. To disentangle the signals of proton-bearing and of quaternary carbons and for spectral editing, two subexperiments with the phase Φ_1 of the initial carbon pulse set to $4(-y)$ and $4(y)$ must be performed, and the corresponding data sets must be added to and subtractacted from each other respectively.

Since the magnetiziations of CH$_n$ signals when compared with DEPT are completely unaffected by these additional elements in the DEPTQ sequence and are identical within experimental error and since the sensitivities for CH$_n$ signals obtained with DEPT and SEMUT are more or less the same, their S/N will not be compared here. The subsequent investigation focuses on complete spectral editing with the aim to obtain the subspectra of all carbon multiplicities and on a comparison of the overall sensitivity per unit time and the editing accuracy in the subspectra of quaternary carbons measured with DEPTQ and SEMUT. The comparison is furthermore restricted to the simplest forms of the experiments and the detection of ^{1}H broadband decoupled spectra since neither DEPT nor SEMUT are suitable for editing of coupled ^{13}C spectra in these circumstances (*17*).

Whether complete editing, using either DEPTQ or SEMUT, partial editing, or no editing at all should be performed and whether the sensitivity or the editing accuracy in the subspectra is of prime importance highly depends on the actual problem and on the spectroscopists personal preferences.

It is well known that with the basic DEPT experiment and with a single scan the signals of all carbon multiplicities—including quaternary carbons—may be detected. In a single scan ^{13}C responses of proton-bearing carbons are the superposition of coherences originating from the initital proton polarization ($\gamma_{\mathrm{H}}I_{\mathrm{H}z}$), transformed into transverse carbon coherence by polarization transfer and the initial carbon polarization ($\gamma_{\mathrm{C}}I_{\mathrm{C}z}$), whereas ^{13}C responses of quaternary carbons originate exclusively from the initial carbon polarization. Since the acquired carbon coherences of different origin are 90° out of phase to each other, they give rise to line distortions for the signals of proton-bearing carbons in single-scan spectra. Phase

cycling is used to get rid of these unwanted line distortions but removes unfortunately the signals of quaternary carbons as well. Applied in this way DEPT exploits exclusively the initial proton polarization, whereas SEMUT and APT exploit exclusively the initial, NOE-enhanced carbon polarization. This is the fundamental difference between the two types of experiments.

Compared to the basic DEPT experiment additional ^{13}C pulses and an additional delay D2 have been introduced in the DEPTQ experiment. These additional elements and an adequate setting of RF pulse phases are responsible for why coherences of quaternary carbons survive and unwanted coherences causing unwanted line distortions for proton-bearing carbons are cancelled in the course of the pulse sequence in a single scan. They have no effect on the initial proton polarization and hence on the intensity of the CH$_n$ signals in the spectrum as outlined above. The carbon pulses act on the initial carbon polarization as two simple spin-echoes, with the Cq signals refocused twice and not affected by the second and third ^{13}C 90° pulses. This is demonstrated in terms of product operators (*19*) for a spin system consisting of a CH and a Cq group (Table 1). The ^{1}H-selection pulse P0 and the delay D2 are set to 135° and $1/(2$ $^1J_{\mathrm{CH}})$ respectively, and proton and carbon chemical shifts are set to zero for simplification. Product operator expressions calculated for the RF pulse phase settings of the first scan are given for selected evolution stages correspondingly marked in Fig. 1.

It is obvious that the responses of CH and Cq may be detected, that the corresponding signals are both absorptive and in antiphase to each other, and that the same theoretical sensitivity for CH as calculated for DEPT is obtained. Furthermore it follows that the corresponding signals originate from single terms—for CH from the initial ^{1}H polarization ($\gamma_{\mathrm{H}}I_{\mathrm{H}z}$),

TABLE 1
Characterization of the DEPTQ Pulse Sequence in Terms of Product Operators

	1	2	3	4	5
CH	$\gamma_H I_{Hz}$	$-\gamma_H I_{Hy}$	$-\gamma_H 2I_{Hx}I_{Cy}$	$-0.7\,\gamma_H 2I_{Hx}I_{Cy}$ $-0.7\,\gamma_H 2I_{Hz}I_{Cy}$	$-0.7\,\gamma_H 2I_{Hx}I_{Cy}$ $\mathbf{0.7\,\gamma_H I_{Cx}}$
	$\gamma_C I_{Cz}$	$-\gamma_C 2I_{Hy}I_{Cy}$	$\gamma_C 2I_{Hy}I_{Cz}$	$0.7\,\gamma_C I_{Hx}$ $0.7\,\gamma_C I_{Hz}$	$0.7\,\gamma_C 2I_{Hy}I_{Cz}$ $0.7\,\gamma_C I_{Hz}$
Cq	$\gamma_C I_{Cz}$	$-\gamma_C I_{Cx}$	$-\gamma_C I_{Cx}$	$-\gamma_C I_{Cx}$	$\mathbf{-\gamma_C I_{Cx}}$

Note. Corresponding expressions for a CH and a Cq group are given for selected evolution stages correspondingly marked in Fig. 1. Both coherence pathways for the CH system originating from initial proton and carbon polarization respectively are shown. Final terms responsible for the measured spectrum are given in bold type. The ^{1}H-selection pulse P0 and the delay D2 have been set to 135° and $1/(2\,^1J_{CH})$ respectively, and proton and carbon chemical shifts have been set to zero for simplification.

for Cq from the initial ^{13}C polarization ($\gamma_C I_{Cz}$)—giving rise to no unwanted line distortions. The same results are valid for the other carbon multiplicities CH$_2$ and CH$_3$ as well and—most important—are achieved in a single scan with no phase cycling at all.

The optional third ^{13}C 90° pulse, which leaves the carbon magnetizations of CH$_n$ unaffected, is applied in conjunction with smaller tip angles for the initial ^{13}C pulse π (Ernst angle) for optimizing the S/N of Cq's with long longitudinal relaxation times. It reestablishes the magnetization of quaternary carbons in the yz plane prior to acquisition and prevents phasing problems with the corresponding signals.

RESULTS AND DISCUSSION

Both pulse sequences DEPTQ and SEMUT have been designed for complete spectral editing, i.e., for the final calculation of multiplicity selective carbon subspectra. Therefore comparative experiments have been performed in a corresponding manner, although the main interest focuses on the behavior of quaternary carbons for the reasons outlined above. However a comparison of the sensitivity and the editing accuracy in Cq subspectra on the basis of equal total measuring times is not so straightforward since DEPTQ, SEMUT, and the basic one-pulse ^{13}C experiment—included as the reference experiment—undergo different mechanisms, and different procedures to measure and calculate carbon subspectra are followed with the editing sequences. Different parameters for data acquisition and processing such as repetition rates or mixing factors, both optimized for maximum S/N and most convenient for data processing respectively, have been proposed for DEPT (*14*) (valid for DEPTQ as well) and SEMUT (*3*). As a consequence of such an individual experimental setup, different number of scans would have to be acquired within the same total measuring time for each experiment, and different noise levels would result for the corresponding spectra. To make the comparison on the basis of equal total measuring times as clear as possible the experimental procedure for

this investigation was as follows. A sample of eugenol dissolved in acetone-d6, covering all carbon multiplicities and covering a wide range of $^1J_{CH}$ coupling constants, was used for the measurements.

First the relaxation delay D1 was varied for all three experiments, DEPTQ, SEMUT, and the one-pulse ^{13}C experiment, in order to study and maximize the S/N per unit time for the Cq's. As a result similar D1 dependencies were obtained with SEMUT and the one-pulse experiment with a maximum—taking into account all three quaternary carbons of eugenol—at D1$_{opt.}$ = 2 s. A higher S/N for the one-pulse experiment than for the SEMUT experiment was observed throughout which must be attributed to additional losses due to relaxation and pulse imperfections with the longer pulse sequence. With DEPTQ on the other hand the highest S/N per unit time for Cq's was obtained with relaxation delays close to zero. The S/N measured for D1 = 0 is almost the same as that with SEMUT but smoothly and steadily decreases with increasing relaxation delays. This different behavior must be attributed to different amounts of heteronuclear NOE built up from the quaternary carbons in the course of the two pulse sequences. For SEMUT the NOE buildup occurs in both the acquisition and the relaxation period but is restricted to the acquisition period with a rapid NOE decrease in the subsequent relaxation period with no ^{1}H broadband decoupling for DEPTQ. Therefore and for quaternary carbons exhibiting NOE in general the sensitivity gain for the corresponding signals achieved with SEMUT compared to DEPTQ depends on the amount of dipolar interactions, i.e., the number and spatial proximity of nearby protons and the relaxation delay D1. With the investigated sample this sensitivity gain (per unit time) is close to zero for D1 = 0 s and increases to about 35% for D1 = 2 s. Because of this different behavior the relaxation delay D1 should be adjusted according to the corresponding T1(C) for SEMUT but should be adjusted—in a way similar to that for the basic DEPT experiment—to the corresponding T1(H) for DEPTQ. For DEPTQ the relaxation delay should be set as short as possible for highest Cq sensitivity, but long enough for adequate CH$_n$ sensitivity. Despite this difference and to not further

complicate the comparison, the same relaxation delay D1 = 2 s, optimized for the NOE-enhanced one-pulse and the SE-MUT sequence, was used for all subsequent experiments. For DEPTQ and SEMUT the delay D2 = $1/(2\ ^1J_{CH})$ was adjusted to a one-bond coupling constant of 145 Hz. For DEPTQ the initial carbon pulse was set to 90°, and the final (optional) 90° carbon pulse was omitted.

The following experiments were then carried out with the same total number of acquired scans for each method in order to compare the results on the basis of exactly the same total measuring time:

1. With DEPTQ two subexperiments solely differing in the phase ($\pm x$) of the initial carbon pulse (Fig. 1) were carried out for all three ^1H selection pulses (P0 = 45°, 90°, and 135°). Equal number of scans (NS = 16) were performed for all six experiments, giving a total of 96 scans, and the corresponding data were stored separately. In a first step the two data sets acquired for each ^1H selection pulse were added and subtracted from each other, yielding two preprocessed data sets with the signals of the CH_n and Cq groups respectively. In a second step the three data sets with the CH_n signals were combined in the usual way to finally obtain the corresponding edited subspectra (not shown) for CH, CH_2, and CH_3 respectively, whereas the three data sets with the Cq signals were simply coadded and processed to give the final Cq edited subspectrum (Fig. 2, spectrum A).

2. Following the guidelines and recommendations given for SEMUT (2) aimed to simplify data processing and to make best use of the measured data, four subexperiments with the ^1H selection pulse P0 set to 0°, 60°, 120°, and 180° were carried out. For the four data sets, an equal number of scans (NS = 24), giving the same total of 96 scans as for DEPTQ, were acquired, were stored separately, and were combined to calculate the subspectra for all carbon multiplicities according to the recipe given in the literature. To calculate the final Cq subspectrum (Fig. 2, spectrum B) the two pairs of data obtained with the selection pulse set to P0 = 0°, 180° and P0 = 60°, 120° were coadded separately in a first step, and the corresponding preprocessed data sets X and U were then combined in a second step according to $U - \cos^2\rho \cdot X$ with $\rho = 60°$. To compare the result with the results of the other experiments on the basis of equal noise levels spectrum B was scaled according to these mixing factors.

3. With the ^1H selection pulse P0 set to 90°, a "Cq-only" experiment, acquiring 96 scans, was performed with SEMUT (Fig. 2, spectrum C).

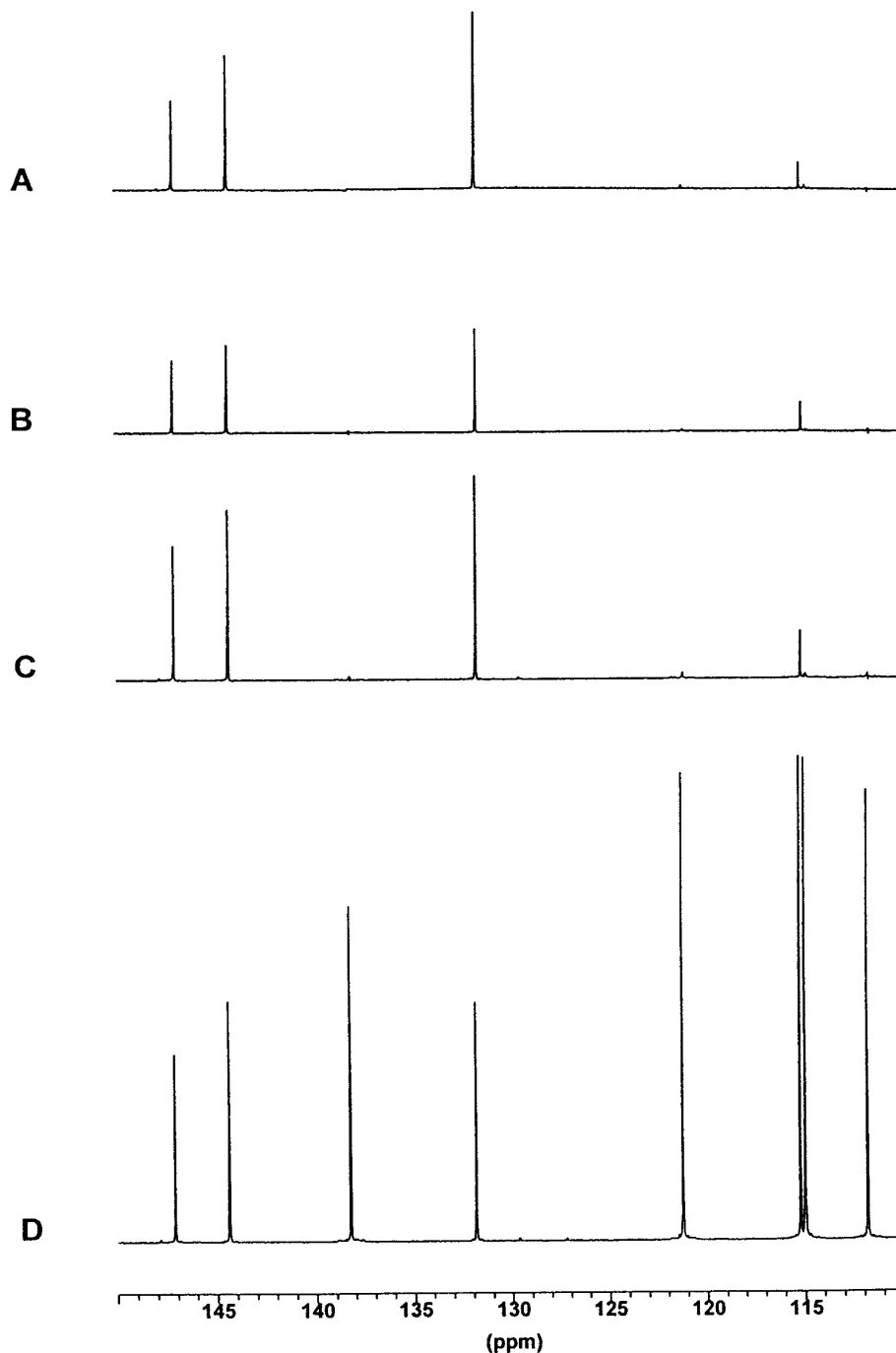4. As a reference a one-pulse ^{13}C experiment with 96 scans was carried out (Fig. 2, spectrum D).

As a result, and most obvious from Fig. 2, improved sensitivities are achieved mainly for Cq—but to a minor extent also for CH_n—(not shown) with DEPTQ compared to SEMUT if applied as single methods for complete spectral editing. Comparable editing accuracies for Cq and CH_n are obtained with these two methods. The lower efficiency of SEMUT observed

for Cq is simply caused by the fact that the signals of Cq and CH_2—in contrast to DEPTQ—both originate from inital carbon polarization and, irrespective of the ^1H selection pulse, appear with the same sign in the corresponding subspectra. This necessitates the unfavorable processing scheme outlined above, heavily affecting the overall S/N in order to completely disentangle Cq and CH_2 signals in the final edited subspectra. As a consequence SEMUT alone is obviously not the best approach for complete spectral editing and as recommended in the literature (2, 12) should be applied in combination with DEPT as a "Cq-only" sequence with the ^1H selection pulse set to P0 = 90° and D1 adjusted accordingly for this purpose. SEMUT-90 yields by far the best S/N per unit time as shown in Fig. 2 (spectrum C).

We believe, however, that with DEPTQ an even better and more efficient strategy for complete spectral editing is now available and that DEPTQ instead of DEPT should be combined with SEMUT-90 to make best use of the available measuring time. The procedure we propose for this most sensitive approach per unit time would be as follows: In a first step and with the relaxation delay adjusted according to T1(H) for CH_n in the same way as for DEPT, DEPTQ spectra are acquired until the S/N is adequate for protonated carbons. Note that short relaxation times D1 are no handicap for the detection of Cq's exhibiting heteronuclear NOE as outlined before. In a second step and only in case the S/N for quaternary carbons is not sufficient, a SEMUT-90 experiment with the relaxation delay adjusted according to T1(C) to most efficiently measure quaternary carbons is performed, and the data are coadded to the edited DEPTQ quaternary data until S/N is adequate as well.

CONCLUSIONS

The proposed DEPTQ pulse sequence is a sensitive and easy-to-use routine experiment for measuring carbon chemical shifts and carbon multiplicities respectively. In contrast to the basic DEPT experiment it yields the signals of all carbon multiplicities, including those of quaternary carbons, without sacrificing any of the well-known advantages of the DEPT experiment such as the low dependence on misset experimental parameters and the dependence on coupling constants in the case of large $^1J_{CH}$ variations. This makes DEPTQ a valuable stand-alone experiment for complete spectral editing, i.e., for obtaining individual Cq, CH, CH_2, and CH_3 subspectra, and makes it superior to corresponding alternatives such as the less sensitive SEMUT experiment or the recently proposed PENDANT sequence hampered by its lower editing quality. Most efficient for complete editing in view of the highest sensitivity per unit time, however, is the combined application of DEPTQ and SEMUT with the latter performed as a "Cq-only" experiment (SEMUT-90). This strategy followed in those cases where the S/N achieved with DEPTQ is adequate for protonated carbons, but is not yet adequate for quaternary carbons, is obviously superior to the

FIG. 2. Comparison of the DEPTQ, SEMUT, SEMUT-90, and the one-pulse ^{13}C (reference) experiments applied to eugenol dissolved in acetone-d6 and performed on a BRUKER DRX-400 spectrometer, with 90° pulse lenghts of 9.1 and 6.2 us for ^{1}H and ^{13}C respectively. The DEPTQ and SEMUT experiments were set up and applied for complete spectral editing as described in the text, whereas SEMUT-90, yielding exclusively the signals of quaternary carbons, was applied for comparison. Several subexperiments with different ^{1}H selection pulses were performed with DEPTQ and SEMUT, and the corresponding data were stored separately. The same relaxation delay (D1 = 2 s) and the same delay for coupling evolution (D2 = 3.45 ms) were used throughout. An equal total number of scans were acquired within the same total measuring time with each experiment. As a result the corresponding Cq subspectra (expansion 110–150ppm) obtained with DEPTQ (A), SEMUT (B), and SEMUT-90 (C) are shown together with the corresponding spectrum of the one-pulse experiment (D). The residual signal at 115.2 ppm, visible in all edited spectra, originates from the olefinic methylene group.

combined application of the basic "CH$_n$-only" DEPT experiment and SEMUT-90 recommended in the literature. Although many of the more routine problems with molecular structures may be solved by partial instead of complete editing of carbon spectra, performing well-established methods such as APT, SEMUT-180, DEPT-135, or no editing at

all and although powerful 2D alternatives exist for this purpose, highly efficent 1D methods for complete spectral editing, including the signals of quaternary carbons, will be valuable tools for daily routine use and will be the prerequisite for a fully automated spectral analysis.

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