

ORSAT and Modifications of SEFT and APT

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Received July 6, 1998; revised October 14, 1998

Up to now, three subspectra are usually needed for the complete assignment of all signals in ¹³C spectrum: two DEPT spectra ($\phi = 90^\circ$ and $\phi = 135^\circ$) and a proton decoupled spectrum. In this paper, we present a method in which a complete assignment becomes possible with merely two spectra. For this purpose, a new pulse sequence (ORSAT) has been elaborated by using off-resonance irradiation. The method described here is a further development of SEFT and APT. The second required spectrum is a DEPT ($\phi = 135^\circ$). Signal assignment of cholesteryl acetate is demonstrated as an example. ¹³C routine spectroscopy can be significantly accelerated by applying this method. © 1999 Academic Press

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Key Words: ¹³C spectroscopy; assignment of signals; off-resonance synchronization; pulse sequence.

An important aim of ¹³C routine spectroscopy is the fast identification of all signals in the spectrum as quaternary, CH, CH₂, and CH₃. For this purpose, three subspectra were previously required, namely a CPD- or BB-decoupled spectrum as well as two DEPT (*I*–*3*) spectra. The CPD-decoupled spectrum contains all resonances, including quaternary carbons. One DEPT subspectrum ($\phi = 135^\circ$) provides CH, CH₃ > 0, and CH₂ < 0. The other ($\phi = 90^\circ$) provides CH signals only.

CPD-decoupled spectra only provide signals with the same (positive) polarity. With SEFT (4, 5), APT (6–8), and PENDANT (9, 10), methods are already known that provide quaternary signals on the one hand and distinguish CH, CH₂, and CH₃ by polarity on the other.

All methods mentioned here can be optimized for a specific value of the coupling constant only. Signals whose couplings differ from this value can only be recorded with loss of intensity.

If the splittings of all CH_{*n*} signals were of the same size, this would mean a much more regular, time-synchronized evolution of the spin systems. The gain in transverse magnetization would be larger. Signal amplitudes would increase.

This task can be achieved in an almost optimal manner by the use of off-resonance decoupling (11, 12). If the proton spectrum is off-resonance stimulated instead of the decoupler pause, the differences in coupling constants can be leveled out.

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By choosing suitable parameters (ν_2 , B_2) in Eq. [1], residual splittings ($J_{i,\text{res}}$) of nearly the same size can be obtained. Here, ν_2 denotes the frequency of the off-resonance irradiation, ν_i is the resonance frequency of the signal in question, J_i is the corresponding ¹J_{CH} coupling constant, and B_2 is the off-resonance decoupler power:

$$J_{i,\text{res}} = \sqrt{\left(\nu_2 - \nu_i + \frac{J_i}{2}\right)^2 + \left(\frac{\gamma_H B_2}{2\pi}\right)^2} - \sqrt{\left(\nu_2 - \nu_i - \frac{J_i}{2}\right)^2 + \left(\frac{\gamma_H B_2}{2\pi}\right)^2}. \quad [1]$$

A rated value for the residual splitting, J_s , must be determined, which applies to $J_s < {}^1J_{\text{CH},\text{min}}$, where ¹J_{CH,min} is the smallest coupling constant to be expected in the spectrum. According to the method of the sum of least squares *Q* (Eq. [2]), the parameters ν_2 and B_2 can be determined for every desired field strength B_0 for a group of some representative proton signals and its typical coupling constants with the directly bound carbon atoms. This was done with the aid of the program Maple V Release 5 (13).

$$Q = \sum_i (J_s - J_{i,\text{res}})^2. \quad [2]$$

Typical coupling constants and ¹H shifts like CH₃ (¹J_{CH} = 122 Hz, $\delta = 0,9$ ppm), CH₂ (¹J_{CH} = 126,3 Hz, $\delta = 1,45$ ppm), OCH₂ (¹J_{CH} = 141,1 Hz, $\delta = 4,1$ ppm), alkenes (¹J_{CH} = 160 Hz, $\delta = 5,5$ ppm), and aromats (¹J_{CH} = 160 Hz, $\delta = 7,3$ ppm) were taken from the standard literature and used for the definition of the parameters for different field strengths B_0 (Table 1).

According to empirical findings, the chemical shifts of the signals in the ¹H spectrum correlate with their ¹J_{CH} coupling constant. The coupling constants increase as the chemical shift becomes larger (Fig. 1). In this approach, carbons with a C–C triple bond provide an exception. They can be considered here to behave just as poor as in the case of the well-known procedures SEFT and APT.

From the parameters to be determined iteratively (ν_2 , B_2) by using Eq. [2], B_2 must be assigned to a value of the decoupler

TABLE 1
Calculated Values of the Residual Splittings $J(x)_{\text{res}}$ ($x = \text{CH}_3, \text{CH}_2, \dots$), Frequency ν_2 , and Power B_2 (Optimized for $J_s = 100$ Hz for Several ^1H Spectrometer Frequencies

$\frac{\gamma_{\text{H}}B_0}{2\pi}$ (field for ^1H in MHz)	$J(\text{CH}_3)_{\text{res}}$	$J(\text{CH}_2)_{\text{res}}$	$J(\text{OCH}_2)_{\text{res}}$	$J(\text{olefines})_{\text{res}}$	$J(\text{aromats})_{\text{res}}$	ν_2		B_2 (CW)	
	$\delta = 0.9$ ppm $J = 122$ Hz	$\delta = 1.45$ ppm $J = 126.3$ Hz	$\delta = 4.1$ ppm $J = 141.1$ Hz	$\delta = 5.5$ ppm $J = 160$ Hz	$\delta = 7.3$ ppm $J = 160$ Hz	ppm	Hz	$\frac{\gamma_{\text{H}}B_2}{2\pi}$ Hz	μT
60	96.4	98.4	100.7	107.0	95.8	16.18	970.78	709.76	16.670
80	96.4	98.4	100.7	107.0	95.8	16.16	1292.79	945.88	22.216
100	96.4	98.4	100.7	107.0	95.8	16.15	1615.07	1182.08	27.763
200	96.4	98.4	100.7	107.0	95.8	16.14	3227.70	2363.43	55.510
250	96.4	98.4	100.7	107.0	95.8	16.14	4034.26	2954.17	69.384
300	96.4	98.4	100.7	107.0	95.8	16.14	4840.87	3544.94	83.260
360	96.4	98.4	100.7	107.0	95.8	16.14	5808.84	4253.87	99.910
400	96.4	98.4	100.7	107.0	95.8	16.14	6454.17	4726.49	111.010
500	96.4	98.4	100.7	107.0	95.8	16.14	8067.53	5908.06	138.762
600	96.4	98.4	100.7	107.0	95.8	16.13	9680.92	7089.63	166.513
750	96.4	98.4	100.7	107.0	95.8	16.13	12101.02	8862.01	208.141
800	96.4	98.4	100.7	107.0	95.8	16.13	12907.73	9452.80	222.017
900	96.4	98.4	100.7	107.0	95.8	16.13	14521.15	10634.38	249.768
1000	96.4	98.4	100.7	107.0	95.8	16.13	16134.57	11815.97	277.520

power by calibration. Knowing the coupling constants, calibration can be achieved by off-resonance decoupling measurements with different values of decoupler power. The calculated off-resonance frequency ν_2 is used for this purpose without further variation. The attenuation of B_2 is varied until all carbon signals show the calculated residual splitting $J_{i,\text{res}}$ (Columns 2 to 6 in Table 1).

Figure 2 shows the dependence of the ν_2 frequency as a

function of the spectrometer frequency. The dependence of the required B_2 radiation power on the spectrometer frequency is represented in Fig. 3.

Replacing the decoupler switch off time in the pulse sequences APT and SEFT by off-resonance decoupling, the evolution of the different spin systems can be synchronized. APT becomes SAPT and SEFT becomes SSEFT, where ‘‘S’’ stands for ‘‘synchronized.’’

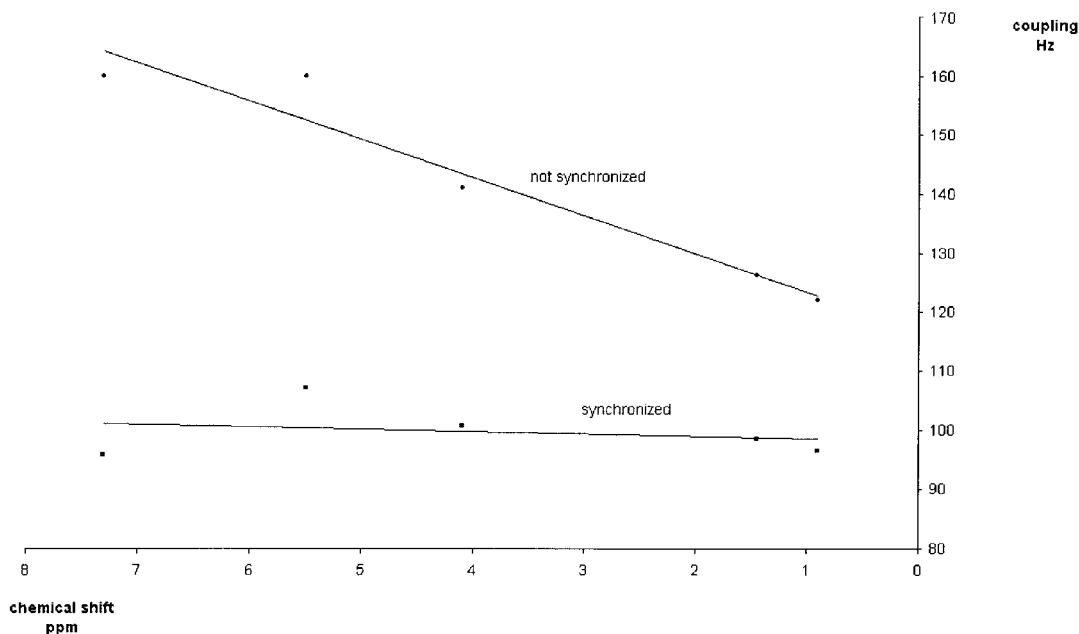


FIG. 1. Couplings and residual splittings ($J_s = 100$ Hz) of synchronized and not synchronized spin systems.

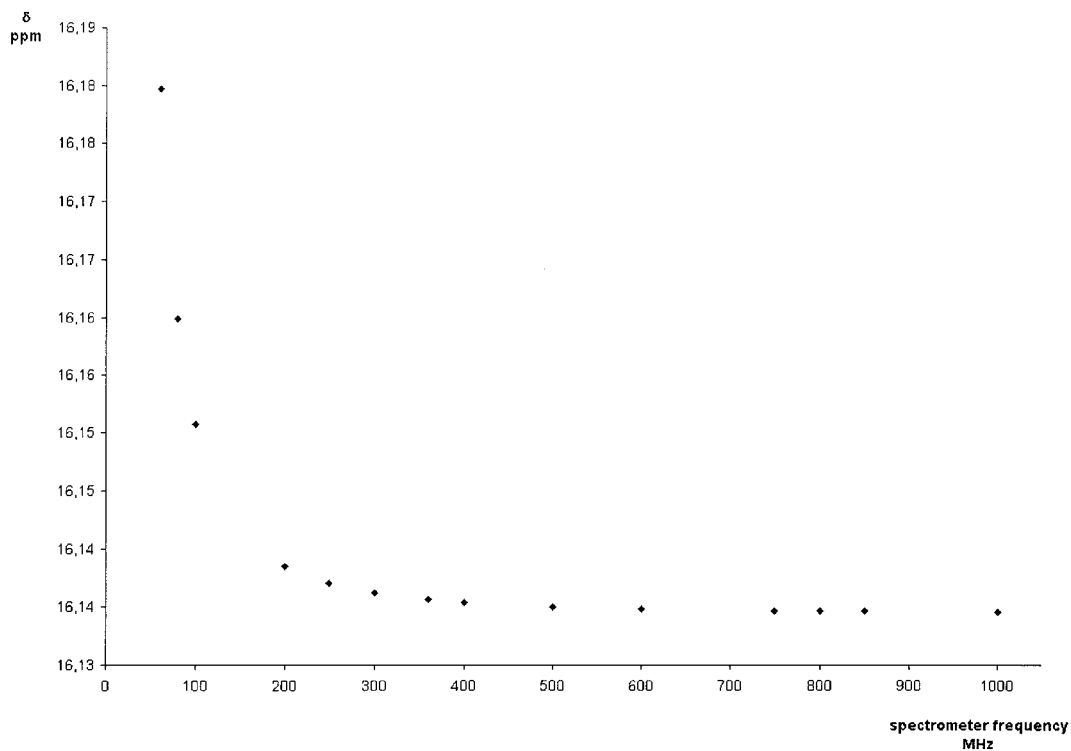


FIG. 2. Chemical shift of the off-resonance irradiation as a function of the ^1H spectrometer frequency ($J_s = 100$ Hz).

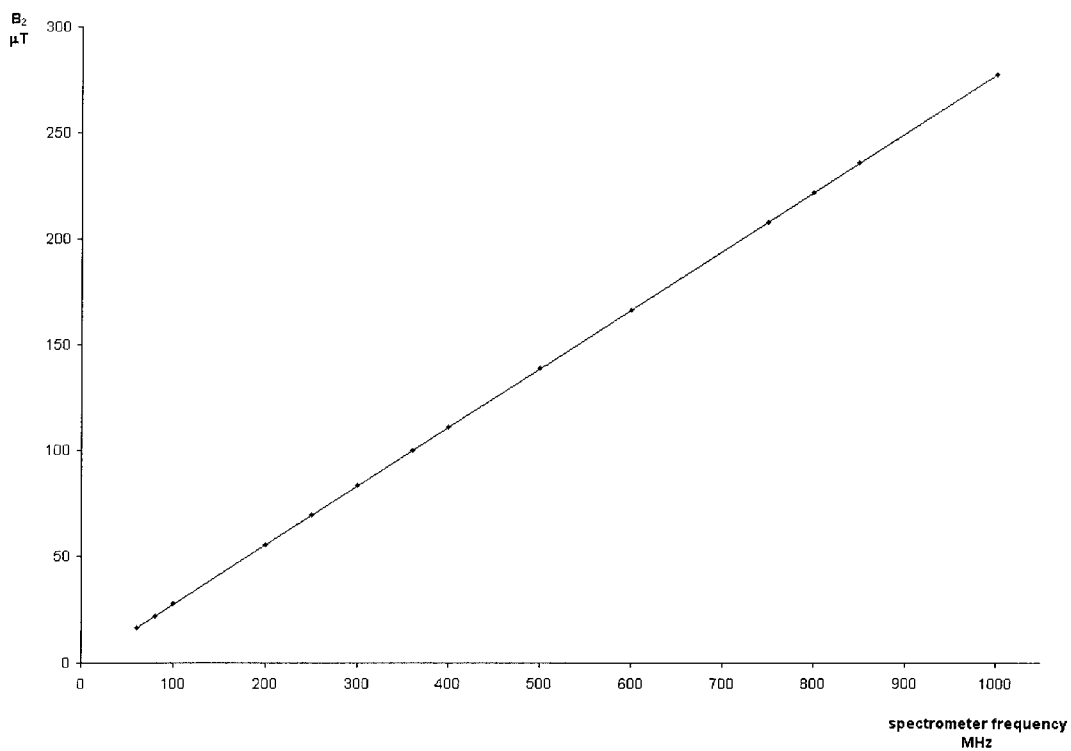


FIG. 3. Power B_2 of the off-resonance irradiation as a function of the ^1H spectrometer frequency ($J_s = 100$ Hz).

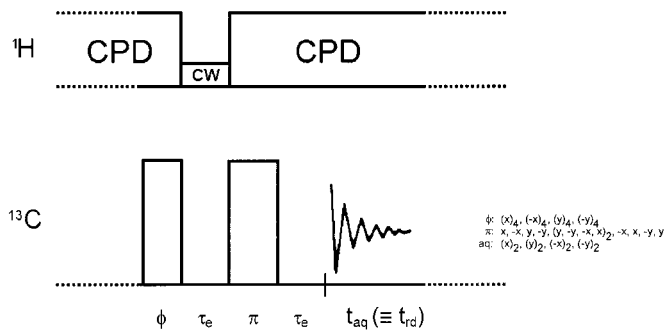


FIG. 4. The pulse sequence of ORSAT, $\phi = \pi - \alpha$ ($\alpha =$ Ernst angle), $\tau_e =$ evolution time, $t_{aq} =$ acquisition time, $t_{rd} =$ relaxation delay.

By synchronizing with the aid of the off-resonance technique, the resulting more homogeneous residual splittings $J_{l,res}$ can be taken into account much better by employing a well-defined length of the evolution time τ_e . The reduction of $^1J_{CH}$ to, e.g., $J_s = 100$ Hz is a random value. This leads to an evolution time of 10 ms.

The APT (respectively, SAPT) pulse sequence differs from SEFT (respectively, SSEFT) by a positive residual magnetization in the z direction after the last pulse. Accordingly, the subsequent relaxation delay can be shorter as compared to SEFT (respectively, SSEFT). Consequently, more magnetization can be recorded within the same period of time.

The task of the second π pulse in the APT (respectively, SAPT) pulse sequence can also be achieved by the variation of the length of the first pulse. When the length of the first pulse is $\pi - \alpha$, where α is the Ernst angle (14), then the subsequent

π pulse fulfills two tasks; it reinverts the magnetization which corresponds to the part being larger than $\frac{\pi}{2}$ ($\sim \cos \alpha$) to the $+z$ direction and simultaneously refocusses the created transversal magnetization ($\sim \sin \alpha$) to a spin echo (after $2\tau_e$). Consequently, with one pulse less, the same result as in the case of APT is achieved qualitatively. The Ernst angle should be estimated for the carbon atom with longest longitudinal relaxation time in the spectrum (in general quaternary C's) with respect to the acquisition time t_{aq} , which simultaneously acts as relaxation delay t_{rd} . Instead of the free evolution of the spin systems during the decoupler switch off time, now off-resonance decoupling (Fig. 4) with the corresponding parameters (Table 1) is switched on. The pulse sequence proposed here has been termed Off Resonance Synchronized Attachment Test (ORSAT, Fig. 4).

The ORSAT results for the signal-to-noise ratio for the quaternary signal of ethylbenzene compared to the other methods (using identical total measuring times in each case) can be taken from Fig. 5. All pulse angles were optimized with the aid of the DEPT sequence. The pulse sequence PENDANT afforded the lowest signal-to-noise ratios, although the pulse lengths were tuned very carefully and the repetition rate for this pulse sequence had been optimized for an estimated relaxation time ($T_1 \sim 60$ s) of the quaternary carbon of ethylbenzene.

If a maximum difference between the amplitude heights of CH and CH_3 is required, the evolution time τ_e results in

$$\tau_e = \frac{0.6959}{J_s}. \quad [3]$$

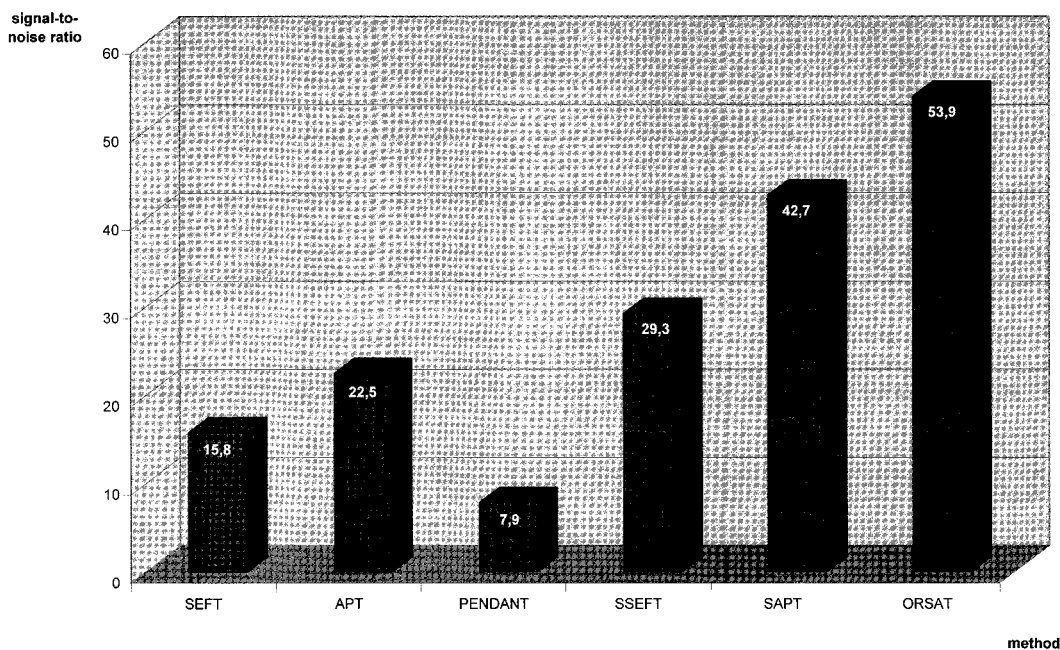


FIG. 5. Amplitude height of the quaternary carbon signal of ethylbenzene.

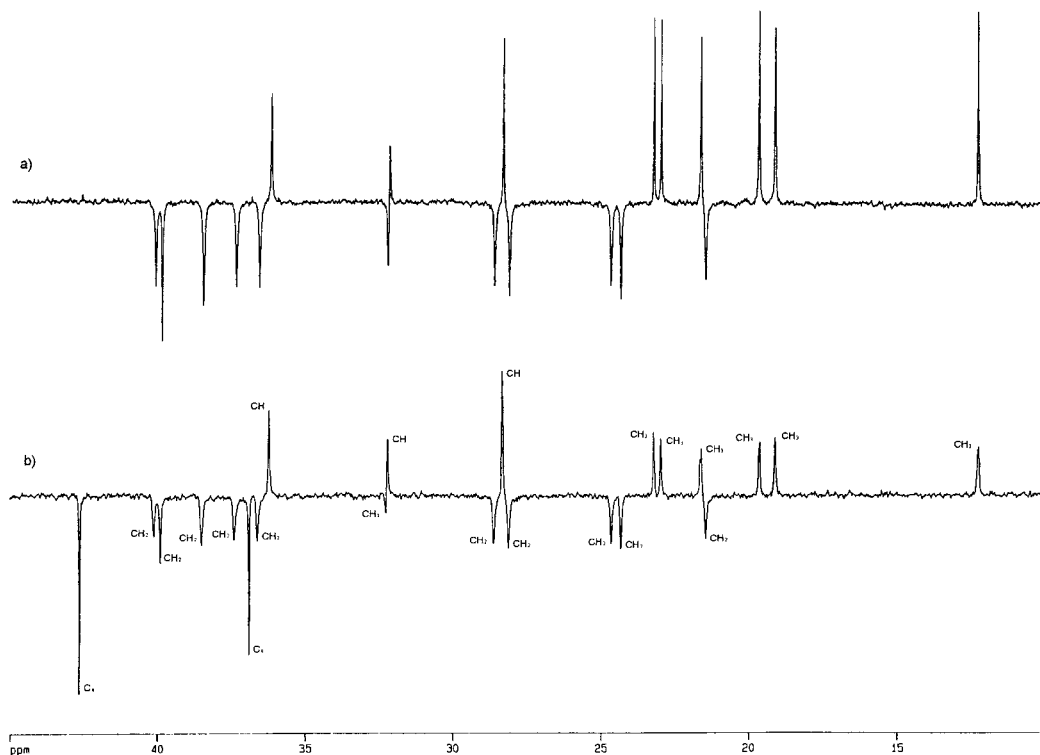


FIG. 6. ^{13}C subspectra of cholesteryl acetate between 10 and 45 ppm: (a) DEPT135, (b) ORSAT.

Consequently, an evolution time of $\tau_e = 6.96$ ms follows from $J_s = 100$ Hz. The intensity of the CH signal is negative and about 57.7% of its maximum value. The polarity of the CH_2 signal is positive and its intensity is 33.3% of the maximum value. The polarity of the CH_3 signals is negative as well as that of the CH and its intensity with $\sim 19.2\%$, exactly $\frac{1}{3}$ that of the CH signals. However, this factor is not achieved completely in practice due to different relaxation times. Because $T_{1c}(\text{CH}_3)$ is usually smaller than $T_{1c}(\text{CH})$ in the same molecule, the relation of $\frac{1}{3}$ can possibly be deteriorated to $\frac{1}{2}$ in the ORSAT subspectrum. The quaternary signals keep their unmodulated intensity. By decreasing the intensity of the protonated signals the quaternary signals are emphasized more clearly. If the phases of the ORSAT subspectrum are additionally inverted, the signals of CH and CH_3 appear with the same polarity in both subspectra. It is now easy to distinguish CH from CH_3 by comparing the signal intensities to those of the DEPT ($\phi = 135^\circ$) subspectrum (Fig. 6). A comparable signal-to-noise ratio in both the ORSAT and the DEPT subspectrum is required. In fact, DEPT gains amplitude because of polarization transfer. Therefore, it needs a smaller number of scans compared to the normal CPD, respectively, ORSAT, subspectrum, which benefits from the NOE, to achieve a comparable signal-to-noise ratio. If the number of scans used for a common CPD spectrum is eight times higher compared to DEPT, this will also satisfy the necessities of the ORSAT subspectrum. The absolute number of scans depends on the appearance of the

quaternary carbon signals in the ORSAT subspectrum. This procedure is less time consuming than recording two DEPTs and one CPD subspectra. Due to the missing additional relaxation delay in ORSAT, accumulation is completed faster and takes approximately the same time needed for one CPD spectrum.

The DEPT subspectrum ($\phi = 90^\circ$) can be abandoned by using an ORSAT subspectrum. With this, the number of spectra required for assignment is reduced from 3 to 2. The remaining DEPT subspectrum ($\phi = 135^\circ$) provides CH , $\text{CH}_3 > 0$, and $\text{CH}_2 < 0$. Additionally, only one ORSAT subspectrum is required.

Experiments were performed on an Bruker WP100 and AVANCE DPX200 spectrometer. The spectra of Fig. 6 were recorded with the use of 16 dummiscans each to achieve a steady state of magnetization. The sample was cholesteryl acetate, 1 g/ml $\text{CDCl}_3 + 5\%$ TMS.

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