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CRAPT: an improved version of APT with compensation for variations in JCH

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A modified version of the attached proton test (APT) sequence for ^{13}C spectral editing, which we call CRisis-APT (CRAPT), is developed and tested on representative organic compounds. CRAPT incorporates ^{13}C compensation for refocusing inefficiency with synchronized inversion sweeps (CRISIS) pulses in combination with ^{1}H broadband inversion pulses to give improved compensation for variations in $^{1}J_{CH}$ along with improved refocusing efficiency. It is shown that CRAPT gives edited ^{13}C spectra with only small losses in sensitivity (between 8% and 15% for strychnine, 1, menthol, 2, cholecalciferol, 3, and isotachysterol, 4), compared with basic ^{13}C spectra obtained on the same compounds. CRAPT also gives significantly better signal/noise than DEPTQ for nonprotonated carbons. Therefore, we conclude that CRAPT is an improvement over APT or DEPTQ or a combination of DEPT135 with a full ^{13}C spectrum for routine ^{13}C spectral editing of organic compounds. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: spectral editing; APT; CRISIS pulses; CRAPT; DEPTQ

Introduction

^{13}C spectral editing is still a valuable NMR technique for characterizing organic compounds.[1] One of the earliest sequences developed for ^{13}C spectral editing was the attached proton test (APT).[2] This gave peaks for all types of carbons with carbons with even numbers (0 or 2) of attached protons giving upright signals while those with odd numbers (1 or 3) of protons giving inverted signals. Thus, it could be run in place of a standard ^{13}C spectrum while providing editing information. However, the main disadvantage of APT was that it was sensitive to variations in $^{1}J_{CH}$, leading to significantly reduced intensities (and, in extreme cases, incorrect signal phases) for carbons with coupling constants differing from the chosen average value (usually 145 Hz).[3] Subsequently, a revised version of APT, called CAPT-3 was developed in an attempt to minimize this problem by incorporating additional pulses and delays during the evolution period.[4] The published results indicated that it did minimize the impact of variations in $^{1}J_{CH}$. However, it had the disadvantage that the evolution time increased from about 8 to 12 ms, which will result in noticeable losses in intensity due to ^{13}C relaxation, loss of NOE and evolution of $^{n}J_{CH}$ couplings, resulting in further loss of sensitivity relative to a standard ^{13}C spectrum.

At about the same time as the original APT sequence, two alternative sequences, INEPT[5] and DEPT,[6] were developed, which involved polarization transfer from ^{1}H to ^{13}C and which gave significantly enhanced signal intensities for protonated carbons. Because DEPT used the final pulse angle rather than a value of $^{1}J_{CH}$ for editing[6] and thus was less sensitive than INEPT or APT to variations in these couplings, it soon became the most widely used spectral editing sequence. If the final pulse angle is 135°, the DEPT sequence yields a similar pattern to APT, except that the CH and CH_3 signals are upright, and the CH_2 signals are inverted. Alternatively, by acquiring three separate spectra with final pulse angles of 45°, 90°, and 135°, followed by appropriate addition or subtraction of pairs of these spectra, one can generate separate CH, CH_2, and CH_3 spectra. However, unlike APT, it does not give signals for nonprotonated carbons and thus still requires measurement of an additional ^{13}C spectrum in order to obtain chemical shifts for all carbons. This problem was later addressed by the development of the DEPTQ sequence, which, by the inclusion of additional delays and pulses, allowed simultaneous acquisition of both a DEPT spectrum and peaks because of nonprotonated carbons.[7] However, in our experience, the intensities of the latter peaks were generally significantly weaker than those from a normal ^{13}C spectrum obtained in the same time.[1]

In recent years, there has also been increasing use of edited 2D HSQC spectra[8] in place of DEPT for spectral editing.[1] An edited HSQC spectrum can be obtained in comparable time to a DEPT spectrum while also providing the ^{1}H chemical shifts of the protons bonded to each carbon.[3] However, like APT and INEPT spectra, edited HSQC spectra are quite sensitive to variations in $^{1}J_{CH}$[9] and, like INEPT and DEPT, give no signals for nonprotonated carbons. Several years ago, Krishnamurthy attempted to minimize the former problem by developing what he called compensation for refocusing inefficiency with synchronized inversion sweeps (CRISIS) pulses, which are variable sweep rate adiabatic ^{13}C 180° pulses, which partially correct for variations in $^{1}J_{CH}$, on the basis of an approximate linear relationship between $^{1}J_{CH}$ and ^{13}C chemical shifts.[10,11] It was shown that CRISIS pulses were more effective than standard adiabatic pulses, particularly in editing mode (Fig. 2 in Ref. [10]). We subsequently demonstrated that an HSQC sequence, which incorporated CRISIS pulses and a number of other improvements,[11] gave significant improvement in sensitivity compared with an HSQC

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sequence without these improvements.[9] Consequently, it occurred to us that incorporation of CRISIS pulses into the original APT sequence might result in a similar improvement in performance for that sequence. Here, we report results for a modified APT sequence incorporating CRISIS pulses, which we call CRisis-APT (CRAPT). CRAPT gives significantly better sensitivity than APT for a variety of organic compounds and only slightly reduced sensitivity relative to a standard ^{13}C spectra obtained on these compounds. It also gives significantly better sensitivity for nonprotonated carbons than DEPTQ. Thus, it provides a viable alternative to either a combination of a ^{13}C spectrum and a DEPT spectrum or a single DEPTQ spectrum for helping to characterize organic compounds.

Results and Discussion

Figure 1 depicts pulse sequence diagrams for a commonly used version of APT (Fig. 1a) and for CRAPT (Fig. 1b). In the APT sequence, $^1J_{CH}$ evolves during the period Δ. Considering that $\Delta = 1/J$, the carbons with odd numbers of protons (CH and CH_3) have inverted phase compared with those with even numbers of protons (CH_0 and CH_2). The first composite 180° pulse serves as a ^{13}C refocusing pulse for the period 2Δ. The second 180° pulse allows one to run the experiment with a small tip angle (typically 45°) for the initial pulse by converting any residual $-Z$ magnetization after the first 180° pulse back to +Z magnetization. The CRAPT sequence uses ^{13}C CRISIS pulses (each of 2-ms duration), very similar to those in the earlier published CRISIS-HSQC sequence, to compensate for variations in $^1J_{CH}$.[9] The compensation is based on an assumed relationship between $^1J_{CH}$ given by $^1J_{CH} = 120 + (\delta_C(\text{ppm}))/3)]$ Hz.[9] The pulse train during the delay, Δ, mimics the multiplicity editing period of the C2HSQC sequence.[11] Because of the double-echo nature of the CRISIS pulse train, ^{13}C chemical shifts are completely refocused at the beginning of the acquisition period. Moreover, the total time duration of the pulse sequence (i.e. the time between the first excitation pulse and the start of data acquisition) is shorter for CRAPT ($\Delta + 4\,\text{ms} + 2$ times the ^1H 180° pulse width) compared with APT (2Δ), potentially decreasing signal losses due to T_2^* relaxation during the pulse sequence. Finally, the use of ^1H broadband inversion pulses (BIP) [12] serves to compensate for both RF inhomogeneity as well as possible pulse miscalibration.

As an initial test, APT, CRAPT, and ^{13}C spectra were acquired for strychnine, **1**, a compound which has been widely used for evaluation of new pulse sequences. APT, CRAPT, and ^{13}C spectra for **1**, obtained with the same total acquisition times, are illustrated in Figs. 2 and 3, whereas Table 1 lists the signal/noise values for each of the carbons of **1** obtained with the three sequences, along with the ratios of signal/noise for these carbons in APT/CRAPT and CRAPT/^{13}C spectra. In addition, the table lists experimental values of $^1J_{CH}$, obtained from a coupled ^{13}C spectrum of **1**, along with the calculated values of this parameter used in the CRISIS pulses. The data clearly showed that the incorporation of CRISIS pulses which compensate for variations in $^1J_{CH}$ leads to significantly improved performance for CRAPT relative to APT. This is most pronounced for carbons nearer the edges of the spectral window, as expected, because these carbons have the

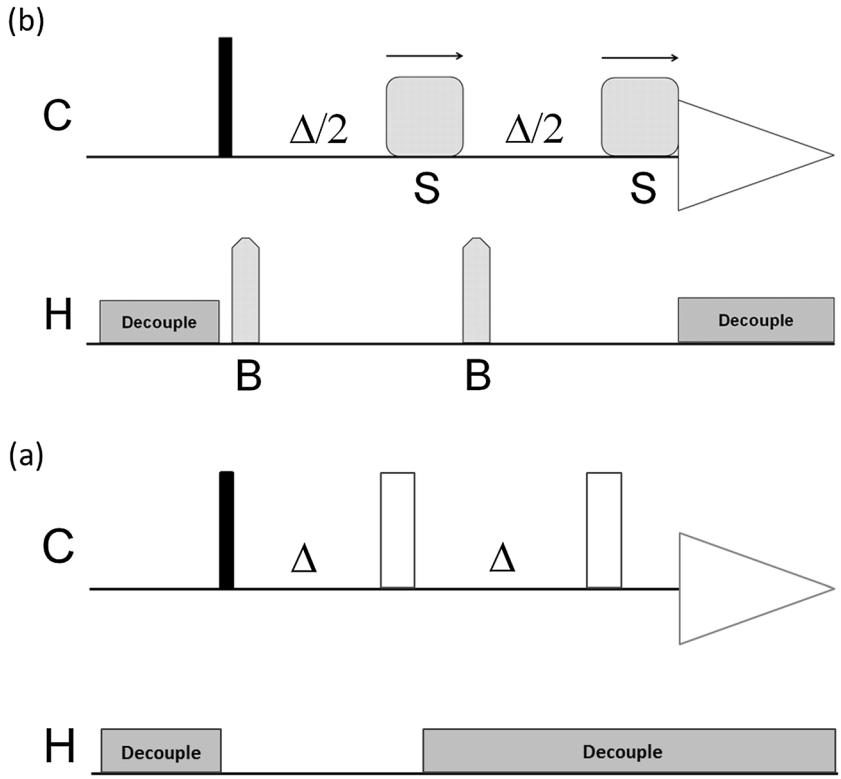


Figure 1. Pulse sequence diagrams for (a) APT and (b) CRAPT. The solid vertical bars represent rectangular pulses of user-selected typical angle (45° in this case). All other pulses are effective 180° pulses. The open vertical bars represent rectangular 90_x-180_y-90_x composite pulses. The adiabatic CRISIS pulses (S) are wurst2i shapes of 2-ms duration. The forward arrows represent a downfield to upfield sweep over an 180-ppm window. The shaped ^1H pulses are BIP[11] pulses of 60 μs duration and 25 KHz γB_2. The delay, Δ, is set to 1/J. A 4-step CYCLOPS phase cycle is used on the ^{13}C pulses, and the receiver and both of the ^1H BIP pulses are along the *x*-axis.

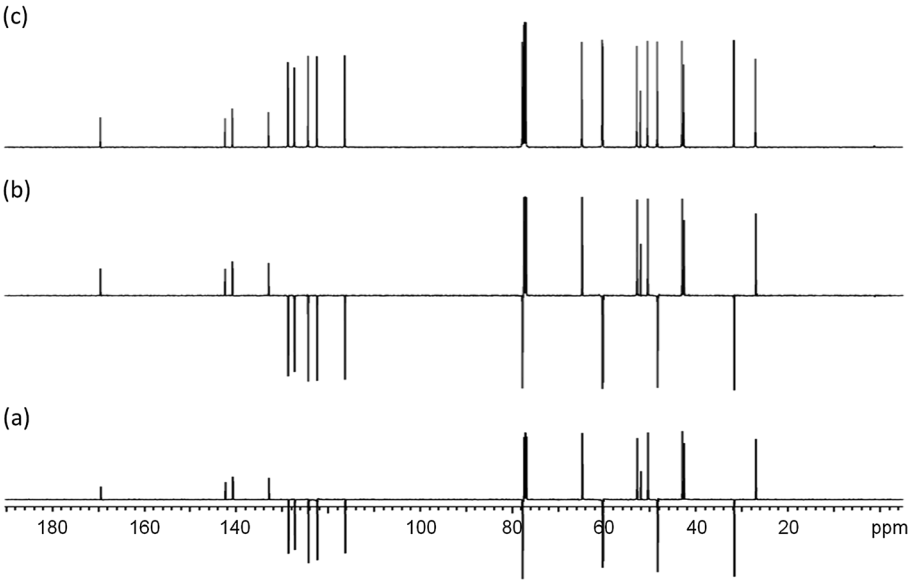


Figure 2. Spectra for strychnine, **1**: (a) APT (b) CRAPT spectrum (c) conventional ^{13}C spectrum. Acquisition and processing parameters are provided in the Experimental section.

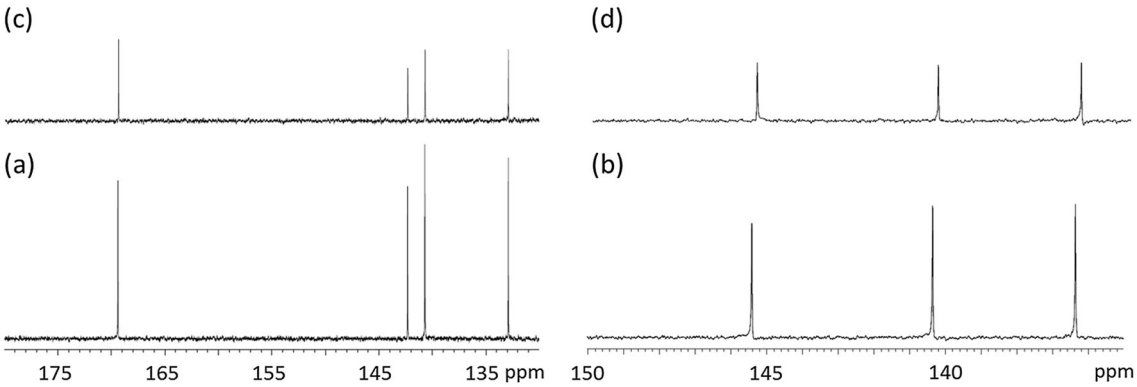


Figure 3. Expansions of spectra showing high frequency nonprotonated carbons of strychnine, **1**, and cholecalciferol, **3**: (a) conventional ^{13}C spectrum of**1**(b) DEPTQ spectrum of **1** (c) conventional ^{13}C of **3** (d) DEPTQ spectrum of **3**. Acquisition and processing parameters are provided in the Experimental section.

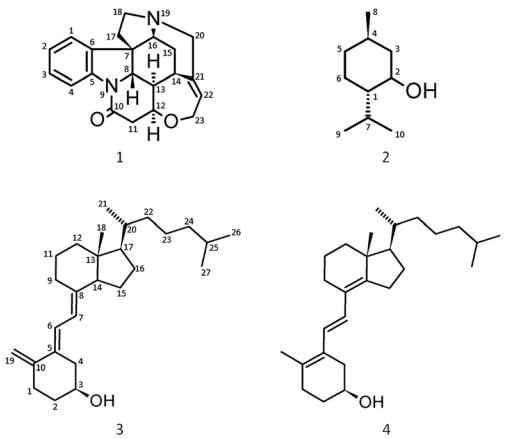
largest variations in $^{1}J_{CH}$ from the average value (145 Hz) used in APT (the root mean square deviation between experimental values of $^{1}J_{CH}$ and the values used in the CRISIS pulses is +/−5 Hz compared with +/−14 Hz between experimental values and 145 Hz). However, the greater tolerance to RF inhomogeneity and improved efficiency of the inversion profiles of the BIP and adiabatic pulses incorporated in CRAPT must also contribute to its improved performance because nonprotonated carbon intensities are also significantly better. The CRAPT/^{13}C ratios showed that, on average, the signal/noise values for the CRAPT carbon peaks were only slightly less than those in the ^{13}C spectrum (averaging 92 +/−4%). Thus, an edited ^{13}C spectrum with CRAPT can be obtained almost as quickly as a standard ^{13}C spectrum. As a further test of the reliability of CRAPT in routine use, additional runs for CRAPT and APT were obtained with the values of both ^{1}H and ^{13}C pulses misset by about 20% to mimic a very badly calibrated probe. The poorly calibrated APT spectrum showed an average intensity loss of 27% compared with the properly calibrated APT spectrum, whereas the corresponding loss with CRAPT was only 17%. A second CRAPT spectrum with both types of pulses misset by 10% showed only an average 5% loss. Thus, CRAPT is a sufficiently robust sequence to be suitable for use in an open access laboratory.

One drawback of using strychnine as a test of ^{13}C spectral editing is that it has no methyl carbons. Because they have three attached protons, methyl ^{13}C signals are particularly sensitive to variations in $^{1}J_{CH}$. As a further test, CRAPT and ^{13}C spectra were obtained for three additional compounds, all of which contained methyl groups (see Scheme 1). The first two chosen were menthol, **2**, and cholecalciferol (Vitamin D3), **3**. However, an initial ^{1}H spectrum of **3** in $CDCl_3$ indicated that it was rapidly isomerizing via double bond rearrangement in the NMR tube, with the reaction apparently catalyzed by traces of acid in the solvent. Consequently, a fresh solution of **3** in DMSO-d_6 was prepared for data acquisition. However, it was also noted that the isomerization of **3** in $CDCl_3$ was almost complete after 24 h and that, after about 5 days, the spectrum displayed a single compound which was more than 95% pure. Using a standard set of 2D NMR (COSY, TOCSY, HSQC, and HMBC) spectra, this

Table 1. Signal/noise measurements for the carbons of strychnine,**1**, obtained using the APT and CRAPT sequences and a standard ^{13}C spectrum

Carbon	δ_C	N_H[a]	$^aJ(o)$[b]	$^aJ(c)$ [c]	Signal/noise[d]				
					APT	CRAPT	^{13}C	CR/A[d]	CR/C[e]
10	169.2	0	—	—	33	59	66	1.79	0.89
5	142.1	0	—	—	45	59	63	1.31	0.94
21	139.8	0	—	—	59	75	83	1.27	0.90
6	132.3	0	—	—	56	72	75	1.29	0.96
3	128.6	1	162	163	138	177	182	1.28	0.97
22	128.0	1	162	163	129	168	171	1.30	0.98
2	124.2	1	163	161	163	190	196	1.17	0.96
1	122.3	1	158	161	156	189	195	1.21	0.96
4	116.2	1	168	159	131	185	196	1.33	0.94
12	77.5	1	149	149	204	206	223	1.01	0.92
23	64.5	2	152	142	171	216	228	1.26	0.95
16	60.2	1	145	140	176	206	229	1.18	0.90
8	60.0	1	143	140	160	194	226	1.21	0.86
20	52.6	2	139	138	159	212	218	1.33	0.97
7	51.9	0	—	—	80	122	135	1.53	0.91
18	50.3	2	132	137	173	214	229	1.24	0.93
13	48.1	1	124	136	187	204	228	1.09	0.89
17	42.7	2	132	134	177	214	229	1.21	0.93
11	42.4	2	131	134	146	167	179	1.14	0.93
14	31.5	1	132	131	199	209	231	1.05	0.90
15	26.7	2	130	129	156	181	197	1.16	0.92
Average					137	167	179	1.25	0.93

[a]Number of attached hydrogens.
[b]Observed value of $^1J_{CH}$ in Hz from a coupled ^{13}C spectrum.
[c]Calculated value of $^1J_{CH}$ in Hz based on $^1J_{CH} = [120 + (\delta_C(ppm)/3)]$ Hz.
[d]Ratio of signal/noise for CRAPT/APT spectra.
[e]Ratio of signal/noise for CRAPT/ ^{13}C spectra.



Scheme 1. Structures and numbering for compounds **1–4**. Compounds **3** and **4** have identical numbering schemes.

reaction product was identified as isotachysterol, **4**, a known acid-catalyzed isomerization product of Vitamin D3.[13] Because **4** has a good distribution of different types of carbons (5 CH_0, 6 CH, 11 CH_2, and 5 CH_3 carbons), it was decided to use it as a fourth test molecule. ^{13}C, APT, and CRAPT spectra were obtained for **2**, **3**, and **4**, again using identical acquisition times for the different spectra. In each case, the results for APT were significantly poorer than with CRAPT, averaging at least 40% intensity losses with particularly bad results for methyl groups. These results parallel the earlier results for strychnine and are not reported in detail here. Instead, the results for CRAPT spectra relative to ^{13}C spectra are highlighted. Results for **2**, **3**, and **4**, respectively, are summarized in Tables 2–4. As shown in the three tables, the average signal/noise ratio for carbons in the CRAPT spectra relative to the same carbons in the ^{13}C spectra was, respectively, 88+/−6% for **2**, 85+/−9% for **3**, and 86 +/−6% for **4**. In addition to this excellent overall performance of the CRAPT pulse sequence, the results for individual carbons for the three compounds (**1**, **2**, and **4**) in $CDCl_3$ are also highly satisfactory. Fifty-four of 58 carbons had CRAPT intensities of 80% or more relative to the intensities for the corresponding carbons in the ^{13}C spectra, and only three carbon nuclei (methylene carbons C-22, C-23, and C-24 of **4**) had intensities of 75% or less.

Signal losses associated with protonated carbons in the CRAPT spectra were often larger for **3** in DMSO-d_6 compared with those for the other compounds in $CDCl_3$. In particular, C-11, C-12, C-20, C-22, C-23, and C-24 showed signal/noise values with CRAPT, which are in the 60–75% range relative to the ^{13}C spectra. Of these, C-20 is a methine carbon whereas the rest are methylene carbons. The lowered intensity of C-20 occurs partly because it is flanked by two peaks of opposite phase (**C**-2 and C-22) ca. 0.1 ppm away on either side, with peak overlap causing some loss of signal intensity for all three peaks. This was confirmed by reprocessing the CRAPT and ^{13}C spectra with only 0.5 Hz line broadening, which brought the three CRAPT peaks closer in relative intensity to the ^{13}C peaks (Table 3). The predominance of methylene carbons among the weakest peaks in **3** and **4** is not surprising because, in our experience, methylene carbons for organic molecules in this molecular

Table 2. Signal/noise measurements for the carbons of menthol,**2**, obtained using the CRAPT sequence and from a standard ^{13}C spectrum

Carbon	δ_C	N_H[a]	Signal/noise		
			CRAPT	^{13}C	CR/C[b]
2	71.5	1	368	401	0.92
1	50.1	1	329	422	0.78
3	45.0	2	439	512	0.86
5	34.5	2	400	494	0.81
4	31.6	1	335	385	0.87
7	25.8	1	332	342	0.94
6	23.1	2	365	454	0.81
8	22.2	3	399	424	0.94
9	21.0	3	392	429	0.91
10	16.0	3	380	414	0.92
			374	428	0.88

[a]Number of attached hydrogens.
[b]Ratio of signal/noise for CRAPT/^{13}C spectra.

Table 3. Comparison of signal/noise for the different carbons of cholecalciferol, **3**, in DMSO-d_6, obtained with the CRAPT sequence and from a standard ^{13}C spectrum

Carbon	δ_C	N_H	Signal/noise[a]		
			CRAPT	^{13}C	CR/C[b]
10	145.9	0	68	71	0.96
8	140.9	0	76	79	0.96
5	136.8	0	76	82	0.93
6	121.4	1	86	92	0.93
7	118.0	1	86	93	0.92
19	112.2	2	72	74	0.97
3	68.4	1	100	113	0.88
17	56.4	1	99	130	0.76
14	56.1	1	103	132	0.78
4	46.4	2	82	94	0.80
13	45.7	0	133	152	0.88
12	40.4	2	76	111	0.68
24	39.4	2	122	162	0.75
22	36.1_2	2	66(55)[c]	108(85)	0.61(0.65)
20	36.0_5	1	95(88)	132(117)	0.67(0.75)
2	35.9_2	2	81(64)	100(76)	0.81(0.84)
1	32.6	2	73	87	0.84
9	28.8	2	52	68	0.76
25	27.8_8	1	158	178	0.89
16	27.6_5	2	70	87	0.81
23	23.8	2	82	113	0.73
11	23.5	2	64	86	0.74
27	23.1	3	171	185	0.92
26	22.8	3	169	188	0.91
15	22.3	2	75	98	0.90
21	19.1	3	135	148	0.91
18	12.2	3	136	145	0.94
Average			96	115	0.85

[a]Number of attached hydrogens.
[b]Ratio of signal/noise for CRAPT/^{13}C spectra.
[c]Values in brackets were measured with 0.5 Hz line broadening.

Table 4. ^{1}H and ^{13}C chemical shift assignments of isotachysterol-**3**, **4**, and signal/noise measurements for the carbons obtained with the CRAPT sequence and from a standard ^{13}C spectrum

Carbon	δ_C	δ_H	N_H[a]	Signal/noise		
				CRAPT	^{13}C	CR/C[b]
14	149.8	—	0	87	102	0.83
10	131.2	—	0	97	114	0.85
5	125.7	—	0	97	116	0.84
7	125.5	6.39	1	168	196	0.86
8	124.4	—	0	104	119	0.87
6	123.5	6.54	1	180	202	0.89
3	67.6	3.92	1	209	226	0.92
17	56.3	1.20	1	229	275	0.83
13	44.0	—	0	143	178	0.80
4	39.5	1.14	2	223	297	0.75
12	37.2	2.00, 1.23	2	157	181	0.87
22	35.9	1.39, 1.07	2	169	229	0.74
4	34.7	2.60, 2.14	2	161	195	0.83
20	34.5	1.49	1	205	227	0.90
1	31.2	2.22	2	176	194	0.91
2	31.1	1.87, 1.61	2	164	175	0.94
25	28.0	1.52	1	203	234	0.87
16	27.0	1.90, 1.45	2	160	174	0.92
15	25.9	2.46, 2.41	2	169	192	0.88
9	24.3	2.25, 2.06	2	151	178	0.85
23	23.7	1.37, 1.18	2	163	228	0.72
26	22.8	0.89	3	212	234	0.91
27	22.6	0.88	3	231	240	0.96
11	19.0_3	1.75	2	111	122	0.91
21	19.0_1	0.96	3	168	196	0.81
19	18.8	1.79	3	131	151	0.87
18	18.2	0.91	3	180	200	0.90
Average				164	191	0.86

[a]Number of attached hydrogens.
[b]Ratio of signal/noise for CRAPT/^{13}C spectra.
[c]Average ^{1}H chemical shift for a pair of overlapped methylene protons.

weight range (384 Da) typically have shorter relaxation times than other protonated carbons. Thus, they should be more susceptible to sensitivity losses due to relaxation and/or loss of NOE during the delay period, Δ, particularly in the much more viscous DMSO-d_6 solution. In some cases, the methylene carbons are comparable in intensity to nonprotonated carbons, because the former lines are significantly broader because of their shorter T_2 values. This could potentially lead to uncertainty in deciding whether a given peak is a methylene or a nonprotonated carbon, but they can easily be distinguished by the differences in line widths. However, the usefulness of CRAPT as a general purpose spectral editing sequence is mainly determined by the signal/noise for nonprotonated carbons because these are consistently among the weakest peaks and thus will determine the total time required to obtain a useable spectrum. For the 14 nonprotonated carbons in **1**, **3**, and **4**, the average signal/noise with CRAPT, relative to a standard ^{13}C spectrum, is 89+/−6% with the worst value being 80% for C-14 of **4**. Interestingly, the signal losses in DMSO-d_6 were comparable with those in $CDCl_3$, confirming that nonprotonated carbons are less sensitive to increased solvent viscosity when compared with protonated carbons. Overall, the signal/noise loss with CRAPT is sufficiently small

that it should generally be possible to obtain a CRAPT spectrum in less time than required to obtain a regular ^{13}C spectrum plus a DEPT-135 spectrum while giving comparable editing information.

Some sensitivity loss for protonated carbons will also be expected with CRAPT in cases where the assumed linearity between ^{13}C shifts and $^{1}J_{CH}$ does not hold. Examples where this will occur are three-membered rings (e.g. cyclopropanes, aziridines, and epoxides), where $^{1}J_{CH}$ is larger than would be predicted on the basis of ^{13}C chemical shifts, with the greatest deviations for cyclopropanes.[14] Nevertheless, the intensities of these carbons should still be larger than for nonprotonated carbons (or they can be distinguished on the basis of line widths) and thus will still allow for spectral editing. However, one case where CRAPT, along with all of the currently widely used spectral editing sequences, will be unreliable is for protonated acetylenic carbons, because of the extremely large values of $^{1}J_{CH}$ (ca. 250 Hz).[14] Furrer has proposed a modified DEPT sequence, based on the accordion principle,[15] which provides reliable results for acetylenic carbons.[16] However, like the original DEPT sequence, this sequence does not give signals for nonprotonated carbons, and it shows reduced intensities, relative to the original DEPT sequence, for many protonated carbons.[15] In any case, acetylenic carbons are relatively rare in natural products, and their presence in synthetic organic compounds can usually be predicted from knowledge of the chemistry involved, whereas the presence of cyclopropyl groups can often be recognized by their unusual ^{1}H chemical shifts below δ1 ppm.

As mentioned in the Introduction section, an alternative sequence that provides editing information for both protonated and nonprotonated carbons is the DEPTQ sequence.[6] However, we have found in the past that it gave relatively poor sensitivity for nonprotonated carbons.[1] To confirm this, we obtained comparison spectra for the DEPTQ and ^{13}C sequences, similar to those reported in the previous text for CRAPT. The analysis of these spectra focused on nonprotonated carbons and used **1** and **3** as test molecules (the sample of **4** had decomposed in the interim, preventing further measurements on this sample). The DEPTQ sequence uses a split relaxation delay with the decoupler turned on during D1 (to build up NOEs for nonprotonated carbons), followed by D2, where the decoupler is off to build up ^{1}H magnetization (for later polarization transfer to protonated carbons). The recommended delays are D1 = 3 s and D2 = 1 s.[7] However, the additional 3 s delay doubles the time per scan, and therefore, the number of scans was changed from 256 for the ^{13}C spectra to 128 for the DEPTQ spectra to keep the total time per spectrum constant at 13 min. The initial ^{13}C pulse has a user-chosen tip angle with a value of 90° being used in the original report.[7] We tried 45°, 60°, and 90° tip angles and confirmed that the best results for nonprotonated carbons were obtained with the latter tip angle. Results for the high frequency nonprotonated carbons of **1** and **3** in DEPTQ and ^{13}C spectra are shown in Fig. 3 with signal/noise data for all nonprotonated carbons of these two compounds are listed in Table 5. The signal/noise values for the nine nonprotonated carbons of **1** and **3** with DEPTQ averaged 44+/−7% of those in the ^{13}C spectra. The best results were obtained for the two aliphatic quaternary compounds, likely reflecting the larger number of protons close to these carbons. Over half (ca. 30%) of the signal loss with DEPTQ can be attributed to the necessity of decreasing the number of scans from 256 to 128 to keep the total measurement time the same as for the other spectra. It is also probable that some of the NOE build up for nonprotonated carbons during D1 is lost during D2. Among the protonated carbons, the sensitivity with DEPTQ was 50–70% of that in the ^{13}C spectra. The sensitivity loss, beyond that attributable to the decreased number of scans, was greatest for methine and methyl carbons, likely because the 1 s D2 delay is too short to allow ^{1}H magnetization of their attached protons to fully recover prior to the DEPT transfer stage. This illustrates what we believe is a fundamental problem with DEPTQ, i.e., that the relative sizes for D1 and D2, which are most favorable for nonprotonated carbons are unfavorable for protonated carbons and vice versa. To confirm this, we tried several additional runs with different values for D1 and D2, but none gave satisfactory results for both protonated and nonprotonated carbons. Thus, in our opinion, DEPTQ is less useful than CRAPT as a routine spectral editing method for organic compounds.

Table 5. Comparison of signal/noise for nonprotonated carbons of **1** and **3**, obtained with a ^{13}C spectrum[1] and a DEPTQ spectrum[2]

Compound	Carbon	Signal/noise		
		^{13}C	DEPTQ	D/C[c]
1	10	65	33	0.51
1	5	64	23	0.36
1	21	81	32	0.40
1	6	76	30	0.39
1	7	151	71	0.47
3	10	69	31	0.45
3	8	79	30	0.38
3	5	80	31	0.39
3	13	170	98	0.57
Average				0.44+/−0.07

[a]The data were obtained on a new run but generally closely agree with those in Tables 1 and 3.

[b]Data obtained with an initial tip angle of 90°. Smaller tip angles gave poorer results.

[c]Ratio of signal/noise from the DEPTQ and ^{13}C spectra.

Conclusions

On the basis of our results for four representative organic compounds, we have shown that CRAPT provides improved results over both APT and DEPTQ while providing the same information in less time than would be required to acquire a ^{13}C spectrum plus a DEPT135 spectrum. CRAPT is also relatively insensitive to errors in probe calibration. Therefore, we conclude that it is a useful pulse sequence for routine ^{13}C spectral editing of organic compounds.

Experimental

Compounds **1**, **2**, and **3** were obtained from Sigma-Aldrich and were used without further purification. The samples for the first three compounds used 10 mg of **1** dissolved in 0.23 ml of $CDCl_3$, 10 mg of **2** dissolved in 0.23 ml of $CDCl_3$, and 10 mg of **3** dissolved in 0.23 ml of DMSO-d6. A second sample of **3** in 0.23 ml of $CDCl_3$ converted almost quantitatively in 5 days to **4**, and this sample was used to obtain the spectra for **4**. All samples were prepared in 3-mm NMR tubes. Spectra were obtained on an Agilent DD2 500-MHz spectrometer equipped with an XSens cold probe. Spectra were obtained with a 25,128 Hz spectral window (δ−5 to δ195 ppm), a 2-s acquisition time (100,512 points with zero

filling to 262,144), a pre-acquisition delay of 1 s, 256 scans per spectrum, and an initial 45° pulse for ^{13}C, APT, and CRAPT spectra. The total acquisition time in each case was 13 min.

Spectra were processed with 2 Hz line broadening. DEPTQ spectra used the same ^{13}C spectral window and acquisition time as the other spectra. The initial pulse was a 90° pulse, the delay, D1, with the decoupler on, was 3 s whereas D2, with the decoupler off, was 1 s, as recommended by the original authors.[6] The number of scans was 128 to keep the total time at 13 min, identical to the previous spectra. Standard Agilent pulse sequences (VnmrJ.4 software release) were used for APT and DEPTQ spectra. For each spectrum, the signal/noise was measured for the tallest peak and converted to signal/noise values for other peaks in the spectrum by multiplying that signal/noise value by the ratio of peak height of a given peak over that for the tallest peak. In each case, noise levels were measured for a 10-ppm region (δ90–100 ppm).

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