Complete Proton and Carbon Assignment of Triclosan via One- and Two-Dimensional Nuclear Magnetic Resonance Analysis

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Abstract: Students from an upper-division undergraduate spectroscopy class analyzed one- and two-dimensional 400-MHz NMR spectroscopic data from triclosan in CDCl₃. Guided assignment of all proton and carbon signals was completed via 1D proton and carbon, distortionless enhancement by polarization transfer (DEPT) 135, ¹H-¹H correlation spectroscopy (COSY), ¹H-¹³C heteronuclear single quantum coherence (HSQC), and ¹H-¹³C heteronuclear multiple bond coherence (HMBC) NMR experiments. The near-symmetry of this diaryl ether provided a challenging structure elucidation problem for early-semester undergraduate spectroscopy students. Experimental ¹H and ¹³C NMR chemical shifts are compared to estimated chemical shifts from computer software.

Introduction

The use of nuclear magnetic resonance (NMR) spectroscopy for structure elucidation is one of the primary methods of characterization for synthesized and/or isolated compounds in organic and analytical laboratories [1, 2]. The number of primarily undergraduate institutions obtaining high-field NMR spectrometers has increased over the past five years [3] and thus provides the opportunity to increase the level of NMR training undergraduates receive. Utilization of a high-field NMR spectrometer in the upper-division undergraduate teaching laboratory and/or classroom allows students to learn advanced topics in NMR theory, structure elucidation of complex organic molecules, and application of NMR instrumentation to current topics of interest. Additionally, NMR may be used in combination with other modern analytical instrumentation, such as high-performance liquid chromatography (HPLC) or gas chromatography coupled to a mass selective detector (GC/MS), to provide a capstone research experience to undergraduates.

Although triclosan has been thoroughly analyzed by HPLC and GC/MS, there are few reports on the NMR chemical shifts for both proton and carbon positions. One investigation assigned the proton chemical shifts of triclosan in an aqueous medium [7], while another study provided only partial proton assignments [8]. Due to its heavy use as a household antimicrobial and its consequential release into the environment, triclosan has stimulated much debate in the scientific literature [9]. Triclosan has also been the subject of undergraduate research in both chemical and environmental disciplines [10].

As part of a senior research project, a student performing analysis on wastewater influent was asked to analyze triclosan via NMR experimentation and assign the appropriate proton and carbon chemical shifts to the correct positions. The student’s results were presented to a spectroscopy class being taken concurrently. The laboratory experiment provided herein describes a step-by-step analysis of one- and two-dimensional high-field NMR spectroscopic material of triclosan [11] in CDCl₃ and is presented as a teaching tool for upper-division undergraduate labs and/or courses. The set-up and evaluation of the experiments ultimately allow the students to learn first-hand the process of assigning all proton and carbon signals in a nearly symmetrical, relatively complex molecule while simultaneously learning about the NMR experiments used to obtain the spectroscopic material. If a high-field NMR is not readily available, the requisite spectroscopic material associated with this report can be downloaded and used to present to an upper-division class and/or laboratory. The upper-division student was able to easily provide assignments of the non-quaternary positions and moved on to the challenge of properly determining the more complex quaternary positions.

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Figure 1. $^1$H NMR spectrum with integration and labeled peaks of triclosan in CDCl$_3$.

Table 1. $^1$H and $^{13}$C NMR$^a$ assignments and observed coupling constants for Triclosan in CDCl$_3$

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H $\delta$</th>
<th>Multiplicity</th>
<th>$J$ (Hz)</th>
<th>Integration</th>
<th>$^{13}$C $\delta$</th>
<th>$^1$H $\delta$</th>
<th>$^{13}$C $\delta$</th>
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<td>-</td>
<td>-</td>
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<td>dd</td>
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<td>7.01</td>
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<td>d</td>
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<td>-</td>
<td>150.5</td>
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<td>6.93</td>
<td>d</td>
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<td>1H</td>
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<td>7.02</td>
<td>120.3</td>
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<td>-</td>
<td>-</td>
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<td>7.02</td>
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<td>OH (2)</td>
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<td>-</td>
<td>1H</td>
<td>-</td>
<td>9.48</td>
<td>-</td>
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</table>

$^a$ $^1$H NMR, 400 MHz and referenced to residual CHCl$_3$ 7.25 ppm; $^{13}$C NMR, 100 MHz and referenced to CDCl$_3$ 77.11 ppm. $^b$ CambridgeSoft ChemDraw Ultra 10.0, 100 CambridgePark Dr., Cambridge, MA 02140.

Results and Discussion

Overview of the $^1$H NMR spectrum (Figure 1; supporting material, pages 1, 2) provided seven signals (less the residual chloroform peak at 7.25 ppm) in or close to the aromatic region. The broad singlet at 5.66 ppm was tentatively assigned to the OH peak at position 2. The near-symmetry of triclosan with its relative ortho and para positioning provided the duplicated splitting pattern of two 1,2,4-trisubstituted aromatic rings, each containing one doublet with a relatively large coupling constant, consistent with ortho coupling ($J \approx$ 9 Hz); one doublet with a relatively small coupling constant, consistent with meta coupling ($J \approx$ 3 Hz); and, a doublet of doublets with corresponding coupling constants for both ortho and meta coupling [1]. Integration of the proton spectrum (Figure 1; supporting material, page 2) provided a total of seven protons, which correlated with the number of protons in the molecular formula of C$_{12}$H$_7$Cl$_3$O$_2$.

If desired, the students can determine the coupling constants and peak multiplicities from the labeled peaks in Figure 1 ($J$ values are also provided in the supporting material, page 3). For example, the peak at 6.65 ppm showed as a doublet with peaks at 6.6587 and 6.6381 ppm. The difference of these peaks can be multiplied by the irradiation frequency of 399.7822 Hz to result in a coupling constant of 8.24 Hz. Similarly, the remaining signals may be evaluated to reproduce the $J$ values listed in Table 1.
Examination of the $^{13}$C spectrum (less the CDCl$_3$ triplet at 77.1) provided twelve signals and the DEPT 135 spectrum verified six methines and six quaternary carbons (Figure 2; supporting material, pages 4, 5). The three quaternary signals between 140 and 155 ppm were in the chemical shift range of an aromatic carbon bearing oxygen and supported the presence of oxygens at C-1, C-2, and C-1'.

The two isolated spin systems of triclosan were readily identified via interpretation of the $^1$H-$^1$H COSY NMR experiment (Figure 3; supporting material, pages 6, 7); however, it was not readily apparent which spin system was associated with ring A or ring B. A tentative assumption was made that the more upfield spin system (signals at 6.65, 6.81, and 7.06) belonged to the relatively electron-rich ring A, which contains the phenolic and aryl ether moieties. Chemical shift estimations corroborated this supposition, although the exact values were not in complete concurrence. With this assumption in place, the tentative proton and methine carbon assignments of positions 3, 5, 6, 3', 5', and 6' were provided by evaluation of the HSQC (Figure 4; supporting material, pages 8, 9). Assigned values are presented in Table 1. The broad singlet at 5.66 ppm was confirmed as belonging to the phenolic OH by its isolation in the COSY NMR spectrum. Additionally, a $^1$H NMR experiment could be performed on the sample with one drop of D$_2$O added to confirm this signal as being the phenolic OH.

The HMBC NMR spectrum provided the ability to distinguish between the two isolated spin systems of triclosan. Cadmium nitrate [12] was added to the sample to reduce the hydrogen bonding of the phenolic hydrogen, and the HMBC
The key link to unambiguously identifying ring A signals was the readily identifiable $J^3$ correlation between the phenolic hydrogen at 5.66 ppm and the C-3 methine at 117.0 ppm (supporting material, page 10). Additionally, the quaternary carbon signals at 142.2 and 147.4 ppm showed weak correlations to the phenolic hydrogen, and although no immediate determinations were made, these correlations implied assignments to C-1 and C-2 (Figures 5, 6). The $J^2$ correlation for OH (2)-C3 confirmed the earlier assumption regarding the electron-rich ring A and solidified the assignments for those positions.

With the key correlation of the phenolic OH to ring A established, attention was turned to the assignment of the quaternary carbons. Working with the HMBC expansion (supporting material, page 11, 12), the carbon signal at 150.5 ppm showed a $J^2$ correlation to H-6', $J^3$ correlations to H-5' and H-3', and was subsequently assigned to C-1'. The next two carbon signals, implicated earlier as C-2 and C-1, at 147.4 and 142.2 ppm provided the expected $J^2$ and $J^3$ correlations to the proton signals in ring A. Notable, was the absence of a correlation between the carbon signal at 147.4 ppm and H-5 providing assignment of C-2 to this chemical shift.
An interesting feature of the HMBC spectroscopic material was the lack of correlations and the symmetry of H-6' to both C-2' and C-4'. The lack of correlations and the symmetry of H-6' to both C-2' and C-4' did not provide unambiguous assignment of these two carbon signals. This ambiguity was worked-out using the electronics of the ring’s substituents. The aryl ether at C-1' provides greater electron density to ortho position of C-2' relative to the para position of C-4' therefore, the ortho position should resonate at the more upfield position. Consequently, C-2' was assigned to 126.4 ppm and C-4' was assigned to 130.2 ppm. This rationalization is corroborated by the chemical shift estimates provided in Table 1. Finally, the 13C signal at 130.2 ppm displayed one strong J correlation to H-6, H-5, and H-3, respectively, thus providing designation to C-4.

Conclusion

In summary, the proton and carbon assignment of triclosan using modern 1D and 2D NMR techniques was demonstrated as a means to instruct the theory and application of high-field NMR spectroscopy to upper-division undergraduate students. The cadmium nitrate/HMBC NMR experiment was essential in that it provided key correlations of OH (2) to the 13C signals of C-1, C-2, and C-3. An interesting feature of the HMBC spectroscopic material was the lack of J correlations for H-3' and H-5' to the quaternary carbons C-2' and C-4' in ring B, but not in ring A. Incorporation of chemical shift estimation was presented as a way of introducing students to an alternative method of approximating chemical shifts.

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Supporting Material. The following supporting material for this article is available: table of contents; 1H and 13C 1D and 2D NMR spectroscopic material used for this project; ChemDraw estimates for proton and carbon chemical shifts, a student worksheet that includes brief descriptions of select NMR experiments and large, numbered structures of triclosan. All supplementary NMR spectroscopic materials are scanned images of actual printed spectroscopic material. This material can be downloaded in a Zip file. (http://dx.doi.org/10.1333/s00897072071a)

References and Notes

3. Search of awards from 2001 to 2006 on NSF web site http://www.nsf.gov/ (accessed Sept 2007). Keywords used: undergraduate and NMR; element code 1189. On average, two to three high-field NMR spectrometers were awarded each year to undergraduate institutions.
11. Triclosan, >97%, (72779-5G-F) was purchased from Sigma-Aldrich Chemical Company.