

Isolating *trans*-Anethole from Anise Seeds and Elucidating Its Structure: A Project Utilizing One- and Two-Dimensional NMR Spectrometry

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The isolation of organic compounds from natural sources continues to be a common type of experiment in undergraduate organic chemistry laboratories. Essential oils are an easily accessible class of such compounds. However, relatively few experiments involving these interesting molecules have appeared in this *Journal* (1–10). Even fewer experiments have utilized NMR spectrometry to determine the structures of these compounds and their derivatives (3, 11–15).

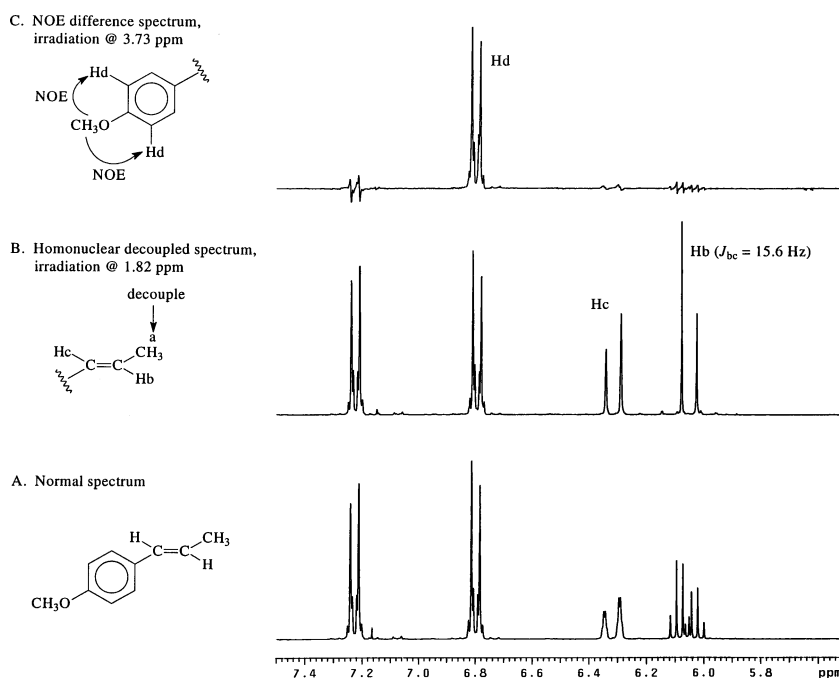
The Project

In an effort to expose my students to the techniques of isolation, purification, and structure elucidation of a natural product, I have introduced a three-week project into our Advanced Chemistry Laboratory at SUNY Oswego. These types of projects are the backbone of our curriculum in this course (16). In this project students isolated *trans*-anethole from anise seeds, purified it by flash chromatography (17), and elucidated its structure by one- and two-dimensional (1- and 2-D) NMR spectrometry. A brief description of the isolation of *trans*-anethole from anise seeds by steam distillation and its conversion to *p*-anisic acid appeared in this *Journal* (4), as did its analysis by HPLC (5). In addition, infrared

(IR) spectroscopy has been used to confirm the structure of the propenyl side chain (18). However, no experiment published to date has used NMR spectrometry to establish the structure of *trans*-anethole.

The project exposes students to a number of important techniques in organic chemistry including steam distillation, thin-layer chromatography (TLC), and flash chromatography. In addition, a wide variety of NMR methods are introduced such as Distortionless Enhancement by Polarization Transfer (DEPT), CORrelated SpectroscopY (COSY), HETeromuclear CORrelated (HETCOR) spectroscopy, homonuclear decoupling, and Nuclear Overhauser Effect (NOE) difference spectroscopy. In fact, it is the extensive use of modern Fourier transform (FT) NMR techniques that makes this experiment so valuable. The relative simplicity of the *trans*-anethole structure, which appears in Figure 1 A, makes this an ideal molecule for a first exposure to the COSY and HETCOR 2-D NMR techniques. The project is well suited for an advanced laboratory in organic chemistry or instrumental analysis. Alternatively, the isolation and purification of *trans*-anethole can be omitted and the emphasis placed solely on the structure determination of a commercial sample in a lab or lecture course in organic spectroscopy.

Figure 1. Partial 300 MHz NMR spectra of *trans*-anethole.



Results and Discussion

The experiment was divided into the following three major sections:

- Steam distillation and isolation of crude *trans*-anethole
- Purification by flash chromatography
- NMR analysis

During the first laboratory period the steam distillation was performed on 10 g of freshly ground anise seed using standard procedures. The crude anethole was extracted into CH_2Cl_2 . While the distillation was in progress, the students performed TLC on a commercial sample of *trans*-anethole to find a suitable solvent system for flash chromatography. They found that with a solvent composed of hexane- CH_2Cl_2 5:1 (v/v) *trans*-anethole had an R_f of approximately .35 (17). The same solvent was then used for the flash chromatographic separation in the second week. The anethole-containing fractions were detected by TLC and combined, and the solvent was evaporated to dryness on a rotary evaporator. In the third week of the project the NMR spectra were generated. The ^1H and ^{13}C spectra were run on each student's sample. The homonuclear decoupled and NOE difference spectra were generated from a single representative sample. Each student was given access to his or her own free-induction decay (FID) patterns for these samples. The FIDs were processed off-line at remote personal computers using a free NMR processing program.¹ The DEPT, COSY, and HETCOR spectra were also generated from a single sample and copies were made available to the students.

Students were given the molecular formula, $\text{C}_{10}\text{H}_{12}\text{O}$, and from this they calculated an unsaturation number of five. Alternatively, the students can be given the mass spectrum, from which the molecular formula can be determined using the molecular ion in conjunction with the $M + 1$ and $M + 2$ peaks (19). The unsaturation number and the four aromatic protons, two at 6.80 and two at 7.22 ppm, supported the conclusion that a para-disubstituted aromatic ring was present, as did a strong IR absorption band at 840 cm^{-1} due to an out-of-plane C-H bending. In addition, the DEPT spectrum showed only two aromatic CH resonances, one at 113.79 and the other at 126.78 ppm, indicating a symmetrically substituted aromatic ring. The aromatic ring accounted for four unsaturation units, leaving one unaccounted for. The fact that two one-proton multiplets were present in the proton spectrum, one centered at 6.06 and the other centered at 6.32 ppm, supported the presence of a disubstituted double bond. These multiplets are shown in Figure 1A. The multiplet centered at 6.06 ppm consisted of two overlapping quartets. This was a challenging pattern for most of the students to interpret. Therefore, a homonuclear decoupling experiment was performed by irradiating the methyl group at 1.82 ppm attached to the double bond. This experiment simplified the spectrum significantly by reducing each of the overlapping quartets of Hb to single lines, as shown in Figure 1B. It clearly revealed the *trans*-coupling constant of 15.6 Hz. In addition, the broad quartets of Hc centered at 6.32 ppm collapsed to single lines with the removal of the allylic coupling to the methyl group. The *trans* stereochemistry was also confirmed by IR spectroscopy, which showed a strong absorp-

tion at 965 cm^{-1} due to an out-of-plane C-H bending. Additional support for the methyl group was the three-proton doublet of doublets centered at 1.82 ppm. The methyl group was split by Hb with a vicinal coupling constant of 6.6 Hz and by Hc with a small allylic coupling constant of 1.8 Hz. The remaining signal was a three-proton singlet at 3.73 ppm typical of an $-\text{OCH}_3$ group. The COSY spectrum clearly showed all the proton-proton couplings. The two sets of aromatic protons were assigned by an NOE difference experiment. Irradiation of the $-\text{OCH}_3$ group caused an NOE enhancement of the multiplet centered at 6.80 ppm, as shown in Figure 1C. This confirmed that these two aromatic protons labeled Hd are ortho to the $-\text{OCH}_3$ group. The remaining two aromatic protons at 7.22 ppm were not enhanced because they are meta to the $-\text{OCH}_3$ group.

With the correct structure and the proton assignments in hand, the students then assigned the ^{13}C spectrum. The carbon types (two CH_3 's, four CH's, and two quaternary carbons) were assigned by DEPT spectroscopy. Specific resonances within each carbon type were assigned by HETCOR spectroscopy. The two aromatic quaternary resonances were assigned by chemical shift arguments: the carbon attached to the oxygen atom resonates further downfield than the one attached to the alkene carbon.

The project has been quite successful. It has been performed for three years and the results are highly reproducible. Yields of *trans*-anethole after flash chromatography average 142 mg. The vast majority of students are able to determine the structure and assign the ^1H and ^{13}C spectra correctly even though this is their first exposure to 2-D NMR spectrometry. The students have acquired numerous NMR interpretation skills. Off-line processing of the FIDs allows students to process their own 1-D NMR data at their leisure. For instructors who do not have access to a modern Fourier transform NMR spectrometer, the spectra are available from me upon request.

Acknowledgments

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^wSupplemental Material

The following supplemental material for this article is available in this issue of *JCE Online*: a detailed student handout, which includes explanations and examples of the NMR techniques; and Instructors' Notes, which contain the ^1H and ^{13}C NMR assignments, answers to questions in the student handout, comments on the experiment, and lists of equipment, supplies, and reagents.

Note

1. The NMR processing software entitled "Magnetic Resonance Companion" (MestRe-C) written by F. J. Sardina, J. C. Cobas, and J. Cruces can be downloaded free of charge at the following address: <ftp://qobruue.usc.es/nmr/MestRe-C>.

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