

Elucidation of Molecular Structure Using NMR Long-Range Coupling: Determination of the Single Isomer Formed in a Regiospecific Reaction

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An undergraduate laboratory experiment in organic chemistry should include interesting organic synthesis and the resolution of a tricky problem related to the synthesis. The second part is important to encourage students' involvement in the experiment. Both parts play a major role in the experiment described in this paper.

With modern spectrometers and the development of new techniques, NMR has evolved into one of the most useful tools available to students in chemistry for investigating structural or mechanistic problems (1). Some examples of potential uses have been described in this *Journal* (2). This experiment introduces students to the main concepts of two essential NMR techniques for molecular structure investigation. Heteronuclear multiple bond connectivity (HMBC) and proton-coupled ^{13}C NMR are used to determine the isomer formed in a regiospecific reaction. The study, based on long-range coupling between quaternary carbons and protons on neighboring carbons, is presented step-by-step with didactic progression. In addition, the regiospecific reaction provides an excellent example of ring-opening in a monosubstituted cyclic anhydride. Nucleophilic attack on carboxylic acid derivatives and problems of regioselectivity are also presented.

Experimental Section

Ethyl 2-(*S*)-acetoxy-3-carboxypropionate (Fig. 1, **2a**) can be prepared in accordance with a procedure previously described in detail (2). 2-(*S*)-Acetoxysuccinic anhydride (**1**) is commercially available but can be easily synthesized from L-malic acid. The two-step reaction requires no special equip-

ment and can be performed as follows. A 50-mL, three-necked, round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, thermometer, and addition funnel is charged with L-malic acid (2.5 g, 18.6 mmol), acetyl chloride (8 mL), and acetic anhydride (4 mL).

• **CAUTION:** acetyl chloride and acetic anhydride must be handled with suitable protective clothing and adequate ventilation.

The reaction mixture is stirred and heated at 60 °C for 2 h under a dry atmosphere. The solvents are then distilled under reduced pressure to afford 2.9 g (quantitative yield) of **1** as a viscous oil, which crystallizes at low temperature; m.p. 55 °C, $[\alpha]_D^{20}$ -24.1° (*c* 1.0, CHCl_3), IR (KBr): ν = 1875, 1790, 1745 cm^{-1} (C=O). The recovered acetyl chloride and acetic anhydride are destroyed after distillation by adding methanol. Then a 50-mL, two-necked, round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and thermometer is charged with 2-(*S*)-acetoxysuccinic anhydride (**1**) (2.9 g, 18.3 mmol) and absolute ethanol (10 mL). The solution is stirred at 50 °C for 30 min and then at room temperature for 6 h under a dry atmosphere. The reaction mixture is evaporated to dryness and the residual syrup is cooled and triturated to afford 3.7 g (quantitative yield) of a white solid; m.p. 52 °C, $[\alpha]_D^{20}$ -26.1° (*c* 1.0, CHCl_3), IR (KBr): ν = 3550, 2600 cm^{-1} (O-H), 1760–1720 cm^{-1} (C=O).

NMR spectra were recorded in CDCl_3 solutions with a concentration of product of about 100 mg/mL on a Bruker ARX 400 spectrometer. However, with a large number of scans, satisfactory spectra showing the C–H long-range coupling pattern can also be recorded on a 250 MHz spectrometer. Students are not usually asked to record the spectra by themselves, as the techniques are complicated and devoid of interest in an undergraduate organic chemistry course.

Results and Discussion

The ring-opening of 2-(*S*)-acetoxysuccinic anhydride (**1**) by ethanol is particularly interesting because of its regiospecific nature (3), even though a mixture of two isomers, the α - and β -acetoxy esters, could be formed. Figure 1 illustrates the two routes of nucleophilic attack by ethanol on the cyclic anhydride (**1**).

This reaction can be carried out in gram-scale amounts and requires no special equipment. The anhydride is treated with an excess of absolute ethanol for several hours at room temperature. After removal of the unreacted alcohol under vacuum, the final product may be submitted to usual NMR analysis. Classic ^1H , ^{13}C , and (C,H) correlation NMR experiments provide sufficient data to suggest that the product obtained is a pure ethyl acetoxy-carboxypropionate isomer,

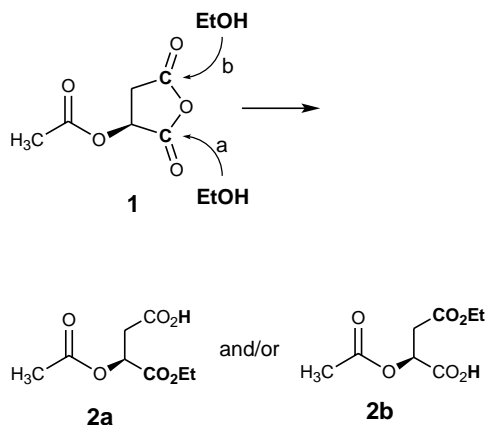


Figure 1. Nucleophilic attack by ethanol on monosubstituted cyclic succinic anhydride.

Table 1. Assignments from ^1H and ^{13}C Spectra

Shift/ppm		Assignment
^1H	^{13}C	
1.2	13.8	ethyl ester methyl
2.1	20.4	acetyl methyl
2.9	35.7	methylene
4.2	61.9	ethyl ester methylene
5.4	—	methine
10.7	—	acid proton
—	168.7	(?)
—	170.1	(?)
—	174.5	(?)

but do not give the regiospecificity of the reaction. The spectral assignments are reported in Table 1.

At this stage it is not possible to know whether the α - or the β -acetoxy ester is formed. Moreover, complete assignment of the carbonyl groups cannot be made. Students are thus made aware of the limits of classic NMR results in such cases and can understand the necessity for more accurate techniques. Until now, the structure of the single isomer described in the literature as α -acetoxy ester had been based only on its chemical reactivity in selective reduction reactions (3).

In the first step, total assignment of the carbonyl signals is required. An HMBC experiment (4, 5) was performed to show the different (C, H) couplings through two and three covalent bonds. Observation of the carbonyl group region reported in Figure 2 provides all the long-range couplings and allows the assignment to be made, as reported in Table 2.

Then, determining which isomer is produced from the regiospecific reaction becomes possible, based on the study of the couplings existing between the carboxylic acid carbon and the protons on neighboring carbons. As illustrated in Figure 3, the two possible isomers differ by the environment of the carboxylic acid function.

In **2a**, the acid carbonyl would exhibit couplings with two hydrogens through two bonds ($^2J_{\text{CH}}$) and with one hydrogen through three bonds ($^3J_{\text{CH}}$), whereas in **2b**, the acid carbonyl would exhibit couplings with one hydrogen through two bonds ($^2J_{\text{CH}}$) and with two hydrogens through three bonds ($^3J_{\text{CH}}$). Note that $^2J_{\text{CH}}$ is always greater than $^3J_{\text{CH}}$. The proton-coupled carbon spectrum, using gated decoupling (6), provides students with the information required to deduce the isomer

Table 2. Carbonyl Assignment from HMBC Spectrum

^{13}C Shift/ ppm	J_{CH} Long-Range Coupling Observed with	Assignment Deduced
168.7	—CH at 5.4 ppm —CH ₂ at 2.9 ppm —CH ₂ (ester) at 4.2 ppm	ethyl ester carbonyl
170.1	—CH at 5.4 ppm —CH ₃ (acetyl) at 2.1 ppm	acetyl carbonyl
174.5	—CH at 5.4 ppm —CH ₂ at 2.9 ppm	acid carbonyl

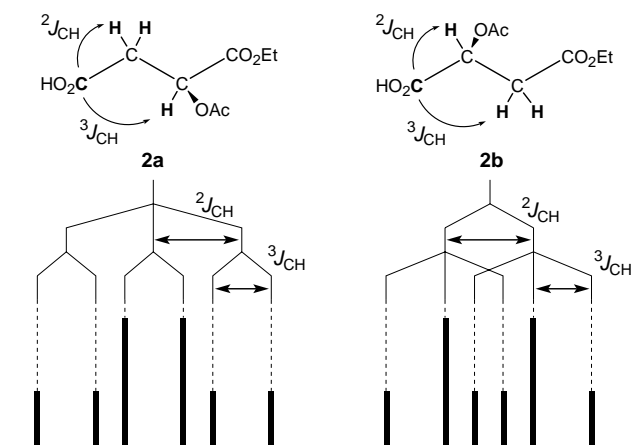


Figure 3. Possible couplings between the carboxylic acid function and protons on neighboring carbons for **2a** and **2b** and related expected spectrum based on relative magnitudes of coupling constants ($^2J_{\text{CH}} > ^3J_{\text{CH}}$).

structure, since each carbon signal splits into a multiplet resulting from coupling with hydrogens directly attached to the carbon ($^1J_{\text{CH}}$) and hydrogens on neighboring carbons ($^2J_{\text{CH}}$ and $^3J_{\text{CH}}$). Thus, the expected signal for the acid function is a double triplet for **2a** or a triple doublet for **2b** (Fig. 3). The spectrum obtained with this technique, presented in Figure 4, reveals that the signal at 174.5 ppm, previously assigned to the acid carbonyl, splits into a double triplet with coupling constants $^3J_{\text{CH}}$ of 5.3 Hz and $^2J_{\text{CH}}$ of 6.9 Hz. This multiplicity confirms that the analyzed product is the α isomer, ethyl α -acetoxy-carboxypropionate ester **2a**.

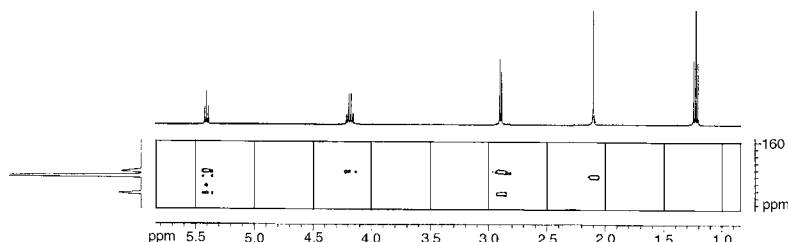


Figure 2. HMBC spectrum of the final product, corresponding to the carbonyl region. Two- and three-bond couplings between quaternary carbons (carbonyls) and neighboring protons are observed.

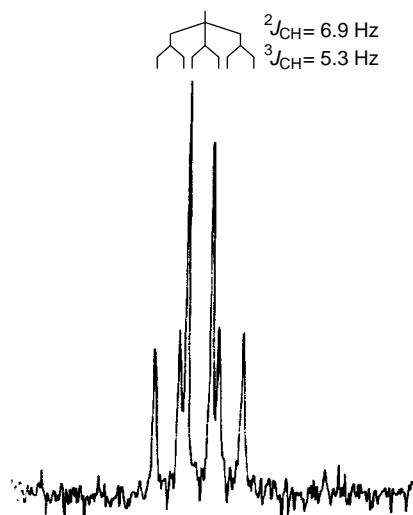


Figure 4. Proton-coupled ^{13}C NMR spectrum of the final product. Only the carboxylic acid carbonyl at 174.5 ppm is presented. This signal splits into a double triplet with coupling constants $^3J_{\text{CH}} = 5.3$ Hz and $^2J_{\text{CH}} = 6.9$ Hz.

Thus, combination of these two experiments allows the unambiguous structural elucidation of the product and should give students a better understanding of the concept of NMR and its potential uses.

Conclusion

The experiments presented in this article highlight a number of points useful for undergraduate students' understanding of organic chemistry and NMR spectroscopy. The reaction considered may be used as an example of a regio-specific reaction. In addition, it may lead to a discussion about why only α -acetoxy ester is formed. In fact, here, the regio-specificity is attributable to the higher electrophilicity of the

carbonyl nearer to the acetyl group, which is due to the presence of the oxygen atom on the α carbon.

The resolution of this structural problem can be used as a laboratory experiment in which the student is first asked to prepare the studied compound and then to analyze the spectra recorded from it by the teaching assistant. The fact that long-range coupling analysis is the only way the structure can be elucidated has to be emphasized. This is why unconventional NMR techniques must be used. Additional analyses could be performed on compounds **1** and **2a** or on other products to enlarge students' experience with analytical techniques. These could include optical rotation measurement and recording infrared spectra (7). Schiff reagents could be used to see if they help interpretation of the ^1H NMR spectra.

^WSupplemental Material

Supplemental material for this article is available in this issue of *JCE Online*.

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