Determination of the *p/o*-Isomer Product Ratios in Electrophilic Aromatic Nitration of Alkylbenzenes Using High Resolution ^{1}H NMR Spectroscopy

Michelle K. Waddell, Charles M. Bump, Edmund M. Ndip, Godson C. Nwokogu[*]

Department of Chemistry and Biochemistry, Hampton University, Hampton, VA 23668, U.S.A.
*Corresponding author: godson.nwokogu@hamptonu.edu

Abstract High resolution ^{1}H NMR spectroscopy, an increasingly available instrumental method, is used in undergraduate organic laboratory practice as a simpler alternative to gas chromatographic method for the direct determination of the *p/o* ratios of the crude reaction product from the nitration of alkylbenzenes. The choice of isopropylbenzene as a substrate illustrates that steric effect can be significant in controlling regioselectivity in electrophilic aromatic substitution reactions.

Cite This Article: Michelle K. Waddell, Charles M. Bump, Edmund M. Ndip, and Godson C. Nwokogu, "Determination of the *p/o*-Isomer Product Ratios in Electrophilic Aromatic Nitration of Alkylbenzenes Using High Resolution ^{1}H NMR Spectroscopy." *World Journal of Chemical Education*, vol. 7, no. 3 (2019): 216-224. doi: 10.12691/wjce-7-3-5.

1. Introduction

Nitration of alkylbenzenes is a reaction used to illustrate the concepts of regioselectivity, directing effects and steric effects of substituents in electrophilic aromatic substitutions (EAS). The earlier method used for the quantitative determination of the regioselectivity of this reaction in laboratory exercises [1,2] and class room demonstrations [3] is the gas chromatographic (gc) analysis of the product mixture. A newer method that has been reported for determining mole ratios of binary mixtures of conformers [4], tautomers [5], stereoisomers [6,7,8,9] and regioisomers [6,7,10,11,12,13] is high resolution ^{1}H NMR spectroscopy. This method is faster, simpler to use, more direct and accurate for the determination of the mole ratio of mixtures than the gas chromatographic method. The most relevant reported examples of this application of quantitative NMR spectroscopy, however, analyzed regio-isomeric ratios of pre-purified solid EAS reaction products. Purification steps such as recrystallization [10] or trituration [12], however, alter the actual product ratios because of different solubilities in the solvent used. We report here the use of high resolution ^{1}H NMR spectroscopy to determine the actual *o/p* reaction products ratios in EAS reactions from which only the reaction solvent has been removed by evaporation.

High resolution/field instruments are necessary for adequate separation of proton signals of components of mixtures and for accurate signal integration. The infusion of modern instrumentation into the undergraduate curriculum by funding agencies in many countries [14] has resulted in the increasing availability of high resolution NMR spectrometers for undergraduate education. Optional autosamplers, automation for running multiple samples, and user-friendly software for spectrometer control and data processing that can be purchased with modern spectrometers make it possible for large undergraduate classes to acquire hands-on skills and experimental data on these instruments.

The example reported here is a laboratory activity that illustrates the determination of the actual regioselectivity of EAS reactions with the following advantages:

(a) The ratio of isomers generated by the reaction is determined directly from the crude after reaction solvent removal without any further purification. Purification steps such as recrystallization, trituration and distillation alter the actual ratio of the reaction product mixture.

(b) The use of isopropylbenzene best illustrates the significance of steric effect on the regioselectivity of EAS reactions in the presence of both statistical and electronic factors.

(c) The relevant aromatic ^{1}H NMR signals for determining mole ratio of products are quite removed from the signals of impurities and unreacted alkylbenzene substrate. This allows for accurate results without purification of the crude even for reactions with low conversion.

(d) This method can be successfully used to determine the *p-/o-* mole ratio of the crude product mixture from

nitration of aromatic compounds even when the products are liquids which are more difficult to purify.

The nitration of an alkylbenzene laboratory activity is preceded by a pre-nitration activity – a discovery-based activity on the use of ^{1}H NMR spectroscopy to determine the mole ratio of compounds of known structures and molar masses in mixtures. This ^{1}H NMR activity acquainted students with matching ^{1}H signals to structures in a mixture, sample preparation, data acquisition procedures and ^{1}H NMR spectral processing with the spectrometer software. Students discover from this activity that under certain conditions, the mole ratios of components of a mixture can be determined using integrals of appropriate signals of the components.

In the second lab period, each laboratory team of three students carries out the mixed acid nitration of isopropylbenzene, the sample preparation and acquisition of proton NMR spectrum of the team's crude product mixture. Each group processes the spectrum of their product on their own time according to instructor demonstrated instructions and calculates the mole ratio of *p*-isopropylnitrobenzene to *o*-isopropylnitrobenzene of their crude product for writing their individual lab reports.

These laboratory activities have been performed by students during two spring and two summer semesters of second semester organic chemistry laboratory courses. The results and answers to post-lab questions in student lab reports indicate that these activities enhanced student skill in instrumental analysis of experimental results and understanding of the factors that control regioselectivity in EAS reactions.

2. Experimental Section

2.1. Pre-Nitration Activity: Determination of the Mole Ratio of Components of a Binary Mixture by ^{1}H NMR Spectroscopy

This pre-nitration activity is an discovery-based exercise which acquaints students with the use of ^{1}H NMR spectroscopy for the calculation of the mole ratio of components of a binary mixture. First, students are provided with processed ^{1}H NMR spectra of pure ethyl ethanoate (EE), pure methyl propanoate (MP) and a mixture of the two compounds or instructor-selected substitutes. These spectra are used to illustrate how proton signals are matched to structures and how this information can be used to assign signals in a mixture to the components. Student groups are then provided samples with known but different masses of ethyl ethanoate and methyl propanoate or any substitutes the instructor chooses, i.e. each lab team in the laboratory section is assigned a quantitatively different mixture of the two compounds EE and MP. Each team prepares an NMR sample of their mixture and all samples are set up for automatic acquisition, processing and printing of the ^{1}H NMR spectra. [15] After obtaining the processed and printed copy of the integrated ^{1}H NMR spectrum of their mixture (Figure 1) each team is required to calculate ratios of integrals of specified signals of components (see SI for description of the calculations) as well as mole ratio of their mixture. The results of these calculations are all

posted on a common lab section table drawn by the instructor on a board. Comparing the ratios from calculations based on integrals of signals of components with the ones calculated from masses and molar masses of components of the different mixtures leads students to discover that the ratio of integrals is equal to the mole ratio of components of a mixture only when the integrals correspond to equal number of protons in the structure of each component! Both the spectral data acquisition for the known binary ester mixture and the processing demonstrations, as described in SI, are completed in one 3h laboratory period. These skills are then applied by students in the data acquisition and independent analysis of the products of the EAS reaction to calculate the *o/p* product ratio.
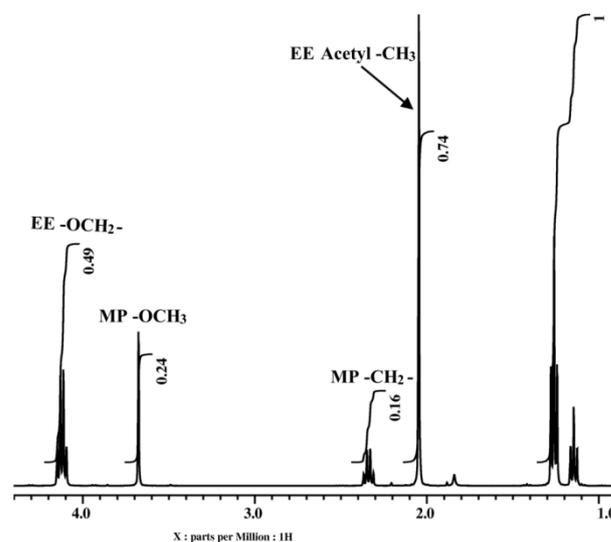

Figure 1. ^{1}H NMR spectrum of a 3:1 mixture of ethyl ethanoate (EE) and methyl propanoate (MP) respectively

2.2. Nitration of Isopropylbenzene and Acquisition of ^{1}H NMR Spectrum of the Crude Product Mixture

When compared with some modern methods [16,17,18] for generating the nitronium ion, the old mixed acid method [19,20,21] is still the simplest one. The procedure involves dropwise addition of 1 mL of conc. sulfuric acid into 1 mL of concentrated nitric acid with stirring and cooling in an ice bath. This acid mixture is then added, in small portions, with stirring using a glass rod or magnetic stirrer if available, to 1 mL of isopropylbenzene dissolved in 5 mL of methylene chloride (CH_2Cl_2). After the addition, the reaction mixture is stirred vigorously for 1 h. Then 10 mL of fresh CH_2Cl_2 is added to the reaction mixture and the organic layer is washed successively with saturated $NaHCO_3(aq)$ and distilled water. After drying over anhydrous Na_2SO_4, the methylene chloride can be removed by using the rotary evaporator, if available or by simple distillation. Reducing the amount of solvent in the product mixture enhances the signals of the products and does not alter the ratio of regioisomers produced.

^{1}H NMR spectrum of the product for each group is prepared in deuterated chloroform ($CDCl_3$). All samples for a lab section are set up on an autosampler and run in automation mode. The acquired NMR data can be made

available to students by three methods: access to a storage computer, e-mailing the data to students and/or posting the data on an instructional platform such as BlackBoard[TM].

3. Hazards

Gloves should be worn for handling concentrated nitric and sulfuric acids, aromatic and all compounds used in the activities. CH_2Cl_2 and $CDCl_3$ are eye and skin irritants. They are also very volatile and should be handled in a fume hood. Methyl propanoate causes skin irritation and ethyl ethanoate causes eye irritation. Nitrobenzenes are toxic substances that should be handled with protective wear.

4. Results and Discussion

A mixture of known mole ratio prepared from pure samples of *ortho-* and *para-*chloronitrobenzenes is used by the instructors to verify that the operating parameters of the 400 MHz spectrometer used in product analysis will ensure close numeric equality between ratio of signal integrals and mole ratio of components of a mixture of aromatic compounds, i.e. that the ratios obtained from integrals is similar to the mole ratio calculated from the masses of components. The ^{1}H NMR spectrum of the mixture of pure samples of *o-* and *p-*chloronitrobenzene and the ^{1}H NMR spectra of each pure component are used also to identify that the signals of the protons adjacent to the nitro-group in the *p-* and *o-*substituted nitrobenzenes can be adequate basis for the determination of the mole ratio of the regio-isomeric products from the nitration of alkylbenzenes (Figure 2).

Figure 2. Structures of p-Nitrocumene and o-Nitrocumene showing the chemical shifts of the protons used to calculate mole ratio of the their mixture in crude product

The suitability of ^{1}H NMR spectroscopy for determining the mole ratio of *p/o-*nitration products of alkylbenzenes at 400 MHz is also based on the fact that the aromatic protons adjacent to the nitro-group of the *p-*product (H_p) and the *o-*product (H_o) (Figure 2) are both well separated from each other and quite distant from the other signals in the crude product mixture at this operating frequency. That this method will work for analysis of the nitration product of most monosubstituted benzenes is illustrated by the observations that the frequency separation between the H_o and H_p signals of *o-*nitrotoluene and *p-*nitrotoluene respectively is 6 Hz at 60 MHz [22] but for the isopropylnitrobenzene analogs, the separation is 170 Hz at 400 MHz. [23] For *o-* and *p-*chloronitrobenzenes, the corresponding *o-* and *p-*proton signals are overlapped at 60 MHz [22] but separated by 145 Hz at 400 MHz [23]. Introductory instructions for processing the spectrum and

determining the integral values for the relevant signals and calculating the mole ratio using aromatic signals may be illustrated to students using the ^{1}H NMR spectrum of the mixture of *p-* and *o-*chloronitrobenzenes.

4.1. ^{1}H NMR Analysis of the Nitration Product Mixture

Student processing instruction for the determination of mole ratio of *para/ortho* nitration products was illustrated with the ^{1}H NMR spectrum of a sample nitration product mixture (Figure 3). Spectral processing should include horizontal and vertical expansions of the region (7.6 - 8.2 ppm) of the spectrum that contains the relevant aromatic proton signals for the *para* and *ortho* product components, as well as the different methods for measuring the integrals. For the *p-*nitrocumene, the two protons adjacent to the nitro-group appear as two triplets centered at 8.15 ppm while the one proton adjacent to the nitro-group in *o-*nitrocumene shows as two doublets centered at 7.76 ppm. Automatic and/or manual integration methods can be used to determine the integral of the relevant signals. The numerical integral value can be read off from the processed spectrum or by using the vertical measure cursor of the processing software if the relevant signal is co-integrated with another signal. Alternatively, the spectrum can be plotted and a ruler can be used to measure the vertical height of integrals for the specific signals. Vertical expansion of signal intensity (abundance) is usually necessary for the signals and integrals for the *para-* and *ortho-*products to be visible when reaction conversion is low. With a well shimmed spectrometer, significant expansions of signal intensity of more than 100 times can be achieved without degrading the spectral baseline. A clean and horizontal baseline is necessary for accurate integral read-out.
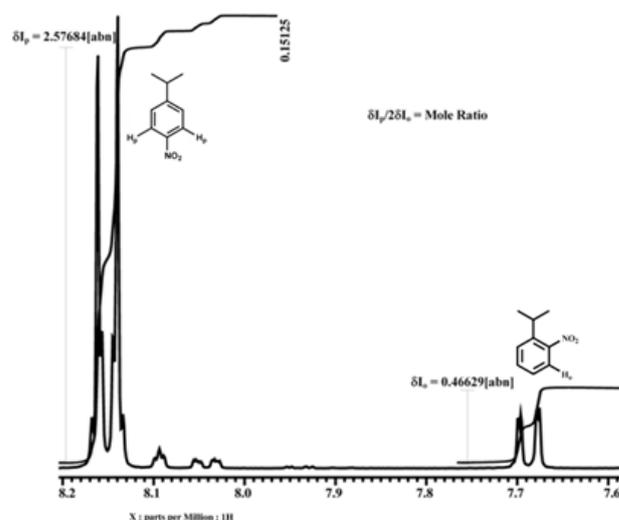

Figure 3. Sample 1H NMR Spectrum of a Nitration Product Mixture Obtained on a 400 MHz Spectrometer

Armed with the information presented above, students are generally able to process their spectra at their own time and use the integrals for the designated signals and the number of protons to determine the *p/o* ratio of their nitration product mixture using the formula:

$$Mole\ Ratio = \delta I_p / 2\delta I_o$$

where δI_p is the integral or integral measure for the two protons adjacent to the nitro-substituent of the *p*-product and δI_o is the integral or integral measure for the one proton adjacent to the nitro-substituent of the *o*-product.

4.2. Statistical Analysis of Students Calculated Mole Ratios

The analysis presented here is based on results obtained by 100 students organized in 33 lab teams in six laboratory sections during the spring and summer of the same year. In each section, students worked in teams of three. Out of the 33 working teams in all the laboratory sections, no nitration product was detected in the ^{1}H NMR spectra for six teams. One probable reason for the failed reactions could be inadequate mixing of the organic and aqueous layers. The spectrum of one of the teams showed only the *para*-product, one group obtained a *p/o* ratio of 3.15:1, another 4.16:1, and another 4.5:1 which are much higher than majority of the other results. Twenty three of the product mixtures obtained *p/o* ratios that were in the range of 2.50:1 to 3.06:1. The average ratio for all samples, excluding the products with ratios of 3.15: 1 and above, was 2.74 ± 0.14. [24] The average of all student reported ratios was 2.88 ± 0.45. Fulkrod [1] reported a value of 2.63 for this ratio by gc. The results with ratios above 3.1:1 as well as the one with only *para*-product might be due to unwitting changes in the procedure such as the amounts of the concentrated acids and temperature of the reaction. The mass ratio of the acids as well as the temperature of the reaction have been reported to lead to product composition varying from a mixture of the *o*-, *m*- and *p*-isomers to only the *para*-isomer. [20]

5. Conclusion

Students in second semester organic chemistry laboratory course used a simple method – high resolution ^{1}H NMR spectroscopy - to determine directly from the crude, the actual mole ratio of regio-isomeric products from the electrophilic aromatic nitration reaction of alkylbenzenes after removing the reaction solvent only. For the nitration of isopropylbenzene, *p/o* ratio varied from 2.50 to 3.06, with the average ratio being 2.74 ± 0.14. Based on the greater than 2 values for the *p/o* ratio obtained by the lab teams, students were able to conclude in their report of this activity, that steric effect was much stronger than statistical and electronic factors in controlling the regioselectivity of the nitration of isopropylbenzene. The activities acquainted students with the use of proton NMR spectroscopy for the simple and direct quantitative analyses of any mixture of known components and applied that knowledge to the analysis of crude product mixtures of EAS reactions.

Acknowledgements

Statement of Competing Interests

The authors have no competing interests

Supporting Information

List of chemicals, student instructions for each activity, lab report questions, lab report guidelines, instructor notes and a table of student reported p- and o- integral values and ratios calculated from them are available.

References

[1] Fulkrod, J. E. A Laboratory Study of Steric and Inductive Effects. *J. Chem. Educ*. 1974, 51(2), 115.

[2] Jarret, R. M., New, J. and Patraitis, C. Electrophilic Aromatic Substitution Discovery Lab. *J. Chem. Educ*. 1995, 72(5), 457-459.

[3] Davis, M.; Deady, L.W.; Paproth, T.G. The Nitration of Alkylbenzenes: A Lecture Demonstration. *J. Chem. Educ.* 1978, 55(1), 34.

[4] Kutateladze, A. G., and Hornback, J. M. Determination of the Position of the Conformational Equilibrium of a Trans 1,2-Disubstituted Cyclohexane by NMR Spectroscopy: An Experiment in Physical Organic Chemistry for Undergraduate Students. *J. Chem. Educ*. 2001, 78(1), 81-82.

[5] Drexler, E. J., and Field, K. W. An NMR Study of Keto-Enol Tautomerism in β-Dicarbonyl Compounds. *J. Chem. Educ*. 1976, 53(6), 392-393.

[6] Markovic, R., Baranac, M., Jovanovic, V., and Dzabaski, Z. Regioselective Synthesis of a Stereodefined Heterocyclic Push-Pull Alkene: ^{1}H NMR Studies and Two-Dimensional TLC Illustrating Z/E Isomerization. *J. Chem. Educ*. 2004, 81(7), 1026-1029.

[7] Friesen, J. B., and Schretzman, R. Dehydration of 2-Methyl-1-cyclohexanol: New Findings from a Popular Undergraduate Laboratory Experiment. *J. Chem. Educ*. 2011, 88, 114-1147.

[8] Saba, S., Clarke, D. D., Iwanoski, C., and Lobasso, T. Using NMR to Probe the Regio- and Stereochemistry of the Hydration of 1-Hexene. *J. Chem. Educ*. 2010, 87(11), 1238-1241.

[9] Centco, R. S., and Mohan, R. S. The Discovery-Oriented Approach to Organic Chemistry. 4. Epoxidation of p-Methoxy-*trans*-β-methylstyrene: An Exercise in NMR and IR Spectroscopy for Sophomore Organic Laboratories. *J. Chem. Educ*. 2001, 78(1), 77-79.

[10] Treadwell, E. M., Lin, T-Y. A More Challenging Interpretative Nitration Experiment Employing Substituted Benzoic Acids and Acetanilides. *J. Chem. Educ*. 2008, 85(11), 1541-1543.

[11] Sen, S. E., and Anliker, K. S. ^{1}H NMR Analysis of R/S Ibuprofen by the Formation of Diastereomeric Pairs. *J. Chem. Educ*. 1996, 73(6), 569-572.

[12] McElveen, S. R., Gavardinas, K., Stamberger, J. A., and Mohan, R. S. The Discovery-Oriented Approach to Organic Chemistry. 1. Nitration of Unknown Organic Compounds: An Exercise in ^{1}H NMR and [13]NMR Spectroscopy for Sophomore Organic Laboratories. *J. Chem. Educ*. 1999, 75(4), 535-536.

[13] Clark, M. A., Duns, G., Golberg, D., Karwowska, A., Turgeon, A., and Turley, J. NMR Analysis of Product Mixtures in Electrophilic Aromatic Substitution. *J. Chem. Educ*. 1990, 67(9), 802.

[14] The chemistry division of the US National Science Foundation (NSF), through its Major Research Instrumentation (MRI) program, funded 82 high resolution NMR spectrometers (67 were 400/500 MHz, 15 were 300 MHz) for undergraduate institutions from 1997 to 2012.

[15] NMR experiments were run on a JEOL ECS 400 MHz spectrometer equipped with a 24-slot auto-sampler using Delta software. Even though the three students in a team obtained the same data, each student submitted an independent report. Working in teams promoted collaborative learning and writing individual reports provided training in writing.

[16] Matthew, S. M., Biradar, A. V., Umbarkar, S. B., and Dongare, M. K. Regioselective Nitration of Cumene to 4-Nitrocumene Using Nitric Acid Over Solid Catalyst. *Catalysis Commun.,* 2006, 7(6), 394-398.

[17] Aridoss, G., Laali, K. K. Ethylammonium Nitrate (EAN)/Tf₂O and EAN/TFAA: Ionc Liquids Based Systems for Aromatic Nitration. *J. Org. Chem.,* 2011, 76(19), 8088-8094.

[18] Augusto, J., Rodrig*ues, R.,* Filho, A. P. D., and Moran, P. J. S. Regioselectivity of Mononitration of Alkylbenzenes by Immobilized Acyl Nitrates. *Synth. Commun.,* 1999, 29(12), 2169-2174.

[19] Knowles, J. H., and Norman, R. O. C. The Transmission of Polar Effects Through Aromatic Systems. Part II. The Nitration of Benzyl Derivatives. *J. Chem. Soc.,* 1961, 2938-2947.

[20] Durst, D. H. and Gorkel, G. W. *Experimental Organic Chemistry,* 2ⁿᵈ Ed., McGraw-Hill, New York, pages 492-xx, 1987.

[21] Haun, J. W., and Kobe, K. A. Mononitration of Cumene. *Ind. And Eng. Chem.,* 1951, 43(10), 2355-2362.

[22] Pouchert, C. J. *The Aldrich Library of NMR Spectra*, Edition II, Vol. I; pages 1131c, 1133b, 1140a, 1143b; Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, 1983.

[23] The reported frequency separations were obtained using our 400 MHz spectrometer on pure samples of the compounds purchased from Aldrich Chemical Company.

[24] A table of measurements of integrals and the calculated ratio from each lab team is provided in Supplemental Information. The reported statistical analysis was based on these results.

Supplemental Information

1. Chemicals Used and Their CAS Numbers:

Compound Name	CAS Registry Number
Ethyl ethanoate (ethyl acetate)	141-78-6
Methyl propanoate (methyl propionate)	554-12-1
Isopropylbenzene (cumene)	98-82-8
p-Isopropylnitrobenzene (*p*-nitrocumene)	1817-47-6
o-isopropylnitrobenzene (*o*-nitrocumene)	6526-72-3
p-Chloronitrobenzene	100-00-5
o-Chloronitrobenzene	88-73-3
Methylene chloride (Dichloromethane)	75-09-2
Deuterated Chloroform	865-49-6
Tetramethylsilane	75-76-3
conc. Sulfuric acid	7664-93-9
conc. Nitric acid	7697-37-2
Sodium Carbonate	497-19-8
Sodium Sulfate	7757-82-6

2. Instructor Notes

2.1. Pre-Nitration Activity: Discovery-based Activity on ¹H NMR Spectroscopy

i. Our activities were carried out on a JEOL ECS 400 MHz NMR spectrometer with a 24-slot autosampler. The control and processing software is Delta v 4.3.6. The software license allows students to install the processing-only software on their personal computers without cost. Processing and automation routines, buttons, and cursors will differ depending on the available spectrometer model but modern spectrometers have identical software capabilities.

ii. Any two compounds can be selected as components for the preparation of the mixtures for the pre-nitration ¹H NMR activity, provided that the chemical shift for at least one ¹H signal for each component is known and separated enough from other signals for separate integration.

iii. For volatile components such as ethyl ethanoate, the mixtures should be stored in snap-cap vials at room temperature. Under these conditions, we found that there is not enough evaporation to alter the initial mole ratio even after a year. In screw-cap vials, ethyl ethanoate can suffer enough evaporation in hours to alter the ratio. Choice of less volatile components will avoid this problem.

iv. It may be necessary to verify that signal separation for the *o*- and *p*-products would be adequate for this laboratory activity on spectrometers operating at frequencies lower than 400MHz.

v. The ¹H NMR spectra of a known mixture of ethyl ethanoate and methyl propanoate and each pure component or substitutes should be obtained and stored on a computer that students have access to. These sample ¹H NMR spectra are then used in the first lab period by instructors to teach and demonstrate the procedures for processing and matching signals to protons in the structures and calculation of integral ratios from the processed NMR spectrum of the mixtures.

iv. Groups should enter their results of the required calculations for their mixture on the same class table. Each student should copy this table for later analysis of all the data to identify the condition when integral ratio is equal to mole ratio for each mixture on the class table.

2.2. Nitration of Alkylbenzenes

i. An alkylbenzene can be assigned for this exercise to all lab sections. Different lab sections or even different teams in a section can also be assigned different alkylbenzenes. The more the number of alkyl substituents on the benzylic carbon, the faster the nitration reaction. The reaction time should therefore be longest for nitration of toluene and shortest for *t*-butylbenzene. We have successfully used toluene, ethyl and isopropylbenzenes over a number of second-semester organic chemistry lab courses. Various alkybenzenes are available from Aldrich Chemical Company. Ratio of *p/o* will differ based on the alkylbenzene used.

ii. The mechanisms for generation of the nitronium ion from the mixed acids and the attack of the nitronium ion on the specific alkylbenzene used can be reviewed to illustrate the mechanisms with a specific example even if these mechanisms may have been presented in lecture class. These mechanisms are found in every undergraduate organic chemistry text book.

iii. In order to avoid the development of a lot of heat and splashing of acid, conc. sulfuric acid must be added to the conc. nitric acid and not the other way round. Other methods for generating the nitronium ion can also be used, with the understanding that product isomer ratios will depend also on the method for generating the nitronium ion.

iv. If magnetic stirring is not available, the reaction mixture can be vigorously stirred with a glass rod or by swirling the reaction flask. These alternatives are however, less effective for creating contact between reactants in the aqueous and organic solvent layers

2.3. Processing of the ^{1}H NMR of the Crude Nitration Product

i. Demonstration of the determination of mole ratio of *para*/*ortho* products is necessary for students to learn how to accurately and independently process the spectrum of their nitration products. The ^{1}H NMR data of the mixture of known composition prepared from pure *p*- and *o*- chloronitrobenzenes is adequate for demonstrating the procedure. The demonstration should stress (a) identification of the region where the signals of interest lie in the complete spectrum (b) software features used to perform horizontal and vertical expansions of the 7.6 - 8.2 ppm region of the spectrum that contains the relevant signals for *para* and *ortho* protons, and (c) how to create the vertical measures of integrals.

ii. For the *p*-isopropylnitrobenzene (cumene), the signals for the two protons adjacent to the nitro-group are centered at 8.15 ppm with each split into three unsymmetrical peaks. The one proton adjacent to the nitro-group in *o*-nitrocumene shows as two signals centered at 7.76 ppm. In the spectrum of the crude product mixture, there are adjacent minor signals centered at 8.097, 8.057 and 8.035 ppm that get automatically co-integrated with the signals of interest. The integration values are therefore higher than what it actually is for the signals of interest. However, the usually identifiable inflection in the combined integral of signals that are separate but close, makes it possible to use vertical measure or a ruler to obtain the numerical height of the integrals of the signals of interest.

iii. Vertical expansion of signal intensity (abundance) is necessary for reactions that produce low yield of product in order for the signals for the *para-* and *ortho*-products to be visible. With a well-shimmed spectrometer and NMR sample tubes rated for the operating frequency of the spectrometer, significant expansion of signal intensity of more than 100 times can be achieved without degrading the spectral baseline.

iv. The acquired spectral data for all nitration samples can be made accessible to students by any or all of the following avenues, if available: (a) a storage computer that students have access to; (b) e-mailing data files to students or (c) upload to campus-wide instructional platform such as BlackBoard. Students should then access their spectra for processing at their convenience.

v. Both the basic demonstrations and exercises on signal matching and quantitative application of ^{1}H NMR experiment can be accomplished in one laboratory period (2h 50m). The nitration reaction and isolation of the crude product mixture can be completed in 1h 30 min. This is followed by preparation of NMR samples, and the set up for automated ^{1}H NMR spectral data acquisition for the crude product mixture of the nitration reaction. The instructor makes all acquired spectral data available to students through the three methods already listed. Students are instructed to download their spectral data and process them according to instructions when they write their lab reports for the activity.

vi. The emphasis on the nitration experiment is the determination of the mole ratio of the isomeric product mixture from the crude, not the percent yield or percent conversion of the reaction.

3. Student Handout: Experimental Procedures

3.1. Hazards and Safety Information

Students should always wear goggles when carrying out experiments in the lab. For safe handling of concentrated nitric and sulfuric acids as well as the alkybenzenes and their nitration products, gloves must be worn. This reaction must

be carried out in a fume hood because vigorous stirring and the exothermic nature of the reaction lead to appreciable evaporation of the CH_2Cl_2 solvent. The ester mixtures should also be handled in a fume hood and with gloves.

CAUTION: *Wear gloves when pouring and measuring concentrated nitric and sulfuric acids! Reaction must be carried out in a hood.*

3.2. Pre-Nitration Activity: *Can ^1H NMR Spectroscopy be Used for Quantitative Analysis of a Mixture of Organic Compounds?*

One of the pieces of quantitative data from ^1H NMR spectrum of a pure compound is the integral value of each signal that provides the ratio of the number of protons responsible for each signal. If a sample is a mixture of two or more compounds, can the integrated ^1H NMR of such a mixture provide any quantitative information about the composition of the mixture? In this exercise, you will investigate whether a quantitative information about a binary mixture can be deduced from the integrated ^1H NMR of the mixture and if so, what are the necessary conditions for such quantitative deduction to hold true.

For accuracy in determination of the integral values of signals, the spectra of the components of the mixture must meet certain basic requirements: (a) the chemical shift of at least one set of protons of each component must be known. This information can be obtained either by looking up the spectra of the compounds in the chemical literature or by running the ^1H NMR sample of each compound; (b) the signals to be used for comparison must be separated from other signals of the same as well as those of the other compound in the mixture so that integrals can be adequately determined; (c) the baseline for the signal of interest in each component must be clean and horizontal. When these conditions are met, integrals are most accurate and values calculated from them are most dependable and reproducible.

In the first part of this activity, you will learn to match signals in the ^1H NMR spectrum of a mixture to protons in the structure of each component compound using the ^1H NMR spectrum of the pure components. The compounds ethyl ethanoate and methyl propanoate and their mixture are adequate for this purpose but your instructor may choose other compounds that may be available in your stockroom. The structures of the two compounds that are used in this write-up are given below. Each compound has a signal that is a singlet, one that is a triplet and one that is a quartet. Labeling of the different proton sets in each structure (a, b, and c) are based on similarity of chemical/magnetic environment. (Figure S1)

Figure S1: Structures and Proton Labels of Methyl Propanoate and Ethyl Ethanoate

The spectra of the two compounds and their mixture will be provided. Using a combination of structural effects on chemical shift, multiplicity and integrals of signals in both spectra of methyl propanoate and ethyl ethanoate, assign and label the corresponding signals for a, b, and c proton sets according to the labeling in the structures above on the spectra of pure ethyl ethanoate and methyl propanoate. Then use the assignments in the spectra of the pure compounds to assign the signal for each proton set – a, b, and c for each compound in the mixture, using the subscript EE and MP respectively to differentiate the signals for ethyl ethanoate from those of methyl propanoate, e.g. a_{EE}, c_{MP} etc.

Do the spectra of the two compounds satisfy the three conditions given above for accurate integration?

Write your name on each spectrum and submit your three signals-labeled NMR spectra and your answer to the question asked above to your instructor before you choose a sample for part 2 of this activity.

In the second part of this lab activity, each group will be assigned a mixture of ethyl ethanoate and methyl propanoate of given mass composition. Each group will prepare an NMR sample of the assigned mixture. ^1H NMR spectra of samples will be collected in automation mode, processed, integrated and the printed spectrum will be provided to your group. Using the given masses of components of the mixture and the processed ^1H NMR spectrum, each group will carry out the following calculations and enter their results on the class table prepared by the instructor so that everybody will have data from all groups.

1. Calculate the mole ratio of your mixture from the masses of components provided
2. Calculate the following ratios of integrals for MP: EE using
   a. The integral for the singlet CH_3 signal for each component of the mixture
   b. The integral for the quartet $-CH_2-$ signal for each component of the mixture
   c. The integral for one hydrogen atom for each compound of the mixture. You may calculate the integral for one H-atom by dividing the integral of a signal with the number of hydrogen atoms responsible for that signal in that compound.
   d. The integral for a CH_3 signal in one compound and a CH_2 signal in the other?

Enter the five ratios (1, 2a,b,c and d) calculated for your mixture on the class table on the board and copy the complete table for the whole class in your lab notebook.

Based on your analyses of the data in the table for each group including yours, answer the following questions in your lab report:

  i.   What is common to all the ratios calculated in 2 a, b and c for each group?

  ii. What is common to all the ratios calculated in 1, 2a, b, and c for each group?

  iii. How do the ratios calculated in 1, 2a, b, and c compare to the one calculated using the integral for a CH_3 in one compound and the integral for a CH_2 in another, i.e. 2d for each group?

  iv. Is there any numerical relationship between some integral ratios and mole ratio for each mixture on the data table? If yes, describe it.

3. From the observations above, provide a yes or no answer to the question that is the topic of this lab activity. If your answer is yes, state the quantitative relationship between the ratio of integrals for signals and the mole ratio of components of a mixture. State the numerical condition that must apply for this trend to hold true for a mixture.

In your lab report for this activity, you must specify the identity of the mixture your group was assigned, including the mass composition.

Show the details of each of the five calculations for 1, 2a, b, c and d.

Provide answer to questions i - iv about the data table and your conclusive statement, i.e. item #3.

Include the class data table and the processed spectrum of your mixture with signals labeled as in the three labeled spectra you submitted to the instructor after the first part of the lab activity. Your spectrum must show assignments for each signal from each compound of the mixture using the a,b,c, and the EE/MP labels as in Figure S1.

3.3. Mixed Acid Nitration of Isopropylbenzene (Cumene) or Any Alkylbenzene:

3.3.1. Introduction:

Nitration of isopropylbenzene is a typical electrophilic aromatic substitution reaction that can yield three possible regio-isomeric products as shown in Eq. 1. The major product isomers for this substrate will be the *p-* and *o*-products since the isoprpyl group is an activating group and therefore, an *ortho-* and *para*-directing substituent. The mechanism for formation of the nitronium ion from the concentrated nitric and sulfuric acid mixture and the two-step mechanism of attack of the nitronium ion on the aromatic ring can be found in your undergraduate organic chemistry textbook.
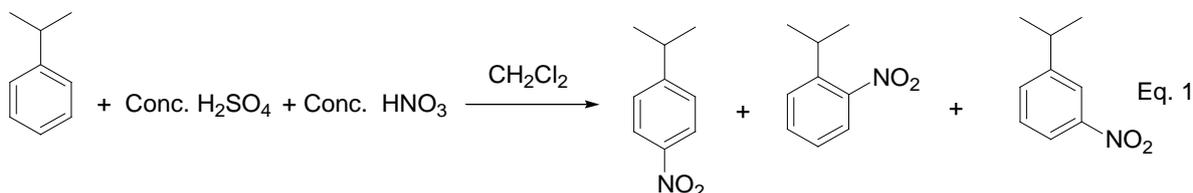
3.3.2. Experimental Procedure:

  i.   Measure into a 125 mL Erlenmeyer flask, 1 mL of conc. nitric acid. Cool in ice/water bath.

  ii.   To this acid, add in small portions, with stirring, 1 mL of conc. sulfuric acid.

  iii. In another 125 mL Erlenmeyer flask, add 1 mL of isopropylbenzene, followed by the addition of 5 mL of methylene chloride (CH_2Cl_2).

  iv.   While stirring the organic solution with a magnetic bar on a magnetic stirrer, add, in small portions, the acid mixture.

  v.   After completing the addition, stir the mixture vigorously for 1 h. Vigorous stirring is necessary to establish large surface contact area between the aqueous and organic layers for the reaction period. Methylene chloride evaporates because of the heat generated in the reaction as well as the vigorous stirring. It is necessary to add additional portions of 5 mL of methylene chloride to maintain at least 10 mL of organic layer at the end of the reaction.

  vi.   At the end of the reaction period, transfer the reaction mixture into a separatory funnel. Rinse the reaction flask with 5 mL of methylene chloride and pour the rinse into the separatory funnel.

  vii. Separate the aqueous acid layer into a beaker for later adequate disposal.

  viii. Rinse the organic layer twice, each time with 5 mL of saturated aqueous sodium bicarbonate solution, followed by two washings each with 5 mL of distilled water.

  ix.   Dry the resulting organic solution over 2g of anhydrous Na_2SO_4 and decant into a clean, dry round bottom flask for solvent removal by rotary evaporation or simple distillation.

  x.   Using a pipette, add one drop of the liquid product into an NMR sample tube followed by the addition of no more than 1 mL of $CDCl_3$ with .03% tetramethylsilane (TMS) as internal reference. If the yield is low, the 1 mL of $CDCl_3$ should be added to the flask containing the product and this solution is then transferred into the NMR sample tube using a pipette.

The acid solution from the reaction should be disposed of either by diluting with water and pouring down the drain or neutralizing with sodium carbonate/bicarbonate, then dissolving/diluting with water and pouring down the drain. The remainder of the product should be handed over to the instructor.

3.3.3. Calculation of p/o Isomer Mole Ratio from ^{1}H NMR of the Nitration Product Mixture:

Reference and phase the spectrum. Expand horizontally and vertically the 7 – 9 ppm region. The signals centered at 7.7 ppm are for the one proton (H_o) of *o*-nitrocumene. The signals centered at 8.15 ppm are due to the two protons (H_p) of *p*-nitrocumene (Figure S2). For low yield products, it is necessary to use the vertical expansion cursor to render both the relevant *para* and *ortho*-product peaks high enough for visibility. After integrating the signals, use the vertical measure cursor to determine the height of the integral for the specific signals of interest. These vertical measures, which are proportional to their integrals, are used to calculate mole ratio of *p*- to *o*- product.

Statistically, the **p/o** ratio should be 1:2 since there is one *para* position and two *ortho* sites for substitution.

Figure S2: Structures of p-Nitrocumene and o-Nitrocumene showing the chemical shifts of the protons used to calculate mole ratio of their mixture in crude product.

Also, electronic factors should favor *ortho* over *para* product since the isopropyl substituent stabilizes the carbocation of the *ortho* σ-complex much better than the carbocation of the *para* σ-complex. Your actual experimental mole ratio should be determined from the proton NMR signal integration using the following equation:

$$p/o \text{ Mole ratio} = \delta I_p / 2\delta I_o$$

where

δI_p = integration or vertical measure units for 2 *para*-H_p;
δI_o = integration or vertical measure units for 1 *ortho*-H_o;

3.3.4. Lab Report for Lab Period #2:

This report should contain the following items: Title of the experiment, equation of the reaction, table of reagents showing structure, name, amount, molecular weight, and number of moles for pure substances, procedure for the nitration reaction including preparation of NMR sample and the calculation of the mole ratio of *p*- to – *o*-isopropylnitrobenzenes. Is your calculated mole ratio in accord with your prediction based on statistical and electronic factors? If not, suggest an explanation for the difference. Attach an annotated copy of the part of the processed ^{1}H NMR spectrum used to calculate the products mole ratio.