Development of a discovery-based organic chemistry lab module: Evaluation of student attitudes and ability to interpret spectroscopy

By

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A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy in Mathematics and Science Education
Chemical Education Concentration

Middle Tennessee State University
December, 2014

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I want to dedicate this dissertation to my mother, husband, and family whose encouragement and faith contributed to the completion of this research.

ACKNOWLEDGMENTS

I first want to thank God for all the many answered prayers during this time, especially for patience. Thanks to my wonderfully supportive family for allowing me to take time away from home to work on this research. Luke Martin has been extremely patient and understanding of my time spent away. Brenden and Gabe Martin have pulled the extra weight at home surpassing my expectations.

I offer my sincere gratitude to Dr. Norma Dunlap, my major professor for her encouragement, patience, and unending support. She has been there from the beginning with kind and persistent words, pushing me towards completion when necessary. This research would not have been possible without her leadership and dedication to education. She is an inspiring educator who truly believes in continually improving students' experiences and their understanding of organic chemistry. I want to thank Dr. Dana Fuller for her service on my dissertation committee, all the extra help with the statistical analysis of the research, and the much needed encouragement. I am very grateful for the multiple meetings of an hour, sometime more, guiding me through the use of SPSS and helping me with the interpretation of data. I also want to thank Dr. Michael Sanger, Dr. Amy Phelps, and Dr. Pat Patterson for being on my committee, helping me prepare for the presentations on this research, the thoughtful comments and professional advice. A special thanks to Dr. Pat Patterson for the many years of mentoring and help with not only this research, but my career and health.

This dissertation could not have been completed without the cooperation of the organic faculty, especially Dr. Handy, Dr. Friedli, and Dr. Stewart, who contributed to the validation of the survey used in this research. They have all been very supportive of conducting this research and improving the organic laboratories. I offer a special thanks to Dr. Handy for his encouragement. I also want to thank his graduate student Zack Taylor, who is always working, for making sure I made it safely to my jeep the many evenings I stayed on campus late.

Finally, I would like to thank the MSE program and Department of Chemistry for financial support during this degree program.

ABSTRACT

Laboratories are a central component of the undergraduate organic chemistry curriculum. For years, educators have questioned the effectiveness of laboratories in science classes, their impact on students' learning, and what constitutes an effective laboratory experience. This research examines the addition of a *discovery-based (guided inquiry)* component to the traditional organic chemistry laboratories at Middle Tennessee State University and its effect on students' abilities to interpret NMR spectroscopy and their attitudes toward the laboratory.

Students in two groups of organic chemistry laboratories were compared. One group used the traditional stand-alone laboratories on the topics of distillation, chromatography and spectroscopy. In the traditional laboratories, students were given the same step-by-step instructions and the outcomes of the laboratory experiments were known. The other group used a three-week *discovery-based* unit on terpene isolation and characterization incorporating the same three topics. Although the students were provided with basic procedures, each group of students used similar, but not identical procedures. The outcomes of this unit were predictable, but often unspecified to the students.

At the end of the term, students' understanding of spectroscopy and their attitudes toward the laboratory were measured using a survey. The survey grouped the spectroscopy content into four categories: predicting number of signals, splitting patterns, fitting spectroscopic data to chemical structures, and assigning signals on a spectrum to the atoms in a given structure. The students' attitudes toward spectroscopy and the laboratory in general were measured using a Likert scale. Although there were no significant differences in the spectroscopy content knowledge of the two groups, students in the *discovery-based* laboratory generally scored higher on the content portion of the survey. Students from the *discovery-based* laboratory also had stronger opinions, whether positive or negative, about their ability to interpret ^{1}H-NMR than the students in the traditional lab. Anecdotal evidence suggests that the nature of the *discovery-based* unit led students and instructors to think more critically about separation techniques and interpretation of spectral data.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I: INTRODUCTION

Introduction

Middle Tennessee State University (MTSU) has the largest undergraduate enrollment of any university in Tennessee. In 2012 the undergraduate enrollment was approximately 22,300 students, which was 88 percent of the university's total enrollment. (Office of Institutional Effectiveness, Planning and Research, 2012).

Each Fall semester approximately 300 students enroll in CHEM 3010, the first organic chemistry class in a two course sequence. The CHEM 3010 course has a required laboratory component, which is CHEM 3011. Students who enroll in organic chemistry are typically pre-professional or science majors in their sophomore or junior year. The overall course is worth four credit hours including the laboratory component, which comprises about 25 percent of the overall course grade. For the laboratory component, students meet once a week for three hours, and they are required to complete pre-lab exercises before coming to lab as well as write a lab report due one week after the completion of lab.

Because of the Complete College Tennessee Act (CCTA) passed in 2010, part of MTSU's funding formula is now linked to student retention and graduation. Since colleges that produce more degrees are rewarded with more money, MTSU and other colleges in Tennessee have a strong vested interest in having students successfully complete courses in which they are enrolled to advance towards degree completion (Kelderman, 2012). Since the academic majors in science, technology, engineering, and mathematics (STEM) are inherently challenging, it is important to keep students engaged in their course work so that they will succeed and eventually graduate (Barr, Gonzalez, & Wanat, 2008). For example, during the 2013-2014 academic year, approximately 20 percent of students withdrew from or failed CHEM 3010. This requires students to either repeat it at a later time or change majors. Indeed, the new funding formula has been a catalyst for the university to explore and promote experiential learning techniques across the disciplines.

Effective organizations are mission driven. The mission of MTSU and the College of Basic and Applied Sciences (CBAS) emphasizes student engagement and experiential learning. Part of the university's mission statement notes that the university is "*committed to preparing students to thrive in their chosen professions and a changing global society*." (Retrieved April 8, 2014, from http://www.mtsu.edu/about/mission.php). Experiential learning is also a central component of the CBAS mission statement. The CBAS mission states:

> "*Within an environment that values excellent teaching and fosters initiatives in research and public service, the mission of the College of Basic and Applied Sciences at MTSU is to:*
>
> - *Prepare individuals for successful careers in scientific and technical fields in industry, teaching, government, and health services.*
> - *Provide general education through exposure and exploration of natural sciences, applied sciences, and mathematics.*

- *Provide "hands-on" experience with state-of-the-art materials and equipment.*
- *Promote the development and practice of critical thought and expression.*
- *Develop basic knowledge and promote attitudinal skills and other abilities needed for further graduate or professional study*."

(Retrieved April 8, 2014, from http://www.mtsu.edu/cbas/mission_statement.php)

The organic chemistry lab provides a perfect opportunity for students to gain "hands-on" experience with a variety of materials and equipment. The nature of the course and lab requires students to think critically. With the exposure to instrumentation and scientific thinking, students should be more prepared to excel in the scientific technical field or continue on in graduate studies. Because of funding and mission, it is imperative to continually examine and assess the structure, pedagogical techniques, and learning outcomes of organic chemistry labs at this time and at this university.

Experiential Learning

Although experiential learning has been practiced to some extent for the past thirty years, its application to higher education has never been more important than in the current academic setting that emphasizes student retention. Made popular by John Kolb in the 1980s, experiential learning is described as "learning by doing". It is based on social and cognitive psychology and philosophy. Kolb developed cycles of the experiential learning model (ELM) based on previous pedagogical and cognitive development research by John Dewey (1938), Jean Piaget (1971), and Kurt Lewin (Marrow, 1975).

Kolb (1984) credited Dewey for his philosophical perspective of pragmatism and his progressive education movement. Dewey has often been recognized for his contributions to the philosophy of learning through experience and its relation to learning theory (Waks, 2013). He was a pioneer of the pedagogical methods of experiential learning and reflective thought. Dewey's progressive education theory places the emphasis of teaching and learning away from the teacher-centered lectures and focuses on the student's experience, ideally in activities that are relevant to their lives. Dewey is often referred to as the father of modern education based on his progressive approach.

Jean Piaget, who is well known for his study of cognitive development in children, proposed that children learn through interactions between themselves and their environment (Kolb,1984). He defined four stages of childhood development from birth to adulthood. *Sensory motor* is the first stage, ranging from birth to two years of age. Children experience and manipulate objects in this stage. The second stage is the *preoperational* stage, where children learn through pretend play. This stage usually occurs around two to seven years of age. During the *concrete operational* stage, age eight to eleven, children think more logically, but their thoughts can still be rigid, and they struggle with abstract and hypothetical thinking. Finally in adolescence to adulthood they reach the *formal operational* stage. This is the stage where they increase their logic and can use deductive reasoning, leading to better understanding of abstract ideas. Piaget’s work stopped at adolescence, but Kolb and others have extended his research to adult learning, as people are lifelong learners (Malerstein & Ahern, 1979).

Kolb also credited Kurt Lewin for his contribution to action research through social psychology. Studying group dynamics, Lewin developed the laboratory training method and the T-group training, which were fact-finding cycles of experience, data collection, and observations (Glassman, Erdem, & Bartholomew, 2013; Kolb, 1984). His ideas of cycles shaped the action research method, which uses a cycle of planning, action, data collection, and reflection.

From these works, Kolb (1984) derived three models of the experiential learning method (ELM). The models outlined a holistic approach to learning and all have four main cycles: concrete experience, reflective observation, abstract conceptualization or hypothesis, and active experimentation. Kolb credits this model for the emphasis on learning through doing, "the process whereby knowledge is created through the transformation of experience” (Kolb, 1984, p. 38). Traditional ELM in formal education has been around for many years. Apprenticeships, internships, work study programs, cooperative education, studio arts, laboratory studies, and field study are all examples of what is considered traditional ELM.

Experiential learning and science laboratories in higher education

Experiential learning is at the heart of science laboratories in higher education. The idea that students are learning how to be scientists by being given the experience in an actual lab setting is a driving force of the importance of science laboratories. Students are experiencing scientific phenomena first-hand rather than merely thinking about scientific principles in the abstract. Chemistry labs are experience-based and are natural places for social interactions and the cycle of ELM. Laboratories provide a hands-on, collaborative environment. The labs are typically designed so that students have smaller groups to actively engage in learning and naturally build educational communities for students. Providing students with a sense of involvement is strongly linked to retention (Tinto, 2005).

Although the purposes of science laboratories in higher education are debated, most will agree that there is a need for students to have an opportunity for experiential learning (Johnstone, 1977; Hofstein & Lunetta, 1982; Kirschner & Meester, 1988; Hofstein & Mamlok-Naaman, 2007; Reid & Shah, 2007). The purpose of labs has shifted from the Industrial Revolution of the 1800s, when the goal was to turn out skilled workers for industry or research laboratories (Reid & Shah, 2007). As education evolved with the advancement of technology and instrumentation, the role of labs has shifted as well. A recent national survey assessed faculty goals for the undergraduate chemistry laboratories (Bruck & Towns, 2013). This survey revealed that there were some universal goals, but the emphasis varied according to discipline. The study categorized undergraduate labs into five disciplines: general chemistry, organic chemistry, analytical chemistry, physical chemistry, and upper-division chemistry. Some goals were universal across disciplines, such as, research experience, critical thinking, and experimental designs. Group work or team work is emphasized less in the organic chemistry laboratories than in other disciplines. However, developing techniques, keeping accurate notebooks, and writing reports are emphasized more in organic laboratories than in other disciplines.

The emphasis for all disciplines is in line with the overall goals of the organic labs at MTSU. The major focus is the hands-on aspect of lab, so that students may think more critically and gain problem-solving skills that follow them in and out of the classroom. This aligns with the CBAS's mission statement that places emphasis on experiential learning and hands-on practice with state of the art equipment.

Background of Study

In the wake of changes in the funding formula and mission, that are designed to promote quality education, educators are continually looking for ways to improve student learning and pedagogical practices. This idea of continual improvement drives research for better teaching methods in organic chemistry laboratories. Can the organic laboratories be more effective? Are the laboratories designed in such a way that students are actively engaged and enjoying the learning process? Are they retaining the knowledge that they have gained? These questions suggest that there are many different ways to improve organic laboratories with experiential learning in the laboratory setting. The scope of this research is to determine if there are better ways to achieve this than the current laboratory curriculum.

Nuclear magnetic resonance (NMR) spectroscopy is the primary tool used by organic chemists for determining molecular structures. In 2004 MTSU received a National Science Foundation (NSF) grant for hands-on-learning to purchase a 300MHz NMR for student use. Since then, interpretation of spectroscopy has become a major component of both the lecture and the laboratory at MTSU. Many of the laboratories in organic chemistry are designed so that the students attain hands-on experience with the latest scientific equipment. The students are getting the hands-on experience, but are they understanding and able to interpret spectroscopy? In an attempt to make the laboratories more effective in reaching this learning goal, this research looks at student outcomes for the interpretation of spectroscopy and their attitudes towards the laboratory. There are many different ways to approach experiential learning in lab, so it is important to determine what makes an effective laboratory.

Laboratory styles

Domin's review of laboratory styles (1999) classified chemistry lab instructional styles based on outcome, approach, and procedure. The outcome is either predetermined or undetermined. If it is predetermined, then it is predetermined by the instructor only or, in some cases, by both instructor and student. Domin (1999) defines the approach as either deductive, "in which students apply a general principle toward understanding a specific phenomenon", or inductive, where "observing particular instances, students drive the general principle" (p. 543). The procedure is either given to the student *via* lab manual or instructor, or it is generated by the student. Domin classified chemistry laboratory instructional styles into four categories: *expository*, *inquiry*, *discovery*, and *problem-based*. These four styles are summarized below in Table 1.

Table 1

Summary of laboratory styles

	Outcome	**Approach**	**Procedure**
Expository	Predetermined	Deductive	Given
Inquiry	Undetermined	Inductive	Student generated
Discovery	Predetermined	Inductive	Given
Problem-based	Predetermined	Deductive	Student generated

Expository, also known as cookbook, verification, or traditional style, is the style used most often in undergraduate chemistry laboratories. Domin (1999) defined this type of lab as a deductive approach where students are given a problem and step-by-step instructions on how to reach a pre-determined outcome that both student and instructor know. The concepts addressed in the laboratory are covered in lecture before the lab is performed. The advantages of this lab style include the ease of lab preparation and training of teaching assistants, however expository labs involve little critical thinking (Gallet, 1998; Pavelich & Abraham, 1979; Hofstein & Lunetta, 1982).

Inquiry style labs have undetermined outcomes and use an inductive approach. The procedure is generated by the students, who take the role of the scientists as undergraduate researchers. Although this lab style has been credited with keeping students engaged and having an overall positive attitude toward the lab, it can be difficult to implement (Merritt, Schneider, & Darlington, 1993; Kern &Carpenter, 1984; Ajewole, 1991). Students are learning new concepts and also using unfamiliar lab equipment and techniques. Because students have not had opportunities to practice novel techniques, this style has been criticized for placing too much demand on the short-term memory (Linn, 1977; Herron & Herron, 1971; Lunetta & Tamir, 1981).

Problem-based labs have predetermined outcomes that generally only the instructor knows and this approach uses a deductive reasoning. The instructor asks a question or series of questions and the students are asked to answer based on their current understanding of the concepts, which have already been covered in the course. Materials are given to students, but they generate the procedure.

Discovery styles have predetermined outcomes that generally only the instructor knows. The approach is inductive by nature, and a general procedure is given to the students. Ideally, students perform the lab before the material is presented in lecture, aiding in the discovery of the results. This style has been criticized because of the extra time needed and the possibility that the desired outcome might not be discovered (Peters, 1967).

Students' attitudes

A person's attitude can affect his or her ability to succeed at tasks and their overall enjoyment or how much they get out of an experience. Nieswandt (2007) stated that "Students' interests and attitudes toward science as well as their perceptions of how well they will perform in learning contexts (self-concept) may play important roles in developing a meaningful understanding of scientific concepts, an understanding that goes beyond rote memorization toward the ability to explain everyday phenomena with current scientific knowledge" (p. 908). Weinburgh (1995) found that students' attitude toward science accounts for approximately 30 percent of variance in achievement in middle school students. Freedman (1997) found that there is a positive correlation between achievement and attitudes toward science.

The noticeable decline in math and science majors stimulated the initiation of research on students' attitudes toward science in the 1980's (Osborne, Simon, & Collins, 2003). Although there was a push for research on attitudes, there were also many challenges in studying attitudes toward science. Identifying what components to measure is challenging because of all the sub-constructs contribute in varying proportions to a student's attitude. Osborne's review summarizes the range of components that contribute to a student's attitude to include (p. 1054):

- The perception of the science teacher
- anxiety towards science and fear of failure in course
- the value of science
- self-esteem at science
- attitudes of peers and friends towards science
- the nature of the classroom environment

- achievement in science

Another notable challenge in studying attitudes toward science is the relationship between behavior and attitudes. Glasman and Albarracín (2006) concluded that attitudes that are easy to access and are predictors of future behavior. This has great implications. If students come into a course confident, their behavior will reflect the confidence and they have more potential to succeed. This also suggests that if they lack confidence, then their behavior will be determined by their negative attitude and, without intervention, they may not have great academic success.

One of the main challenges in research of student attitudes is determining or developing an appropriate instrument. With so many factors that influence attitudes toward science including gender, personality, structural variability, and curriculum variables, researchers must consider many different aspects when choosing the correct instrument and there are many limitations in developing an appropriate instrument. Instruments have been criticized because they only measure one aspect of an individual's view, and they usually do not take into account most of the factors that can be influential (Osborne et al., 2003).

Bandura (1997) defines self-efficacy as a person's belief in their ability and capabilities. So in academia, this translates to a student's expectation or belief in their ability to achieve, often in terms of grades (Bong & Skaalvik, 2003). Students tend to overestimate their ability in terms of grades. This is particularly true of students with lower GPA’s and students in lower division courses compared to ones with higher GPA’s or ones in upper division courses (Nowell & Alston, 2007).

Statement of Purpose

The purpose of this research was to investigate potential improvements to the organic chemistry laboratory. This research looked at the specific learning objective of interpretation of spectroscopy in the organic chemistry laboratory. The control group used the current laboratory curriculum at MTSU, which is a series of stand-alone expository labs designed to teach several separation techniques, and NMR spectroscopy. The treatment group participated in a multi-week *discovery-based* unit that covered the same techniques. The *discovery-based* unit was designed in a multi-week series, not stand alone individual laboratories.

This research also looked at students’ attitudes toward the laboratory in general and specifically toward the spectroscopy content component. Students’ predictions of their expected final lab grades and lecture grades were also compared to their actual grades earned in both the laboratory and course to see how their opinions compared to their actual ability. Although most studies on curriculum variables show little if any meaningful information on effect (positive or negative) attitude (Osborne et al., 2003), this research was designed to determine if there was a difference in attitudes between the two groups and if there was an impact on achievement, as perceived from the student, to the actual grade earned.

CHAPTER II: REVIEW OF RESEARCH IN ORGANIC CHEMISTRY LABS

Research on Lab Styles in Organic Chemistry

Dunlap and Martin (2012) published an overview of *discovery-based* labs in organic chemistry that have occurred in the last fifteen years. The entire article is listed in Appendix A. In summary, the article placed the organic *discovery-based* laboratories into three categories:

- Identifying unknowns
    - “Unknowns” are samples given to the students with no identification. Students use various means to determine the identities of the unknowns.
- Reaction analysis
    - This category is the analysis of the conversion of one compound to another based on reaction type.
- Isolation and purification
    - Techniques are used to purify mixtures and isolate pure compounds.

Identifying unknowns can be expository in nature, but there was an example lab published in which the design made the laboratory *discovery-based*. In the discovery-based lab, multiple structures were given that were structurally similar, giving rise to more discussion and critical thinking. Melting point, infrared spectroscopy (IR), and NMR were used to determine the identity of the unknown (Glagovich & Shine, 2005). There were also several “unknown” laboratories reported that involved reactions where the reactant was an unknown and, based on the identification of the product obtained through spectral data such as IR and NMR, the reactant was identified. These reactions included oxidation of unknown alcohols, nitration of unknown arenes, dehydration of unknown alcohols, and nucleophilic additions to unknown carbonyl compounds (Dunlap, Mergo, Jones, & Martin, 2006; Dragojlovic & Nielsen, 2003; McElveen, Gavardinas, Stamberger & Mohan, 1999; Rosenberg, 2007).

The second category, reaction analysis, involves analyzing a product and proposing a mechanism. This category has the most works published within the academic literature of organic chemistry laboratories. The published laboratories that are appropriate for the undergraduate organic laboratory include:

- Epoxide ring openings and alkyl halide formation from alcohols (Moroz, 2003; Christensen, Huddle, Rogers, Herbie, & Mohan, 2008; Kjonaas, 2008)
- Investigation of stereoselectivity and regioselectivity, such as prediction of possible reaction products for stereoselective addition of Grignard reagents (Ciaccio, et al. 2001)
- Regioselective reduction of aldehydes (Bara & Mohan, 2005)
- Regioselective epoxide ring-opening (Centko & Mohan, 2005)
- Regioselective electrophilic aromatic substitution (Eby& Deal, 2008)
- Regioselective hydrogenation of a series of chalcones (Mohrig, Hammond, Schatz, & Davidson, 2009)

The final category of organic *discovery-based* labs is isolation and purification. The common techniques taught are distillation, recrystallization, and chromatography. Most labs involving isolation and purification are extensions of traditional labs that have been designed so that students use different reagents or use different techniques to purify their samples, and then they compare their results (Horowitz, 2003). Stoub (2009) published a laboratory where students purify a "poisoned" Excedrin sample using these isolation and purification techniques. Another laboratory has students isolate two isoflavones, osajin and pomiferin, from osage orange fruits (Whaley, et al., 2007).

From the published *discovery-based* laboratories in organic chemistry, few had thorough assessments of the effectiveness. Although it was speculated that students were more enthusiastic in discovery-based labs, and that they were more independent in lab, most had only informal assessments based on observations of students' questions in class. (Horowitz, 2003; Whaley, et al., 2007). Stoub (2004) used informal observations, but also used student evaluations, end of the year assessments, and notebook reflections to assess the effectiveness of his laboratories. Perhaps the most in-depth assessment was done by Mohrig (2009), who had students take an anonymous online survey giving their perceptions and their opinion of the effectiveness of the laboratory. It also addressed the effectiveness of the teaching assistants.

Research on Students' Attitudes in Organic Chemistry Labs

There are few actual studies that focus only on students' attitude in the organic chemistry laboratory. Most studies in organic chemistry focus on attitudes in the chemistry course or in general science laboratoriess. For example, there have been studies comparing the effect of anxiety on students' overall chemistry attitude, organic chemistry achievement, and self efficacy (Kurbanoğlu, 2013; Kurbanoğlu & Akin, 2012), research on the effect of Process-Oriented, Guided-Inquiry (POGIL) lecture techniques and students' attitudes on their performance in the POGIL class (Chase, Pakhira, & Stains, 2013), and gender differences related to attitude (Turner & Lindsay, 2003). Two heavily studied areas related to student attitudes in organic chemistry courses involve the use of computers and on-line homework (Parker & Loudon, 2013), and the use of attitudes as predictors for computer-based education (Kevin, Liberty, & Texas University, 1975).

Studies looking directly at attitudes in the organic chemistry laboratory are limited. Cooper and Kerns (2006) studied how students' attitudes and perceptions were affected by type of lab they participate in, whether traditional or problem-based. They used qualitative assessments such as observations, video tapes, interviews, and open-ended questionnaires to determine effectiveness. Ten participants from the problem-based laboratories were randomly chosen to be interviewed for approximately thirty minutes and were asked about laboratories, science classes, teaching assistants, professors, and laboratory course they were taking. Example questions were "What has been your best lab experience? Why? What did you enjoy the most or least?" (Cooper & Kerns, 2006, p. 3). From theses interviews, a theme about student groups and instructors emerged. The students perceived both peer groups and instructors as either good or bad.

Students from both groups answered the open-ended questionnaire. Both problem-based and traditional groups liked the overall atmosphere and hands-on aspect of the laboratory. Students in the problem-based labs viewed the role of the instructor and students differently than that of the traditional laboratories. The problem-based group viewed the role of the instructor as a guide to help students toward the correct conclusions, whereas the traditional laboratory viewed the role of the instructor as supervisor. The role of the student in the problem-based laboratory was to *figure out why* and to *understand* versus the traditional laboratory where the student role was much more passive in nature. The researchers concluded that students in the problem-based laboratory group were more confident and self-reliant than in the traditional group.

A study at Seattle University looked at organic chemistry laboratory students' attitude toward safety in the laboratory (Alaimo, Langenhan, Tanner, & Ferrenberg, 2010). The traditional laboratory safety training was presented the first day of class with a four module lay out. The safety modules included topics of risk management, chemical toxicity, chemical spill and waste collection, and safety equipment. In the traditional laboratory, the instructor included lab safety and waste disposal as part of the laboratory briefing for each individual experiment at the time they were performed.

The safety-team curriculum used the same modules the first day of class, but added a hands-on activity. There were different samples of chemicals at each laboratory station that the students had to dispose of properly. Each student was provided with gloves that had been treated with commercial glow powder, of which the students were unaware. After their waste disposal activity and lab station clean up, a black light was used to see contamination on door handles, student clothes, notebooks, and other areas in the laboratory. Instead of having the instructor do the safety briefing as part of the weekly experiment, two to three students were assigned to safety teams weekly. Their duties were to present a five minute safety presentation that detailed hazards and risks associated with that day's experiment. They also made handouts that outlined the four safety areas: chemical hazards, procedural hazards, personal protective equipment (PPE), and waste collection. Although the laboratory instructor was responsible for the overall laboratory safety, the safety team would take turns walking through the laboratory to ensure appropriate PPE, proper labeling, and no experiments were left unattended. They were in charge of post laboratory inspection which entailed that all areas of the laboratory were properly cleaned and equipment was put away.

The instrument used to measure attitudes was a 37-item questionnaire composed of two parts; multiple choice/true-false questions, and five-point Likert-scale questions. The participants were from three groups: 1) the safety-team organic lab section, 2) the traditional organic lab section from the previous year, and 3) a section of general chemistry that used the traditional style for safety. Participants voluntarily and anonymously took the survey. The safety-team group performed either better or the same as the two traditional groups. Breaking it down by discipline, the safety-group did thirty percent better than the traditional organic laboratories and fifty-seven percent better than the traditional general chemistry laboratories. All other questions were not significantly different, indicating there was no differences between the groups. Based on informal observations, the researchers concluded that students who participated in the safety-team group exhibited “increased sophistication with respect to PPE use, waste collection, and lab cleanliness” and they sensed an overall more professional atmosphere in the laboratory (p. 860).

This next study could fall under computer animations research however, the animations used were directly related to organic chemistry lab and were designed as a pre-lab exercise for an extraction of caffeine from an aqueous solution lab. Supasorn, Jones, and Vibuljan (2008) designed two very similar interactive animations on organic extractions to show the molecular level of extractions. One was narrated only and the other used written text. This research compared advantages of different delivery strategies, text or oral narrative information, within simulations on content knowledge of extractions and attitudes.

First semester organic chemistry students were randomly assigned to each group, where they watched the animation as a pre-laboratory exercise. A six question pre and post-cognitive test about organic extraction was used to assess content knowledge on lower-order cognitive skills involving simple recall or simple application, and higher-order cognitive skills which require synthesis and analysis. A sixteen item Likert-scale questionnaire and two open-ended questions were used to assess students’ attitudes toward the animations and lab. It was found that students in the written text group performed higher on the lower-order cognitive skills section, the students in the narrative had more positive attitudes toward the value of the instructional animation tool in general.

As summarized in the preceding section, there has been a great deal of research in students' attitude in lab in general chemistry. However, the research is limited in the organic chemistry lab. Some of the research from general chemistry may be transferrable, but more research is needed in this area.

CHAPTER III: METHODOLOGY

Introduction

In an effort to determine how different pedagogical styles in laboratory influence learning outcomes and student attitudes, this study compared groups of standard sections of organic chemistry labs. One group was enrolled in the traditional labs that have been used at the University consistently for the past sixteen years. The other group replaced three of these traditional labs with a three-week *discovery-based* unit on terpene isolation and characterization. The study utilized the traditional laboratory format in the Fall semester of 2012, and Spring and Summer semesters of 2013 ($N = 78$). The *discovery-based* terpene unit component was included in the Summer 2011, Fall 2012, and Spring 2013 semesters ($N = 82$). All participants were randomly assigned to laboratory sections with varying lecture instructors, with the exception of the summer sessions, where the same professor instructed both lecture and the laboratory. Although there were more than 160 students enrolled in these sections of CHEM 3011, the participants were chosen according to voluntarily signed consent forms and by laboratory sections. In order to avoid instructor bias, the study compared only the lab sections in which the same instructor taught a section using the traditional lab format and a section using the *discovery-based* unit. Table 2 contains a summary of the individual weekly lab experiments used in both groups. The experiments in italicized bold print indicate topics that are common to both lab styles. At the end of the term, students' understanding of spectroscopy and their attitudes toward the laboratory were measured using a survey.

This research format was approved by seven members of the MTSU Chemistry Department as well as by the Institutional Review Board (see Appendix B).

Table 2

General lab schedules

Traditional	**Discovery-based**
CHECK IN, including safety worksheet	CHECK IN, including safety worksheet
Crystallization and Melting Point	Crystallization and Melting Point
Distillation of Cyclohexane/Toluene and Gas Chromatography	Distillation of Cyclohexane/Toluene and Gas Chromatography
Column Chromatography/Isolation of Caffeine	Column Chromatography/Isolation of Caffeine
Molecular Modeling of Isomers	Molecular Modeling of Isomers
Infrared/NMR Spectroscopy	***Steam distillation of terpenes/TLC***
Synthesis of 1-Bromobutane	***Column chromatography/data collection***
Elimination of Cycloalkanols/NMR	***NMR analysis/product identification***
Spartan Modeling of Subst. and Elim	Spartan Modeling of Subst. and Elim
Spartan Modeling of Alkene Reactions	Elimination of Cycloalkanols/NMR and Alkene Epoxidation
Alkene Epoxidation	

The experiments in italicized bold print indicate topics that are common to both lab styles

Both group's lab grades were determined by formal lab report write-ups and three quiz grades. The reports were formatted with an introduction, table of reagents, procedure, observations, calculations, results and conclusion, and they were graded on a ten point scale. Both groups were graded using the same scale.

Overview of Population Used for Study

Although there are typically a total of nine CHEM 3011 sections in the Fall and two in the Spring and Summer sessions per year at MTSU, only four sections were used for each group in this study. The four sections of CHEM 3011 assigned as the traditional laboratory were taught by four separate instructors, all of whom also taught a section of the *discovery-based* laboratory. The instructors were coded using letters A through D. The Fall 2012 sections were taught by instructor A ($N = 21$) and instructor D ($N = 21$). The Spring semester 2013 laboratory was taught by instructor C ($N = 21$), and the Summer sections both 2011 and 2013, were taught by instructor B ($N = 15$).

At the end of each semester, the students filled out a survey that was designed to measure their ability to interpret spectroscopy as well as their attitudes about the laboratory. Regardless of their laboratory section and instructor, all students in all sections received five extra credit points for submitting a fully completed survey to ensure that the students would take the survey seriously.

The sections of CHEM 3011 that participated in the *discovery-based* laboratory data were taught by the same four instructors who taught the traditional sections applied in this study. The Fall 2012 sections were taught by instructor A ($N = 16$) and instructor D ($N = 19$). The Spring semester 2013 laboratory was taught by instructor C ($N = 32$), and the Summer semester section was taught by instructor B ($N = 15$). At the end of each semester, the students were given the same survey designed to measure their ability to interpret spectroscopy and their attitudes about the laboratory. These students also received the five extra credit points for total completion of the survey.

Traditional Labs

The traditional laboratories covered separation techniques and spectroscopy in separate laboratory activities. These were unrelated individual experiments performed in one three-hour lab period per experiment. In the distillation laboratory, students separated a 50:50 mixture of cyclohexane and toluene *via* simple or fractional distillation. The laboratory experiment provided step-by-step instructions, and students knew what the outcome should have been. Gas chromatography was used to obtain percent composition of the separated components (see Appendix C).

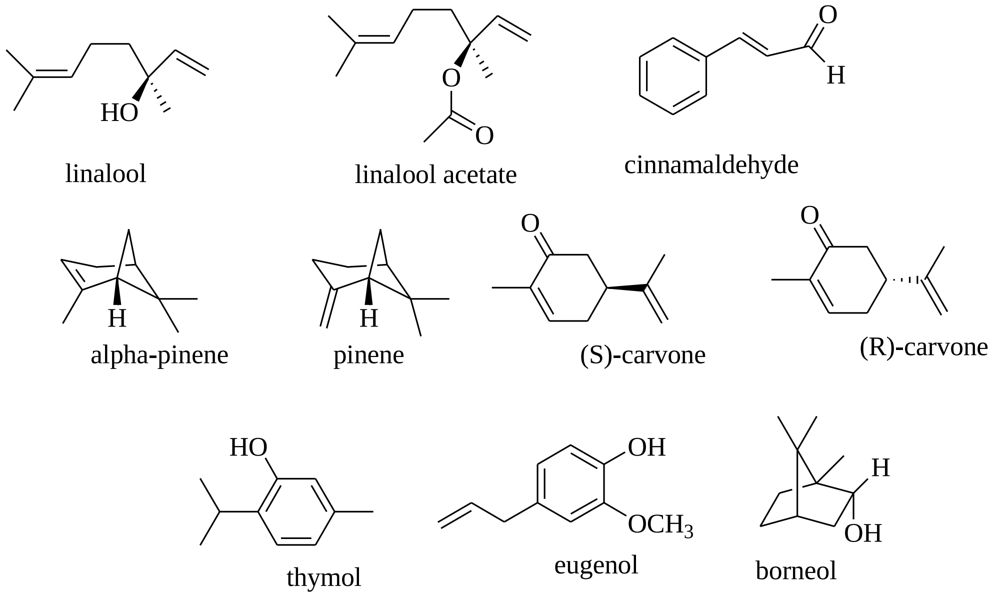
Extraction and chromatography were the other separation techniques students learned in CHEM 3011. In the traditional laboratory, students extracted caffeine from instant tea and purified the caffeine using column chromatography (see Appendix D). They used thin-layer chromatography (TLC) to calculate a retention factor (R_f), which was used to validate that the recovered compound was caffeine. Again, students were given step-by-step instructions, and they knew that they were all going to isolate the same compound, caffeine.

In these traditional laboratories, lab instructors taught spectroscopy using a "cookbook" approach to identify an unknown (see Appendix E). A table of possible structures was given, and students systematically ruled out possibilities by process of elimination based on data obtained by infrared spectrometry (IR) and nuclear magnetic resonance (NMR) spectroscopy. Functional groups were identified using IR, and NMR was used to further characterize and identify their unknown.

Discovery-Based Lab

The *discovery-based* unit (see Appendix F) focused on separation and purification methods as well as spectroscopy, which are typical components of the undergraduate organic chemistry laboratory curriculum. Rather than three unrelated experiments, students carried their initial material through a three week sequence. This laboratory sequence combined some separation and purification methods and spectroscopy into one three-week guided inquiry sequence. Techniques included steam distillation, extraction, TLC, column chromatography, and NMR spectroscopy. After conducting some initial research, students chose common herbs and spices used in kitchens, grown in gardens, or purchased from local grocery stores. In the first week students carried out steam distillation of an herb or spice, using short-path distillation equipment, to separate the volatile components or essential oil from the plant material. Students were encouraged to bring in materials of interest to them, as long as there was some distinguishable odor. They were also required to carry out pre- laboratory research to find two to five expected volatile compounds from their chosen plant material. At the end of the distillation, the organic material was separated from the water by liquid-liquid extraction.

The second week focused on chromatography, both TLC and column. Lab instructors guided students to find a TLC system in which the component from week one had an R_f of 0.25-0.75. They had access to several ethyl acetate/hexane mixtures to aid them in identifying the number of components present. Based on their TLC data, they chose which solvent to use for a gradient column, and then isolated the individual compounds. In the second week, students also collected ^{1}H NMR, ^{13}C NMR, and in some cases Distortionless Enhancement by Polarization Transfer (DEPT), Correlation Spectroscopy (COSY), and Heteronuclear Multiple Quantum Coherence (HMQC) data. Going back to the original pre- laboratory assignment, they determined which compounds they had isolated. Some common compounds isolated are shown in Figure 1. For instance, linalool and linalool acetate were found in lavender, basil was a source of eugenol, and rosemary has several components: α-pinene, pinene, and borneol.



linalool

linalool acetate

cinnamaldehyde

alpha-pinene

pinene

(S)-carvone

(R)-carvone

thymol

eugenol

borneol

Figure 1. Common compounds isolated in the *discovery-based* unit.

In the third week, students presented their spectral data, as well as their methods, to each other in class. This included crude percent recovery and the percent recovery of each component of their crude sample, R_f of each component, and the structure(s) of major components based on spectroscopy. Like the traditional laboratory grade, the grade was based on written reports and quizzes.

Instrument

The survey used to measure students' ability to interpret spectroscopy and attitudes toward organic chemistry laboratoriess was internally validated by five organic chemistry professors and two chemical education professors at Middle Tennessee State University. The survey originally contained nine short answer/multiple choice questions, two actual ^{1}H spectra of *para*-anisaldehyde and eugenol, and fifteen Likert-scale questions about students' attitudes in lab. The entire survey can be found in Appendix G.

The survey items were carefully designed to represent four sub-sets of skills: predicting number of NMR signals, splitting patterns, fitting data to a possible structure, and assigning signals on a given spectrum to a given chemical. The first sub-set was based on the ability of students to predict the number of signals expected for a given compound for both ^{1}H-NMR and ^{13}C-NMR (see Figure 2). These questions were designed to measure students' ability to identify planes of symmetry and chemically equivalent carbons or hydrogens in given molecules.

The second sub-set required students to recognize splitting patterns in ^{1}HNMR (see Figure 3). The hydrogens on carbon five are next to two equivalent hydrogens.

For each of compounds, A, B, and C, indicate how many signals would be seen in either the ^{1}H or ^{13}C-NMR spectrum, whichever is listed. (Just give the number of signals, **NOT** the splitting or chemical shift).

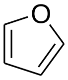
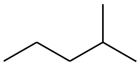
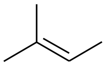
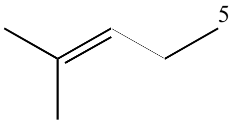
Compound A ^{1}H-NMR	Compound B ^{1}H-NMR	Compound C ^{13}C-NMR
		

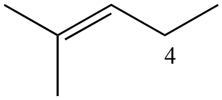
Figure 2. Example questions on predicting number of signals.

What would be the splitting pattern for the hydrogens on carbon 5 in a ^{1}H-NMR?



1- doublet
2- triplet
3- quartet
4- multiplet (complex)

What would be the splitting pattern for the hydrogens on carbon 4 in a ^{1}H-NMR?



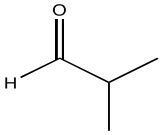
1- doublet
2- triplet
3- quartet
4- multiplet (complex)

Figure 3. Example questions on splitting patterns in ^{1}H-NMR.

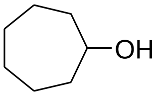
The hydrogens on carbon four are next to non-equivalent hydrogens. If students understood the "n + 1" rule, ideally they chose "triplet" and "multiplet" respectively as their answer.

The third sub-set of questions required students to fit ^{1}HNMR or ^{13}C NMR data to a given structure (see Figure 4). Students were given a chart for their use of chemical shifts for both ^{1}H-NMR and ^{13}C-NMR. They were not being measured on their ability to memorize chemical shifts but rather to apply them. This sub-set required more higher-order thinking skills on the part of the student. They needed to recognize not only the number of signals, but they were also expected to be able to understand the chemical environment to determine an estimate of the chemical shift. Splitting patterns and integration values were also included in the data set for the ^{1}H-NMR question.

Which ^{13}C-NMR data matches the following compound?

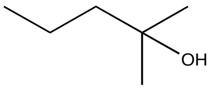


1- δ 16, 21, 41, 205
2- δ 16, 41, 75
3- δ 16, 41, 205
4- δ 16, 21, 41, 75



1- δ 20, 25, 28, 29, 32, 35, 62
2- δ 20, 25, 32, 64
3- δ 20, 25, 28, 69, 72, 110, 120
4- δ 20, 25, 32, 110

Which ^{1}H-NMR data matches the following compound?



1- δ 1.3 (t, 3H), 1.6 (t, 2H), 3.8 (t, 2H), 2.0 (s, 6H), 2.8 (broad s)
2- δ 1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0, 3.8 (t, 6H), 2.8 (broad s)
3- δ1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0 (s, 3H), 2.1 (s, 3H), 2.8 (broad s)
4- δ 1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0 (s, 6H), 2.8 (broad s)

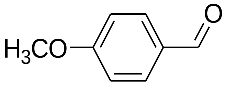
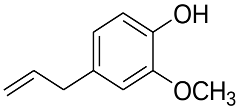
Figure 4. Example questions on fitting spectral data to structure.

The final sub-set of questions dealt with assigning signals on a given spectrum. For this, the survey provided a ^{1}H NMR spectrum of *p*-anisaldehyde and eugenol (see

Appendix G). Students identified as many signals as they could based on the labeled compounds. These questions were assessed separately because there was not a single correct answer. Of the two spectra, *p*-anisaldehyde was considered to be the easiest to interpret. This compound had six assigned groups of hydrogens to label whereas eugenol had eight. The chemical shifts are summarized in Table 3.

Table 3

Chemical shifts of p-anisaldehyde and eugenol.

p-anisaldehyde 	δ 3.6 (s, 3H), 6.9 (d, 2H), 7.8 (d, 2H), 9.8 (s, 1H)
Eugenol 	δ 3.2 (d, 2H), 3.9 (s, 3H), 5.1 (m, 2H), 5.5 (s, 1H), 6.0 (m, 1H), 6.7 (d, 2H), 6.9 (d, 1H)

In addition to the survey questions that measured spectroscopy content, the survey also included questions designed to assess student attitudes about organic chemistry laboratoriess. There were thirteen Likert-scale questions, broken down into two factors: 1) students' attitudes about spectroscopy content, and 2) students' attitudes about the laboratory in general. The Likert-scale was based on a scale of one to five, in which one was strongly disagree, two was disagree, three was undecided, four was agree, and five was strongly agree. All questions are presented in Table 4, where the asterisk indicates questions that were worded negatively, where "strongly disagree" is favorable and coded reversely to reflect that in the analysis.

Table 4

Likert-scale questions related to attitude.

Factor: Students' attitude about spectroscopy content
The labs enhanced my ability to interpret ^{1}HNMR The labs enhanced my ability to interpret ^{13}CNMR The labs enhanced my ability to interpret IR *The labs did not help me learn spectroscopy
Factor: Students' attitude about the lab in general
The lab design made interpreting spectra meaningful and gave a sense of purpose How much did you like the overall lab I found this semester labs to be fun *I found this semester labs to be confusing *I found this semester labs to be frustrating This semester, lab has increased how much I like science in general This lab has helped me gain useful knowledge I have benefited from taking this lab I learned interesting new things in lab
Questions cut from analysis
The labs were easy The labs were challenging

* indicates statements that were reverse coded

Data Analysis

Statistical results were generated using Microsoft EXCEL and SPSS software and the significance was set at an alpha level of 0.05. The multiple choice/ short answer questions were coded with one being a correct response and zero being an incorrect response. Cronbach's alpha was used for reliability on the multiple choice/short answer content questions. This test measured the internal consistency of the instrument. Desirable alpha values are close to one and indicate greater internal consistency; values of 0.7 or higher are preferred (Nunnally, 1978, p. 245). The item-total correlation was used to determine if any questions in the survey had responses that varied differently than other questions, which could affect the overall reliability of the instrument. One of the original short answer/multiple choice questions was not included in the survey analysis based on the correlated item-total correlation (0.030). Items that correlate near zero are generally discarded (Nunnally, 1978, p. 263). Once the question was removed, the Cronbach alpha went from 0.65 to 0.68.

Content knowledge

The independent samples *t*-test was used to analyze the differences in the total percent correct score on the multiple choice/short answer section of the survey between the traditional laboratory and *discovery-based* laboratory. The independent samples *t*-test was also used for the two questions that involved assigning signals on a spectrum of *p*-anisaldehyde and eugenol. Cohen's *d* analysis was used on the content-based questions, for the multiple choice/short answer and the two spectra items to measure effect size. Cohen's *d* examines the ratio of the differences in means to the pooled variance for practical significance, no longer using sample size, which could affect the power of the study. Cohen (1992) defines a small *d* effect size as 0.20, medium as 0.50, and a large as 0.80.

A chi-square test of independence was used to test each multiple choice/short answer item. This test was used to test for differences in distributions of responses between the two groups. A Cohen's *w* was used on each chi-square test of independence to measure effect size. Cohen (1992) defines a small *w* effect size as 0.10, medium as 0.30, and a large as 0.50.

Attitudes

For the Likert section of the survey, the questions were coded numerically, one was strongly disagree, two was disagree, three was undecided, four was agree, and five was strongly agree. Factor analysis with a Promax rotation was used to group the survey questions. Two factors were selected based on the eigenvalues (see Appendix I) (O'Connor, 2000). The questions were categorized into two factors: attitudes about lab in general (nine questions) and attitudes about spectroscopy content (four questions). Two questions were not highly correlated with either factor, so they were not used in the analysis. See Table 4 for the factor items. Independent samples *t*-tests were used to compare the means of the two factors between traditional and *discovery-based* lab students. The Cohen's *d* was also reported.

A chi-square test of independence was used to see if the distributions of item responses for a given item differed between the traditional lab group and the *discovery-based* lab group (Howell, 2013). For the questions with significant chi-square test statistics, the residuals with an absolute value near or greater than two were used to determine how the responses differ between the two groups. The Cohen's *w* was also reported.

Grades

The independent samples *t*-test was used to analyze the difference in students' self-reported expected grades in both CHEM 3011 laboratory and CHEM 3010 lecture and their actual grades earned. The grades were given quality points based on the scale outlined in Table 5 which ranged from zero for an F to four for an A. The Cohen's *d* was also reported.

CHAPTER IV: RESULTS AND CONCLUSIONS

Assessment of the effect of the *discovery-based* unit was carried out using a survey that included content-based questions measuring students' abilities to interpret spectroscopy and attitude-based questions. The statistical analysis was divided into three categories: content-based questions (multiple choice/short answer and assigning signals), attitude-based questions, and content-based questions analyzed by each instructor involved in the study.

Content-Based Questions Results

Multiple choice/short answers

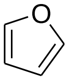
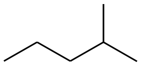
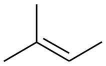
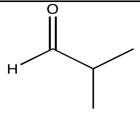
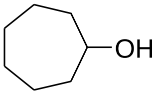
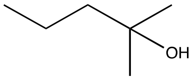
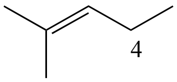
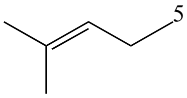
There were eight multiple choice/short answer content-based questions included in this analysis. No significant differences were found between the traditional laboratory participants' overall score (N = 78, M = 47.92, SD = 27.58) and the *discovery-based* laboratory participants' overall score (N = 82, M = 49.39, SD = 27.14), $t(158) = 0.34$, p = 0.865, d = 0.05). Additionally when each multiple choice/short answer item was compared, there were no significant differences between the two groups' responses to any question. The results for the individual items are summarized in Table 5.

Assigning signals on given spectrum

The two spectra questions were analyzed separately then the multiple choice/short answer section because these had multiple correct responses. The *p*-anisaldehyde spectrum was scored on a scale from zero to six, with six indicating that all signals were identified correctly. The eugenol spectrum was scored in a similar way, with eight indicating all signals identified correctly.

Table 5

Chi-square of independence: Multiple choice/short answer content items

Question	Structure	% Correct		χ^2	*p*	*w*
		Traditional	*Discovery-based*			
Students indicate how many signals would be seen in either the ^{1}H or ^{13}C-NMR		57.7 %	70.7 %	2.96	0.085	0.14
		48.7 %	41.5 %	0.85	0.357	0.07
		52.6 %	43.9 %	1.20	0.273	0.09
Students match ^{13}C-NMR data to the compounds		52.6 %	54.9 %	0.09	0.769	0.02
		38.5 %	36.6 %	0.06	0.806	0.02
Students match ^{1}H-NMR to the compound		35.9 %	34.1 %	0.05	0.826	0.02
Students predict the splitting pattern for the hydrogen on the numbered carbon 4 or 5 for a ^{1}H-NMR		34.6 %	48.8 %	3.30	0.069	0.14
		62.8 %	64.6 %	0.05	0.811	0.02

df = 1 *N* = 160

There was no significant difference between the traditional laboratory participants' score for the *p*-anisaldehyde spectrum (*N* = 78, *M* = 55.77, *SD* = 31.99) and the *discovery-based* laboratory participants' score ($N = 82$, $M = 63.82$, $SD = 28.43$), $t(158) = 1.69$, $p = 0.122$, $d = 0.27$. The results were similar for the eugenol spectrum; traditional laboratory ($N = 78$, $M = 24.04$, $SD = 19.92$) and the *discovery-based* laboratory ($N = 82$, $M = 29.73$, $SD = 25.20$), $t(158) = 1.58$, $p = 0.093$, $d = 0.25$, indicating no significant difference.

Content-based analysis by instructor

To avoid instructor bias, the groups were also compared based on individual instructors. This study only included data from paired sections with the same instructor teaching a traditional laboratory and a *discovery-based* laboratory. Although there were no significant differences for the multiple choice/short answer part of the survey, all the means, with exception of instructor B, are higher for the *discovery-based* group than the traditional group. The results are summarized in Table 6.

Table 6

Results for multiple choice/short answer items based on instructors

	Traditional	Discovery-based	t	p	d
Instructor A	$M = 44.64$ $SD = 31.52$ $N = 21$	$M = 48.44$ $SD = 24.10$ $N = 16$	0.40	0.691	0.14
Instructor B	$M = 71.67$ $SD = 20.30$ $N = 15$	$M = 68.33$ $SD = 21.06$ $N = 15$	-0.44	0.662	0.16
Instructor C	$M = 36.90$ $SD = 24.52$ $N = 21$	$M = 41.41$ $SD = 30.53$ $N = 32$	0.57	0.574	0.16
Instructor D	$M = 45.24$ $SD = 21.82$ $N = 21$	$M = 48.68$ $SD = 21.61$ $N = 19$	0.50	0.619	0.16

The interpretation of spectral data on the survey was also analyzed by instructor. There were no significant differences for the interpretation of *p*-anisaldehyde or eugenol spectra when comparing each of the two sections taught by the same instructor. The data for each instructor are summarized in Table 7.

Table 7

Results for interpretation of spectra based on individual instructors

	Compound	Traditional	Discovery-based	*t*	*p*	*d*
Instructor A	*p*-anisaldehyde	*M* = 59.92 *SD* = 34.79 *N* = 21	*M* = 66.67 *SD* = 25.09 *N*= 16	0.69	0.492	0.24
	eugenol	*M* = 22.62 *SD* = 19.61 *N* = 21	*M* = 26.56 *SD* = 19.83 *N* = 16	0.60	0.550	0.20
Instructor B	*p*-anisaldehyde	*M* = 57.78 *SD* = 31.41 *N* = 15	*M* = 67.78 *SD* = 23.96 *N* = 15	0.98	0.335	0.36
	eugenol	*M* = 27.50 *SD* = 18.41 *N* = 15	*M* = 39.17 *SD* = 29.45 *N* = 15	1.30	0.204	0.47
Instructor C	*p*-anisaldehyde	*M* = 53.17 *SD* = 29.64 *N* = 21	*M* = 62.50 *SD* = 30.23 *N*= 32	1.11	0.274	0.31
	eugenol	*M* = 19.64 *SD* = 15.60 *N* =21	*M* = 24.61 *SD* = 23.00 *N* = 32	0.89	0.380	0.25
Instructor D	*p*-anisaldehyde	*M* = 53.17 *SD* = 33.59 *N* = 21	*M* = 60.53 *SD* = 32.49 *N* = 19	0.70	0.487	0.22
	eugenol	*M* = 27.38 *SD* = 24.88 *N* = 21	*M* = 33.55 *SD* = 29.18 *N* = 19	0.72	0.425	0.22

Attitude Question Results

Students' attitudes were measured using Likert-scale questions. From the factor analysis Promax rotation, the questions were divided into two factors: attitudes about the laboratory in general, and attitudes about ability to interpret spectroscopy. For the factor of attitudes about the laboratory in general, there were no significant differences between the traditional laboratory participants' responses (N = 78, M = 3.42, SD = 0.65) and the *discovery-based* laboratory participants' responses (N = 82, M = 3.50, SD = 0.77), t(158) = 0.72, p = 0.473, d = 0.16. The same is true regarding the factor for students' attitude about interpretation of spectroscopy in the traditional laboratory (N = 78, M = 3.57, SD = 0.71) and the *discovery-based* laboratory participants responses (N = 82, M = 3.35, SD = 1.04), t(158) = 1.60, p = 0.111, d = 0.29.

Each Likert-scale item was compared individually. These results are summarized in Table 8, with the significant items in bold face. Of the nine questions, two were found to be significantly different: the item regarding students' ability to interpret ^{1}H-NMR, χ^2 (4, N = 160) = 16.01, p = 0.003 and the item regarding students' ability to interpret IR, χ^2 (4, N = 160) = 18.47, p = 0.001. The residuals for these significant items are listed in Table 9.

Table 8

Chi-square test of independence on students' attitude items

Attitudes toward the laboratory in general	χ^2	*p*	*w*
The lab design made interpreting spectra meaningful and gave a sense of purpose	3.09	0.543	0.07
How much did you like the overall lab	3.18	0.528	0.07
I found this semester labs to be fun	4.60	0.331	0.08
I found this semester labs to be confusing	6.71	0.152	0.10
I found this semester labs to be frustrating	2.35	0.642	0.06
This semester, lab has increased how much I like science in general	4.81	0.308	0.09
This lab has helped me gain useful knowledge	4.72	0.317	0.09
I have benefited from taking this lab	9.39	0.052	0.12
I learned interesting new things in lab	3.75	0.441	0.08
Attitudes toward spectroscopy			
The labs enhanced my ability to interpret ^{1}HNMR	**16.01**	**0.003**	**0.16**
The labs enhanced my ability to interpret ^{13}CNMR	8.97	0.062	0.12
The labs enhanced my ability to interpret IR	**18.47**	**0.001**	**0.17**
The labs did not help me learn spectroscopy	3.16	0.532	0.07

Bold items are significantly different; *df* = 4, *N* = 160

Table 9

Residuals of individual significant Likert items

		strongly disagree	disagree	un-decided	agree	strongly agree
The labs enhanced my ability to interpret ^{1}HNMR	Traditional lab	-1.85 (*N* = 0)	-0.19 (*N* = 12)	0.96 (*N* = 15)	1.05 (*N* = 45)	-1.67 (*N* = 6)
	Discovery-based lab	1.80 (*N* = 7)	0.18 (*N* = 14)	-0.94 (*N* = 9)	-.102 (*N* = 34)	1.63 (*N* = 18)
The labs enhanced my ability to interpret IR	Traditional lab	-2.18 (*N* = 2)	-0.98 (*N* = 13)	0.47 (*N* = 19)	1.78 (*N* = 40)	-0.59 (*N* = 4)
	Discovery-based lab	2.13 (*N* = 15)	0.96 (*N* = 22)	0.46 (*N* = 16)	-1.73 (*N* = 22)	0.57 (*N* = 7)

Predicted Grades Compared to Actual Grades

The difference in students' perceptions of their abilities based on self-reported expected grades in laboratory and lecture actual abilities based on course grades was not significant. The grades were assigned quality points based on the scale outlined in Table 10, which ranged from zero for an F to four for an A. The results for the comparison of the expected laboratory grade to the actual laboratory grade for traditional laboratory and *discovery-based* laboratory grade are shown respectively: ($N = 78$, $M = 0.20$, $SD = 0.80$); ($N = 82$, $M = 0.04$, $SD = 0.59$), $t(158) = 1.46$, $p = 0.145$, $d = 0.23$. The comparison for the overall expected lecture grade for the traditional ($N = 78$, $M = 0.38$, $SD = 0.97$) compared to the *discovery-based* ($N = 82$, $M = 0.29$, $SD = 0.80$), $t(158) = 0.70$, $p = 0.486$, $d = 0.10$ also indicated no significant differences in the groups.

Table 10

Quality points assigned for grades

Letter grade	Percentage	Quality Points
A	90-100 %	4
B	80-89 %	3
C	70-79 %	2
D	60-69 %	1
F	below 60 %	0

Limitations of the Study

The participants in this study came from the population at Middle Tennessee State University, which is a regional comprehensive university in the Southeastern United

States. Most of MTSU's undergraduate students are from Tennessee, with the largest population coming from public high schools in the Middle Tennessee region. As a result the results of this study may not be generalizable to other populations.

Although all students enrolled in CHEM 3011 filled out the survey, the sample size was limited to students in sections where the instructors taught both a section of the *discovery-based* lab and the traditional lab. This was done to avoid laboratory instructor bias when analyzing the results. One of the laboratory instructors taught a section of the *discovery-based* laboratory in the summer of 2011 and taught the traditional laboratory in the summer of 2013. The time delay may have muted some differences. Also, during the summer sessions, the course instructor was the laboratory instructor and a professor with years of teaching experience that the other instructors in the study did not have. The study did not take into account the different lecture instructors during the Fall and Spring semesters. Since student enroll in lab separately from lecture, this study focused only on laboratory instructors. There could be some lecture instructor influences in the results; however answering this question was not in the scope of this research.

The reliability of survey based on the Cronbach alpha was 0.68, which was close to the preferred minimum 0.70. Having more questions on the survey may have increased the reliability, but the survey was designed to be given in lab where time was limited. The students' had to fully complete the survey for the extra credit points, take a final comprehensive test, and check out in one class period.

The teaching experience of the GTAs is another limitation. Although the GTAs who were instructing these laboratories were extremely competent, it is clear based on instructor B's survey averages that more teaching experience may enhance student learning. The GTAs were not as comfortable commenting on the presentations, so an organic professor was present for the final laboratory period in the *discovery-based* sections. It is unclear whether this impacted student learning.

Discussion

Content-based portion of the survey

For the multiple choice/short answer section of the survey, the individual questions were analyzed; two questions approached statistical significance and had small effect sizes. One question asked students to predict how many signals would be seen in the ^{13}C-NMR for furan ($p = 0.085$, $w = 0.14$). The other question asked them to predict the splitting pattern for the hydrogen on carbon four on 2-methylbut-2-ene ($p = 0.069$, $w = 0.14$). For both questions, the *discovery-based* laboratory participants scored higher, 13.0 % higher and 14.2 % higher than the traditional laboratory participants, respectively. These questions represented different sub-sets of skills; predicting number of signals and splitting patterns. Since the other multiple choice questions did not approach statistical significance or had small effect sizes, the students' performance on these questions were comparable for the two laboratory groups. It was, however, interesting that the *discovery-based* laboratory performed higher on the two questions exhibiting a small effect size.

Although there was no significant difference on the multiple choice/short answer survey overall, when the results were analyzed based on individual laboratory instructors, the mean percent correct for three of the four (Instructors A, C, and D) instructors was higher for the *discovery-based* laboratory. For instructor B, however, the traditional laboratory scored higher. There are several differences between instructor B and the other three instructors that could account for the different student performances. First, the two sections taught by instructor B occurred in the Summer of 2011 (*discovery-based*) and in Summer of 2013 (traditional), a much larger time interval than the other three instructors. Second, instructor B taught the lecture and the laboratory for these students, the other three laboratory instructors did not teach the lecture. Third, instructor B was a tenured faculty member with many years of teaching experience in the organic chemistry class and laboratory; the other three laboratory instructors were graduate students.

The *discovery-based* laboratory had a higher percentage correct on the spectra assignment portion of the survey. Although there was no significant difference between the groups, both spectra scores were higher for the *discovery-based* group and both comparisons had a small effect size, indicating that there was a practical difference. For the *p*-anisaldehyde spectrum, the *discovery-based* group scored 8.05 % higher than the traditional laboratory ($t(158) = 1.69$, $p = 0.122$, $d = 0.27$). For the spectrum of eugenol, the *discovery-based* participants scored 5.69 % higher than the traditional laboratory ($t(158) = 1.58$, $p = 0.093$, $d = 0.25$). These differences in percentage correct could be the difference of letter grade in many cases. When analyzed by individual instructors, the improved performance of the *discovery-based* group was true for all the instructors, so this effect seems to be independent of instructor. The improved performance ranged from 3.94 % to 10.0 % for the four instructors.

Attitude portion of the survey

The individual Likert questions about students' attitudes were analyzed, and only two questions were significantly different. Both of the significant questions were in the factor of attitudes towards spectroscopy. Although there were no significant differences between the groups, there was a small effect size for this factor, indicating a practical significance ($t(158) = 1.60$, $p = 0.111$, $d = 0.29$).

Based on the residual results, the *discovery-based* participants were more likely to strongly agree or strongly disagree with the statement that suggested the laboratory enhanced students' ability to interpret ^{1}H-NMR. This could be due to the students' experience with the presentation aspect of the *discovery-based* laboratory. Many students identified their components and interpreted their spectral data correctly. However, there were many students who prepared for the presentation, but their spectral data did not confirm the component they claimed to isolate. These students received feedback from their peers and instructors, and they had the opportunity to re-analyze their data. Due to the pressure of presenting, and the nature of the *discovery-based* laboratory, students could not just use the process of elimination to identify what compound was represented in the spectra. The added pressure may be the reason why students in the *discovery-based* laboratory have stronger opinions about their ability to interpret spectroscopy, whether negative or positive.

The residuals on the question dealing with interpretation of IR indicated that the traditional laboratory had a higher confidence for interpreting IR. The traditional participants had exposure to the IR in the unknown laboratory. The *discovery-based* participants could not collect an IR spectrum on their oil because the amount of oil recovered was too small. The only exposure these students would have had with IR was in their lecture course. This result then, is not surprising, and indicates that the participants in both groups took the survey seriously.

There was no significant difference in students' self-efficacy of grades in either group. This was surprising as literature indicates that students' tend to overestimate their grades. This was the final question on the survey, and after the intensity of trying to answer questions based on spectroscopy, it may be reasonable to suggest that students' confidence in their grade was lowered.

Conclusions

In conclusion, a *discovery-based* laboratory component was introduced into several sections of the organic chemistry laboratories at MTSU and compared to sections of the traditional laboratory. The goal was to continue to provide experiential learning and improve the laboratory in a way that would lead students to think more critically. The learning objective measured was students' ability to interpret spectroscopy.

Traditional laboratories are practical. Because of the ease in preparation and the mass number of students that come through organic chemistry laboratory, the majority of the organic laboratories at MTSU have been predominantly the traditional laboratories. Traditional laboratories have been criticized for not being as effective as other laboratory styles in aiding students to reason more critically (Domin,1999). Gaddis and Schoffstall (2007) suggest that *discovery-based* laboratories can be easily incorporated for large laboratory sections. Mohrig (2004) suggests recasting traditional laboratory experiments so that a question or problem comes first, and he goes on to suggest practical ways of transforming many traditional laboratories to *discovery-based* laboratory format.

The results from this research indicate that there is an advantage to including *discovery-based* laboratory components into the organic chemistry curriculum at MTSU. Although there were few significant differences, the means for spectroscopy questions were consistently higher in the *discovery-based* groups. This indicates that the trend is a better understanding of NMR spectroscopy. Also student's attitudes were significantly different for the *discovery-based* group for their overall confidence in interpreting spectroscopy, whether negative or positive. This could indicate that students' were thinking more critically. Freedman (1997) found a correlation between achievement and attitudes toward science. This would be interesting to analyze as future research for this study.

With so few publications about the effectiveness of organic laboratories, there is a need for research on different laboratory styles in this field. Students can be influenced by their laboratory experiences. Cooper and Kerns (2006) point out the change in students' perception of their role in a problem-based laboratory was less passive than in a traditional laboratory setting. Mohrig et al. (2009) found that the post-lab discussions in a *discovery-based* chalcones laboratory really added to his students' critical thinking skills as they analyzed their results. The research findings presented here support the overall idea that other laboratory styles are more effective than the traditional laboratories, and this research is a needed addition to the current literature.

During the semesters that this research was conducted, there were very competent GTAs. Informally, they expressed that they thought the students enjoyed the laboratories more as a whole, and they enjoyed teaching the laboratories more. However, there was more chaos because not everyone was doing the same thing at the same time, especially during week two. They also observed the critical thinking process in week three when the spectral data was presented and analyzed. Students exhibited greater critical thinking skills in the *discovery-based* laboratory presentations than the simple process of elimination required in the traditional unknown laboratory, where they turned in a written repost without class discussion. Anecdotal evidence suggests that not only did the students think more critically in the *discovery-based* laboratory, they also seemed more engaged in the laboratory.

The ability to interpret NMR varied among the GTA’s, and new GTAs without much research experience may find the interpretation aspect of this laboratory challenging. With this in mind, this laboratory would be better suited if the choice of herbs and spices was limited to ones previously tested so the GTA’s could better be prepared.

Future work

There are areas of this research that can be expanded. Now that the laboratories at MTSU are equipped with more sensitive infrared spectrometers, it is possible to add IR analysis to this laboratory. The addition would give the students access to more state of the art equipment that is now available. For GTA training purposes, the lab also needs to be revised to limit the herbs and spices for student use.

Anecdotal evidence shows heightened interest in the lab for the instructor and higher levels of student engagement in the *discovery-based* sections. A formal assessment such as interviews or open-ended questionnaires given to instructors and students regarding their perceptions of the effectiveness of the laboratories would be an interesting addition. The attitude portion of the survey indicates students in the *discovery-based* laboratories had stronger opinions in their ability to interpret spectroscopy. It is speculated that this could be due to the pressure of presenting data to their peers and instructors. It would be interesting to incorporate a presentation in a traditional laboratory and see if the attitudes are similar.

There will be continued research on the effectiveness of the organic chemistry laboratories at MTSU. This is a student-centered university and it is imperative that there is ongoing research to ensure that students are engaged, thinking critically, and having opportunities to use state of the art equipment in science.

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APPENDICES

APPENDIX A

Publication in Advances in Teaching Organic Chemistry

Discovery-based labs for organic chemistry: overview and effectiveness

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Although more common in general chemistry courses, a number of discovery-based or guided-inquiry laboratory experiments in organic chemistry have been reported over the past fifteen years. These are generally believed to be an improvement over traditional "cookbook" experiments, with increased student interest and engagement. A survey of the chemical education literature gives many examples, with most falling into one of just a few categories. Examples from each of these categories are summarized, as well as examples that focus on assessment of student learning and perceptions.

Introduction

Laboratories are a central component of the undergraduate organic chemistry curriculum, where students are taught techniques, research skills, and support for lecture material. For years educators have been looking at the effectiveness of science laboratories and the impact on student's learning, and there are many opinions on what constitutes an effective lab. Chemistry labs have been classified as expository, problem-based, inquiry or discovery (1). The types of lab share similarities but differ in respect to outcome, approach and procedure, and there have been debates on which type of lab is most effective (2,3,4,5). *Expository*, also known as cookbook, verification, or traditional style, is the predominant laboratory style used in undergraduate organic chemistry laboratories. This type of lab has been defined as a deductive approach where students are given a problem and step-by-step instructions on how to reach a pre-determined outcome. The concepts covered in the laboratory will have been covered in lecture before the lab is performed. Although the majority of undergraduate labs use an expository approach, the method has been criticized by many educators and researchers. Advantages include ease of lab preparation and training of TAs, however expository labs involve little critical thinking (6,7,8). Increasingly, organic laboratories have incorporated some *discovery*, or *guided inquiry-based* labs. These are seen as more practicable labs than *open inquiry* and *problem-based* experiments, where students are expected to develop a procedure. In a typical discovery or guided-inquiry experiment, students follow a given procedure, collect their data, make observations and draw conclusions based on their results. The outcome varies from predetermined to undetermined. This is a more inductive approach than the expository labs, and develops critical thinking skills.

In discovery-based labs, the instructor does not give step-by-step instruction, but may give a general procedure. Students are playing the role as the discoverer in lab with less "guidance" from the instructor (1). Some evidence suggests that students learn more and are more engaged in a guided-inquiry lab or a discovery based lab than in the traditional, cookbook lab setting (9). Discovery labs are inductive in nature, illustrate the scientific method, and connect theory with empirical data (10). Admittedly, students' attitudes towards the labs vary; most would agree that their "problem solving skills" were used more, but they also found the laboratories more frustrating and difficult (11). Most of these studies have been conducted in general chemistry labs, but these open the idea that changing the traditional lab structure in organic chemistry may deepen students' understanding of the subject.

As there are educators that are "pro-discovery", there are criticisms of the style as well. It has been argued that if a student does not have basic knowledge of the material to be learned, they are unable to make the "correct" discovery, and it is unclear how a group of students can discover the same thing. Also, discovery labs are more time consuming and more challenging in regard to training of teaching assistants (11).

Although most of the research in the area of effectiveness of different lab types on student learning has been focused in the general chemistry laboratory courses, some studies have been published for the undergraduate organic chemistry laboratory. The goal of this chapter is to summarize representative examples of published discovery-based organic chemistry labs that can be implemented into the undergraduate curriculum, as well as the scant research that has been done on the *effectiveness* of discovery labs in the organic chemistry laboratory.

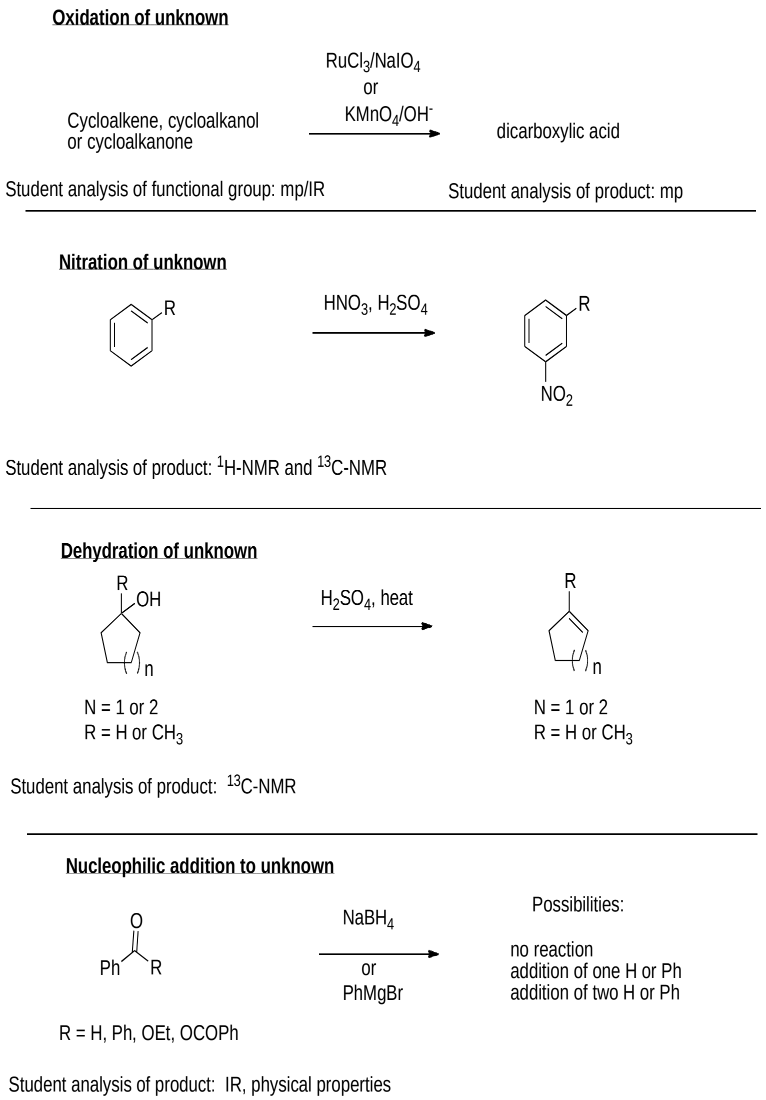
Summary of discovery and guided-inquiry labs

A survey of discovery and guided-inquiry labs specific for organic chemistry fall, for the most part, into a few different categories. These are: labs involving identification of unknowns, labs involving reaction analysis, and labs involving isolation and/or purification. Several published laboratory manuals have incorporated multi-step and guided-inquiry experiments; however the focus of this chapter is on experiments published in journals (12, 13).

Labs involving identification of unknowns

Identification of unknowns lends itself well to discovery and guided-inquiry. The extent of critical thinking on the part of the student depends on how much is "unknown". For instance a common expository lab involves giving a table of compounds with ten different melting points and asking students to identify an unknown by a melting point. This would involve learning lab techniques, but little in the way of critical thinking. However, expanding the number of compounds in the table, as well as the extent of analysis, and including compounds with similarities leads to a more discovery-based approach. An example of this is the identification of a series of unknowns based on melting point analysis as well as IR and NMR spectroscopy (14). From a list of eighty-one compounds, students narrow down the possibilities based on melting point or boiling point. An IR is taken and analyzed in order to further narrow the possibilities by functional group. Final determination is based on NMR spectroscopy.

More advanced use of unknowns involves the reaction of an unknown, and then analysis of spectral data for identification of the product, and therefore of the starting material. A number of labs have been published using this approach, including functional group oxidation, aryl nitration, alcohol dehydration and nucleophilic addition to carbonyls. These are summarized in Figure 1.

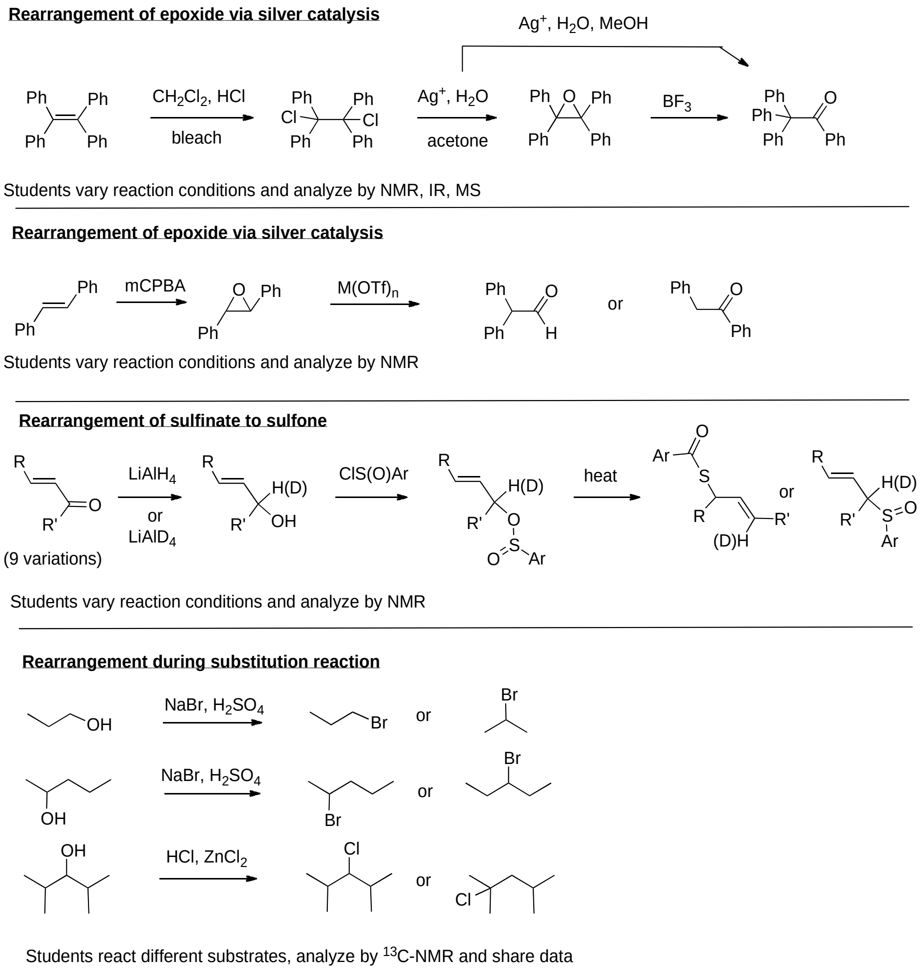


Fi*gure 1. Examples of labs involving reaction and analysis of an unknown.*

In the first case, students are given an unknown that may be either an alkene, alcohol or ketone, with twelve possible structures (15). The first task is to identify the functional group by chemical tests and IR. The oxidation method proposed by the student is dependent on the functional group, and analysis of the carboxylic acid product is done by NMR. Although students are all carrying out the same reaction, they will obtain different products, and will need to analyze properties of the products and then work back to identify the starting material. The next two are variations of typical expository labs done in nearly every undergraduate organic chemistry lab. Nitration of methyl benzoate, as well as dehydration of cyclohexanol are standard labs. Modification of both of these to include four unknown starting materials, and analysis of the product by NMR adds the element of discovery (16,17). Students must analyze the spectroscopic data of the product in order to work back to the identification of the starting unknown. A final example is that of sodium borohydride and Grignard addition to unknown carbonyl compounds. The unknowns include an aldehyde, a ketone, an ester and an anhydride. Students carry out both reactions and analyze whether or not a reaction has occurred, as well as identity of a product in order to work back to identification of the unknown (18).

Labs involving reaction analysis

Most of the published labs fall into this category and vary with the extent of discovery by the student. Several procedures result in various products based on mechanism, and the student's "discovery" involves both product analysis and proposal of mechanism. There are a few examples in which a rearranged product may be observed, either in an epoxide ring opening, or in an alkyl halide formation from an alcohol (19,20,21). Several more advanced labs have been reported, including a sulfinate to sulfone rearrangement, and a ring-closing metathesis (22,23). These are perhaps best suited for an upper level advanced synthesis lab, as they both deal with reactions not commonly covered in the typical two-semester organic sequence. Figure 2 gives examples of published labs that involve inquiry on the students' part on reaction mechanism, due to rearrangement that occurs during the reaction.



Rearrangement of epoxide via silver catalysis

Students vary reaction conditions and analyze by NMR, IR, MS

Rearrangement of epoxide via silver catalysis

Students vary reaction conditions and analyze by NMR

Rearrangement of sulfinate to sulfone

(9 variations)

Students vary reaction conditions and analyze by NMR

Rearrangement during substitution reaction

Students react different substrates, analyze by ^{13}C-NMR and share data

Figure 2. Labs involving analysis of a rearranged product.

Several other labs involve the investigation of stereoselectivity and/or regioselectivity of a reaction, and are summarized in Figure 3.

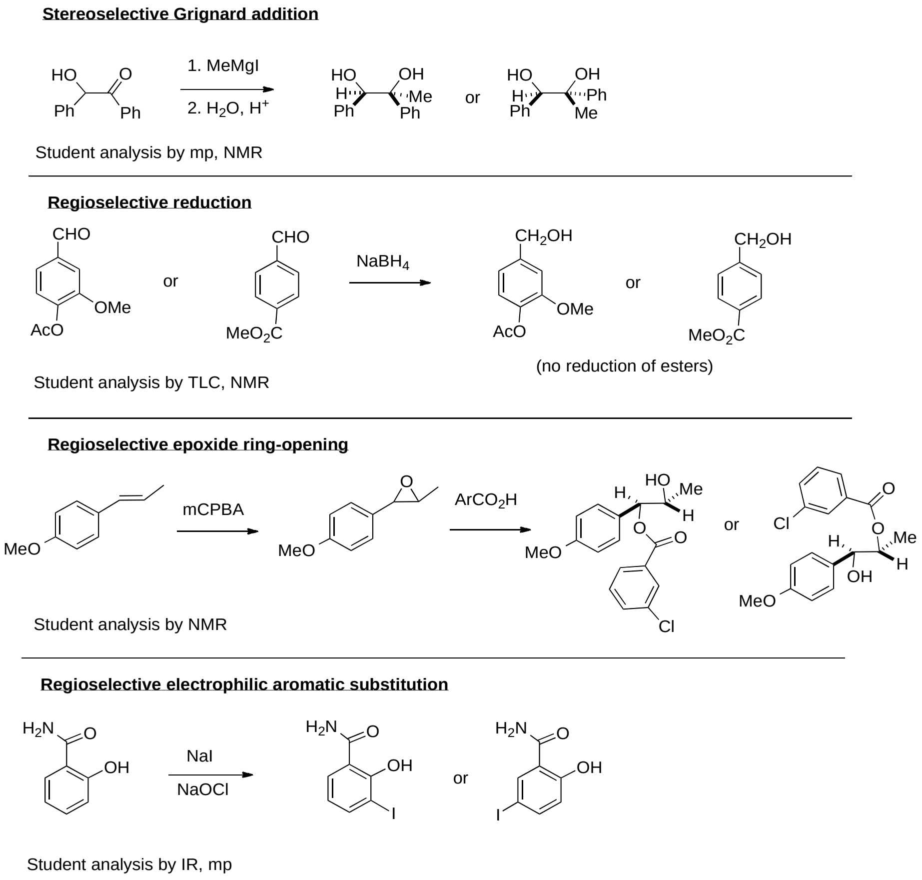


Figure 3. Labs involving analysis of stereoselective and regioselective reactions.

The discovery on the part of the student in these labs relies mainly on prediction of possible reaction products, and use of physical or spectroscopic techniques for verification. For example, in the addition of a Grignard reagent to racemic benzoin, all students carry out the same reaction, and determine the product based on melting point, with a discussion of diastereoselectivity (22). An example of a regioselective reaction has students using one of two possible substrates, each with an aldehyde and an ester funtional group. Analysis of the product by NMR spectroscopy is used in order to identify which group(s) were reduced (23). Other examples of prediction and verification of regioselectivity in reactions include epoxide ring-opening and electrophilic aromatic substitution (24,25). Another example that falls into this category is the synthesis and regioselective hydrogenation of a series of chalcones (26).

Labs involving purification

Several labs have been published that are an extension of the traditional purification labs involving distillation, recrystallization and chromatography. Varying techniques and/or samples lends the element of discovery to these labs. One example combines extraction, recrystallization and distillation into one experiment. Students perform the experiments with variable conditions for each technique, and share and discuss results (27). Another example involves the purification of "poisoned" Excedrin using extraction, chromatography and spectroscopy (28). Students' interest is heightened by the use of a familiar medicine. Another example is that of the isolation of components of plants by extraction, purification by chromatography and spectroscopic identification (29).

Discovery-based or research-like laboratory courses

Most ambitious are the reports of entire courses developed on the basis of guided-inquiry labs. Of the published reports, a common feature is the development of technique using expository-type labs, followed by a multi-week combined experiment. By using the expository labs first, students gain confidence in their abilities before proposing and carrying out a multi-week discovery-based project. In one example, after gaining experience, students propose a multi-step synthesis, carry it out, and then write a formal report on their results (30). In another example, all students carry out a multi-week synthetic research project using a Wittig reaction, halogenation, elimination and then formation of metalloles (31). While perhaps the most interesting for students, these are challenging for the instructor.

Summary of effectiveness of discovery and guided-inquiry labs

In many of the labs outlined in this chapter, informal observations were used to assess the effectiveness of the guided-inquiry labs and student learning. The instructors observed the questions that students asked during the lab, and concluded that the students in the guided-inquiry labs exhibited more independent thinking than in a traditional lab setting. It was also observed that students took more responsibility for what they were learning, felt the labs were more entertaining, and found the labs more rewarding than traditional labs (27,13,29).

Others, such as Stoub's purification lab, not only used informal observations, but also used student evaluations, end of year assessments, and reflections in a notebook to assess the effectiveness of the lab (28). Again, it was found that students asked deeper questions based on a deeper understanding of the laboratory, they took ownership of their work, and generally enjoyed the lab. There was no statistical analysis reported, but the laboratory handouts, student discussion questions, and instructor notes are provided in the supplemental material.

Miller and Leadbeater measured student learning versus students' perception of learning in their guided inquiry lab (32). A WebCT pre-laboratory test was given as a measure of prior knowledge of the material covered, which was broken down into three components: microwave energy, biodiesel, and esterification. The same test was given as a post-laboratory test to see if knowledge was gained. There was a statistical difference in scores on the pre-test vs. post-test, suggesting that the laboratory had a positive impact on students' understanding of the content. Along with the pre and post-test, a five point Likert-scale survey was administered which linked the test results to students' perception of their content comprehension. This survey was administered *via* WebCT before and after the laboratory as well. Their confidence from participating in this lab gave mixed results. In comparing the pre- and post-test, a statistical significance was observed for: comprehension of action of microwave energy in heating a reaction, knowledge of differences in microwave equipment, the concept of biodiesel, the actual synthesis and reaction conditions, and the students' abilities to interpret ^{1}H NMR. However, there was no statistical significance shown for: properties of biodiesel, mechanism of esterification, and trans-esterification.

Another very thorough assessment was carried out in Mohrig's three-week inquiry-based project for the synthesis and hydrogenation of disubstituted chalcones (26). Students synthesized and purified a disubstituted chalcone the first week. The second week was based on the regioselective hydrogenation of the chalcones, including analysis by TLC, IR, NMR, and GC-MS. For the final week students presented their data to their peers. The instructor acted as a research facilitator, and asked probing questions to assess understanding. Other students in the section typically added to the discussion for possible interpretations and general laboratory procedures. After input from others in the class on the presentations, the students wrote a formal lab report. Upon completion of the unit, students took an anonymous online survey to reflect their perception of the effectiveness of the laboratory. From the 547 students that participated in the free response survey, only 10% didn't like the experiment, while the aspects that students like the most were: use of spectroscopy (29%), diversity of lab skills (14%), approach allowing time for repetition (10%). The study also addressed issues involved in TA training. Although there was a weekly meeting and most TA's had taught guided-inquiry labs prior to this lab unit, the enthusiasm and amount of preparation influenced the results of this study. Results of questioning the participating students about the TA's showed that 32% of TA's made the student reason through problems on their own, 62% asked questions and "guided" the student in the proper direction, 3% answered all questions and corrected all problems, and 3% of the TAs did not know how to answer the questions.

Although there are many informal observations about the effectiveness of inquiry-based labs summarized in this chapter, there are few examples with formal assessment of the effectiveness. There is clearly a need for further research on the effect on student learning and attitudes toward organic chemistry, as well as a need to investigate TA training and their perceptions, as well as faculty. More research documenting and statistically analyzing aspects of the published guided-inquiry organic chemistry labs would also be helpful.

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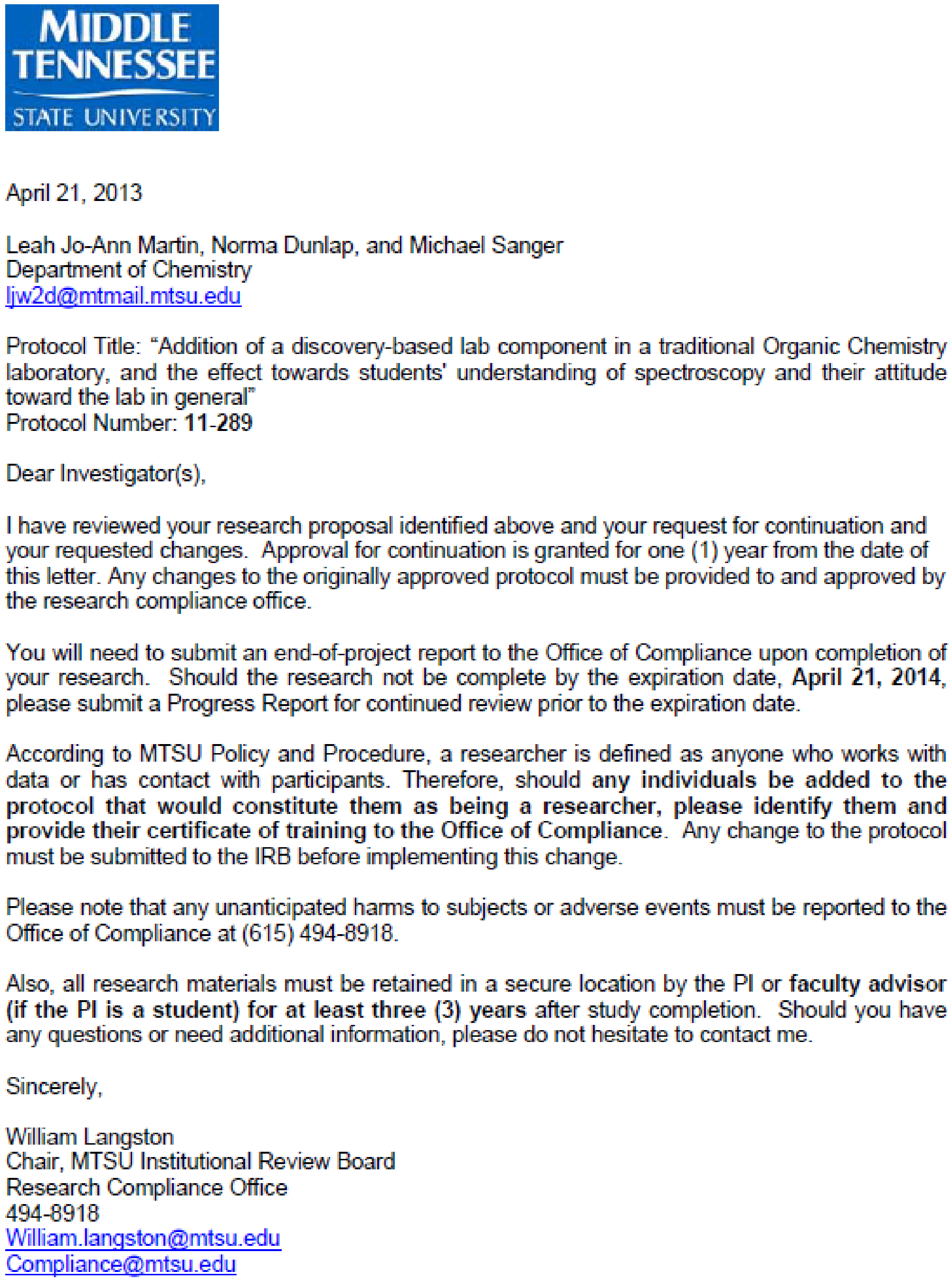
APPENDIX B

Instructional Review Board Approval



MIDDLE TENNESSEE
STATE UNIVERSITY

April 21, 2013

Leah Jo-Ann Martin, Norma Dunlap, and Michael Sanger
Department of Chemistry
ljw2d@mtmail.mtsu.edu

Protocol Title: "Addition of a discovery-based lab component in a traditional Organic Chemistry laboratory, and the effect towards students' understanding of spectroscopy and their attitude toward the lab in general"
Protocol Number: **11-289**

Dear Investigator(s),

I have reviewed your research proposal identified above and your request for continuation and your requested changes. Approval for continuation is granted for one (1) year from the date of this letter. Any changes to the originally approved protocol must be provided to and approved by the research compliance office.

You will need to submit an end-of-project report to the Office of Compliance upon completion of your research. Should the research not be complete by the expiration date, **April 21, 2014**, please submit a Progress Report for continued review prior to the expiration date.

According to MTSU Policy and Procedure, a researcher is defined as anyone who works with data or has contact with participants. Therefore, should **any individuals be added to the protocol that would constitute them as being a researcher, please identify them and provide their certificate of training to the Office of Compliance**. Any change to the protocol must be submitted to the IRB before implementing this change.

Please note that any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

Also, all research materials must be retained in a secure location by the PI or **faculty advisor (if the PI is a student) for at least three (3) years** after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

William Langston
Chair, MTSU Institutional Review Board
Research Compliance Office
494-8918
William.langston@mtsu.edu
Compliance@mtsu.edu

APPENDIX C

Traditional Distillation Lab

Distillation: Separation of a Mixture of Cyclohexane and Toluene

Summary: In this experiment, you will carry out both a simple distillation and a fractional distillation of a mixture of cylohexane and toluene. The distilled product will be analyzed for purity by gas chromatography.

Techniques learned: purification by distillation
analysis by gas chromatography

While crystallization is the most important technique for purifying solid compounds, distillation is the most important technique for purifying two liquid compounds. Distillation is one of the oldest and most important operations of chemistry. The origins of distillation are as old as civilization: it was discovered many years ago that more potent beverages could be made by heating a dilute alcohol solution and condensing the vapors.

Distillation is the process of vaporizing a liquid out of one container and then condensing the vapors back to liquid in a second container, known as the **receiver**. The liquid that is condensed is the **distillate**. Distillation exploits the difference in boiling points between two liquids. The **boiling point** is defined as the temperature at which the vapor pressure of a liquid is equal to the external pressure applied to the surface of the liquid. In an open container this external pressure is atmospheric pressure.

Consider the behavior of a mixture of two miscible (soluble) liquids. According to Dalton's Law, the total vapor pressure exerted by a mixture of two liquids is given by the sum of the partial pressures exerted by the individual components. Using Rauolt's Law, the pressure exerted by the individual components is equal to the product of the vapor pressure of the component times its mole fraction. Combining Rauolt's and Dalton's Law yields:

$$P_T = X_A P_A + X_B P_B$$

where P_T = total pressure
X_A = mole fraction of compound A

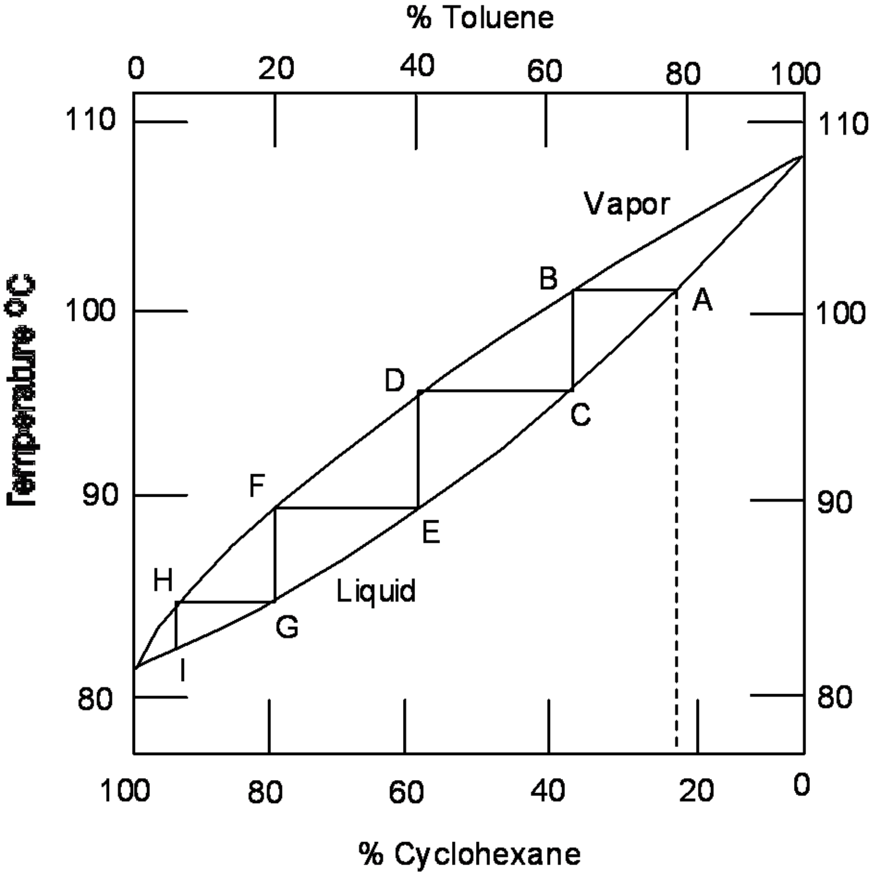
P_A = vapor pressure of compound A

X_B = mole fraction of compound B

P_B = vapor pressure of compound B

When a mixture boils, the composition of the vapor is different from the composition of the liquid. This is the key to distillation. At a given temperature the vapor phase is richer in the more **volatile** component than is the boiling liquid with which the vapor is in equilibrium.

Using the vapor liquid curve shown below consider the separation of a mixture containing 22% cyclohexane and 78% toluene.



Vapor composition diagram for a cyclohexane-toluene mixture

Cyclohexane has a boiling point of 81 °C and toluene a boiling point of 111 °C. When the mixture containing 22% cyclohexane/78% toluene is heated to its boiling point of 101 °C (point A), the vapor contains 35% cyclohexane/65 % toluene (point B). If this vapor is condensed, the condensate (point C) would contain 35% cyclohexane/65% toluene. The condensate contains a much higher percentage of the more volatile cyclohexane and the material left in the original flask has been enriched in toluene. The boiling point of the material in the original flask will rise. As the distillation continues the boiling point of the liquid in the flask will continue to rise and approach the boiling point of toluene. If the vaporization process takes place only one time, this process is known as **simple**

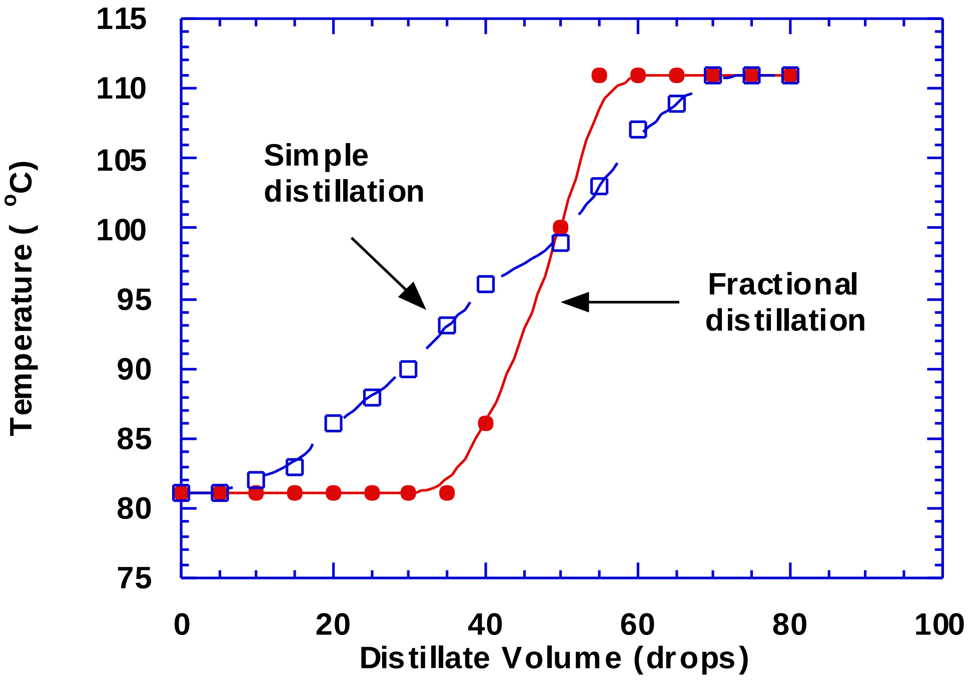
distillation. For samples that contain compounds that differ in boiling point by less than 40 ^{o}C, simple distillation will not result in separation into pure components. Separation of these mixtures requires a technique known as fractional distillation.

Fractional distillation

Let us return to the original condensate and consider what happens to it. *If the lower part of the distilling column is maintained at a higher temperature than the upper part of the column, the condensate will be partially revaporized as it flows down the column.* As the original condensate (point C) is revaporized, the composition of the vapor becomes 58% cyclohexane and 42% toluene (point D). If this vapor were condensed, it would have the composition of point E. If this condensate were vaporized, the vapor (point F) would have a composition of 78% cyclohexane/22% toluene. If this process were repeated over and over again, pure cyclohexane would eventually emerge from the distillation column and be condensed in the receiver vial.

This repetitive process is equivalent to performing a number of simple distillations within the column and is known as **fractional distillation**. With each vaporization/condensation cycle, the vapor phase produced in each step becomes increasingly richer in the **more** volatile component. The condensate that flows down the column correspondingly becomes richer in the **less** volatile component. Each of these vaporization/condensation cycles is known as a **theoretical plate**. A measure of the efficiency of a fractional distillation column is **HETP** or **height equivalent to a theoretical plate** and is the vertical length of a column required to achieve one theoretical plate. Using a column of sufficient length, packed with an appropriate material, it is possible to separate mixtures whose component boiling points differ by only 2-3 ^{o}C degrees.

During a fractional distillation of a binary liquid mixture, the head temperature (the temperature at the top of the column) ideally should rise to the normal boiling point of the more volatile component and remain there until that component is completely removed. The head temperature may then drop somewhat, indicating that the more volatile component has been removed. If additional heat is provided, the less volatile component will begin to distill, and the now higher head temperature should remain constant until all of the second component has distilled. A distillation should not be stopped if this small drop in temperature occurs. In fact, your experimental results are likely to look different from the ideal graph on the next page due to poor insulation in the microscale apparatus.



In a laboratory fractional distillation apparatus, a column is packed with an inert material on which successive condensations and vaporizations take place. You will be using a copper sponge.

To ensure a successful distillation a number of details must be considered. One of the most important considerations in a fractional distillation is the rate at which distillation occurs. The tendency is to distill the sample as fast as possible. This impulse must be resisted. Implicit in the above discussion is that equilibrium is established between the vapor and liquid phase. If the distillation is carried out too fast, liquid-vapor equilibrium will not be established and the components will not separate cleanly. Another consideration to ensure a smooth distillation is to include a boiling chip in the distilling flask. Sometimes local superheating occurs that causes the solution to bump or splash. To avoid this, the use of a boiling chip is required. The boiling chips are made of a porous inert material. Small air bubbles trapped in the pores prevent superheating by providing nuclei for bubble formation. A third consideration is the size of the distilling flask. This flask should be no larger that twice the volume of liquid to be distilled. Use of a larger flask will result in excessive loss of material due to the large vapor volume and high glass surface area. The additional glass area also acts as a condenser making it difficult for the material to rise in the column. A final consideration is the correct placement of the thermometer. The thermometer should be positioned where the top of the mercury-containing bulb is level with the bottom of the side arm leading to the receiver vials. If the position of the thermometer is incorrect the measured boiling point will be wrong. If it is too high, the temperature that is shown on the thermometer will be low. You will be measuring the temperature of the air rather than the temperature of the vapor phase.

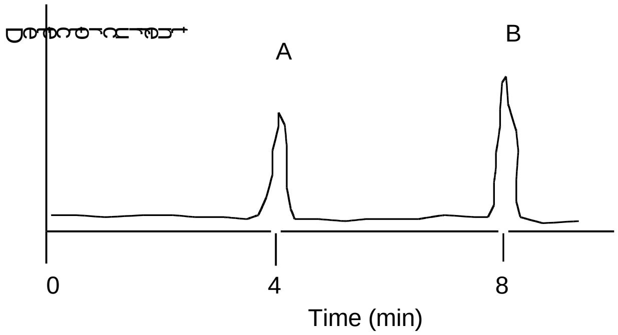
Finally two safety precautions must be observed. First **never** heat a closed system. As you heat the apparatus, the gas within the system begins to expand. As the gas continues to expand there may be a rapid increase in pressure that could result in the system coming apart. Therefore in adding the copper sponge to the fractionating column, make sure you do not plug the column. A second consideration is to always stop a distillation before the flask becomes completely dry. When the flask is dry, its temperature may rise quickly. Some organic substances, especially alkenes and ethers, may contain peroxides as impurities that could be concentrated and can explode when dry.

In today's experiment you will be conducting two distillations. In the first you will separate a mixture of toluene and cyclohexane to become familiar with the technique of distillation. In the second distillation you will carry out a fractional distillation. You will graph both distillations, and will analyze the collected fractions by gas chromatography.

Gas Chromatography (GC)

This is the first example of chromatography that you will be exposed to in this lab. Other types that will follow are thin-layer chromatography (TLC) and column chromatography.

<u>All of these follow the same basic principle for separation of mixtures</u>: the mixture is dissolved in a mobile phase (either a gas or liquid) and passed through a stationary phase (the column). Based on physical properties of the components of the mixture, such as boiling point and polarity, the components will pass through the stationary phase at different rates. Thus, they elute from the column at different times. The gas chromatograph instrument consists of a high-temperature injection port (where the sample is volatilized), an oven that contains a coiled metal or glass column packed with firebrick, and a detector at the end of the column. This column is an inert support for a low molecular-weight polymer such as Carbowax (the stationary phase). The mobile phase is helium gas, which acts as a carrier for the mixture being analyzed. In practice, a small (1µl) dilute sample is injected. It dissolves in the Carbowax, and the volatile components evaporate and are swept along the column by the helium, where they redissolve in the Carbowax, reevaporate and are carried further along the column until they eventually reach the detector, an electrically heated tungsten wire. When an organic sample contacts the wire, it heats up, affecting the electrical resistance. The chromatogram records the current vs. time. The time for a particular component to elute is the <u>retention time</u>. The integrator then integrates the area under the signal to give relative percentages of each component of the mixture. An example of the separation of a mixture of cyclohexane and toluene is shown below.



The retention time of component A is 4 minutes, and of B is 8 minutes.

Procedure

Note: Half of the lab will do the simple distillation and the other half will do the fractional distillation. Your TA will tell you which one you are doing (the only difference is the use of a Vigreaux column for the fractional distillation). You will find a group and share data for whichever distillation you are NOT doing.

Distillation

1. Obtain a mixture of cyclohexane/toluene (5.0 mL) from the front of the lab and transfer it to the 10 mL round bottom flask. Add a boiling chip to the flask and assemble the distillation apparatus as directed by your TA. For the fractional distillation, assemble the distillation apparatus with a Vigreaux column inserted between the distillation flask and the distillation head. Placement of the thermometer bulb is critical in this experiment as discussed earlier. The bulb of the thermometer should be just below the sidearm of the distilling head.

2. Lower the distillation apparatus into the sand bath to heat the sample. The receiver vial should immersed in an ice bath to cool the distilled vapors and the end of the condenser side arm should be inside the receiver vial to minimize the vapors released to the lab.

3. Continue heating the sample until distillation begins. Two conditions are required for a successful experiment:

a. The distillation rate should be slow in order to ensure the temperature registering on the thermometer does not lag behind the vapor temperature. A steady distillation rate of 3-4 drops per minute is good. If a drop of liquid cannot be seen suspended from the end of the thermometer, the rate of distillation is too fast. The rate of distillation may be controlled by adding or removing sand from around the flask with a glass stirring rod (NOT METAL and NEVER use a thermometer!!).

b. A constant distillation rate. Do not continually adjust the distillation rate once you have started collecting data.

4. Record the temperature as every other drop distills (note: you are counting the drops coming out the end of the vacuum distillation adapter, NOT the drops on the thermometer). Continue collecting data until approximately 0.5 mL are left in the round bottom flask. (Note: approximately 21 drops/mL) To stop the distillation raise the apparatus out of the sand and allow it to cool. After the data has been collected, plot the drop number vs. temperature.

You should have three vials ready and labeled (F1-F3). Collect the distillate as it condenses into receiver vials. Discard the first few drops as the temperature is rising (this is called the fore-run). Once the temperature is constant (within about 3-4˚C), collect the first fraction into a vial labeled F1. When the temperature drops and then begins to rise, switch to F2 until the temperature levels out again. Once it has leveled out, collect the drops into a vial labeled F3.

5. Take a GC trace of vials F1 and F3. Your TA will explain how to use the GC. The GC trace should have two peaks: one for cyclohexane and one for toluene. It will also integrate the area under each peak. To calculate your % composition, add up the total area for the cyclohexane and toluene peaks and calculate the % of each. Identify the components based upon the GC trace (and bp), and report the percent composition of the two components. Turn in the GC trace of F1 and F3 with your lab report.

Lab Report: use the worksheet provided, which includes the following sections:

I. Introduction: a summary of the concept of distillation and the purpose of the lab, including structures of toluene and cyclohexane and the bp of each one

III. Procedure and Observations (parts I, II and the Procedure part of III should be completed before lab). Observations should include any deviations from procedure and relevant observations-this is what you will use to write up your results. The observations are recorded during lab and don't need to be complete sentences.

IV. Results: This is basically a summary of your observations and should be written after the lab, in complete sentences. This should include the graphs for the simple and fractional distillations (drop # vs. temperature), GC trace and percent compositions of F1 and F3.

V. Conclusions: a brief summary of what you learned from this lab, and discussion of the purity of recovered cyclohexane and toluene, including what you may have done to improve the purity.

Bring the worksheet to lab and turn in the completed worksheet as your lab report for this experiment.

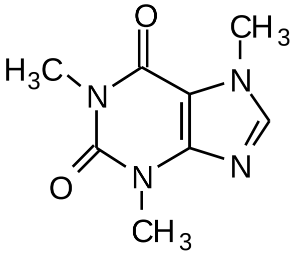
APPENDIX D

Traditional Column Chromatography Lab

Isolation of Caffeine by Column Chromatography

Summary: Caffeine will be isolated from tea using liquid-liquid extraction and then purified by column chromatography
Techniques: liquid-liquid extraction, TLC and column chromatography

The isolation of compounds with pharmacological properties from plants is a vital area of activity in the pharmaceutical industry. Many of these isolations are based on the differences in solubility of various compounds for different solvents. In today's experiment you will first take advantage of caffeine's solubility in water to extract it from either instant tea or tea leaves and then separate it from other water soluble compounds such as tannins by extraction with an organic solvent. A final purification of the caffeine utilizing column chromatography will be carried out and the purity of sample determined.

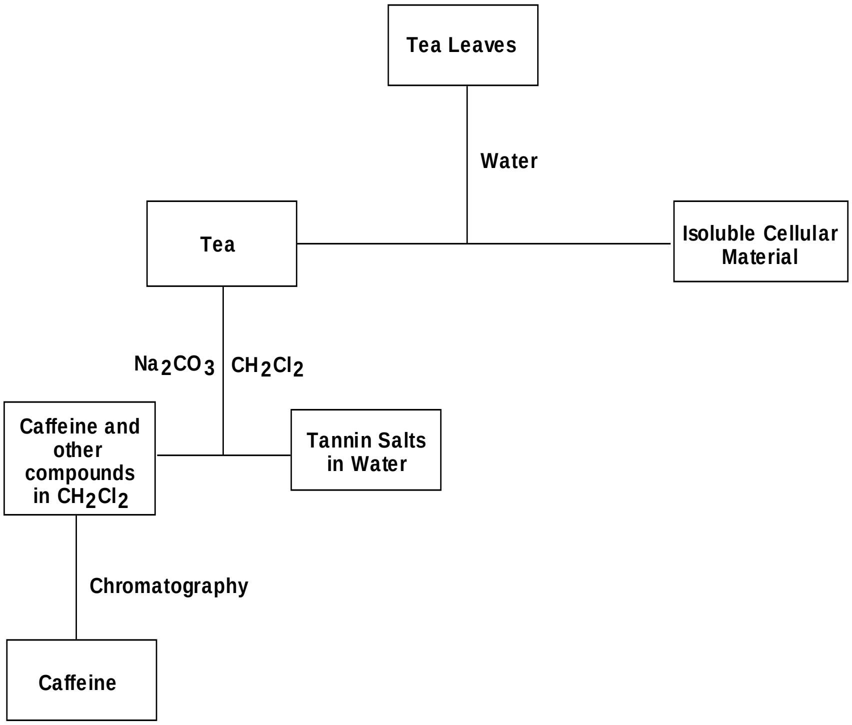


Caffeine

Caffeine is one of the most widely used drugs in the world. Its role as a stimulant has been known for thousands of years. However the pure compound was not isolated until 1821 by Pierre Jean Robiquet. It is present in coffee beans, tea leaves, and cocoa beans, where it may represent up to 5% of the mass. It has a number of physiological effects including stimulating respiration, heart rate and the central nervous system. In addition it acts as a vasodilator and a diuretic. Many popular stay-awake preparations contain caffeine as the main ingredient. Overuse of caffeine has several side effects such as insomnia, restlessness, headaches, and muscle tremors.

Caffeine is a member of a class of bitter tasting compounds known as alkaloids. Alkaloids are compounds isolated from plant sources that possess a basic nitrogen. Many alkaloids have pharmacological properties and include nicotine, cocaine, morphine, and strychnine. Structurally caffeine belongs to a class of nitrogenous bases called purines. Other purines include the DNA components adenine and guanine.

Isolation of Caffeine from Tea Leaves



We have already covered two methods of purifying organic compounds: recrystallization and distillation. Today's experiment introduces two new purification techniques, extraction and column chromatography. Extraction is a chemical operation based on the distribution or partitioning of a substance between two phases. The two most common types of extraction are liquid-liquid and solid-liquid. In liquid-liquid extraction a sample is placed in a mixture of two immiscible (insoluble) solvents. In many cases the two solvents are water and some organic solvent such as ether or methylene chloride. The substance will partition itself based on its relative solubility in the two solvents. A measure of this distribution is known as the partition coefficient (K).

$$K = \frac{S_o}{S_w} = \frac{C_o}{C_w} = \frac{\text{solubility of X in organic layer (g/mL)}}{\text{solubility of X in water layer (g/mL)}}$$

For example, the solubility of caffeine in water is 2.2 mg/mL at 25 ^{o}C and in dichloromethane (methylene chloride) is 89 mg/mL. The partition coefficient is therefore 40. Knowledge of the distribution factor allows one to determine the efficiency of a given extraction. As an example suppose 50 mg of caffeine was removed from 1 g of tea leaves into 50 mL of water during an initial extraction process. We now wish to purify the caffeine by extracting it into 20 mL of dichloromethane. How much of the caffeine is transferred to the dichloromethane layer and how much remains in the original water layer? If X is the caffeine extracted into the dichloromethane layer then 50 - X is the caffeine remaining in the water layer.

$$K_D = 40 = \frac{\frac{X}{20}}{\frac{50 - X}{50}}$$

Solving for X reveals that 47.1 mg of caffeine is transferred to the dichloromethane layer while 2.9 mg remains in the water layer.

There are a number of practical considerations in picking an extraction solvent. The extracting solvent must **not** react chemically with the components of the mixture. Also the extracting solvent must be insoluble in the original solvent. The extracting solvent must selectively remove the desired compound from the original solution, and finally the solvent must be easily removed from the solute.

In addition to liquid-liquid extraction, another form of extraction is solid-liquid in which a solid is treated with a solvent in a beaker or Erlenmeyer flask followed by filtration of the extracted solvent from the solid. This is the procedure for the first extraction of caffeine from the tea leaves.

Even after the second extraction, the isolated caffeine is still not pure. This can be seen in the slight green tinge of the isolated caffeine, as pure caffeine is a white crystalline solid. Therefore the caffeine requires at least one more purification step. Several are available. One is recrystallization, and another is **sublimation**. During sublimation a solid samples vaporizes into the gas phase without passing through the liquid phase. Sublimation is not a general technique since many compounds do not sublime. However if sublimation is possible, the purification proceeds with using a solvent or any transfer steps resulting in a reduction in sample loss.

Another more general method is chromatography, particularly **column chromatography**. Column chromatography is similar to thin layer chromatography in that it is a liquid-solid adsorption technique. Its main purpose is to separate the components of a complex mixture. However column chromatography is easily scaled up for preparative applications unlike thin layer chromatography. In column chromatography, a vertical glass tube is packed with a polar adsorbent along with a solvent. Many of the packing materials used for column chromatography are the same as those used for thin layer chromatography. The sample is added to the top of the column; then additional solvent is passed through the column to wash the components of the sample down the column. As the sample descends the column the sample interacts with the stationary and mobile phases. The relative strengths of those interactions determine the speed at which the sample moves down the column. Fractions are collected as the column is eluted and analyzed for the presence of the desired compound. For further information on various mobile and stationary phases see the discussion on thin layer chromatography in the preceding experiment.

Reference

Taber, Douglass F. and Hoerrner R. Scott "Column Chromatography: Isolation of Caffeine" *Journal of Chemical Education,* **1991**, *68(1),* 73.

Procedure

1. Weigh 2.0 g of instant tea and place in a 50 mL beaker.
2. Add 10 mL of distilled water and 1.1 g of anhydrous Na_2CO_3. Mix thoroughly and allow the mixture to sit for 10 minutes.

3. NOTE: liquid-liquid extractions are usually carried out in a separatory funnel, however in this case the mixtures forms intractable emulsions; so for ease of separation this will be carried out in a centrifuge tube - centrifuging the mixture facilitates separation of the layers. Transfer the contents of the beaker to your centrifuge tube and add 2 mL of CH_2Cl_2 (dichloromethane). After putting the snap-cap on the centrifuge tube, extract the caffeine into the organic layer by inverting the tube several times to mix the two layers.
4. In the event that the two layers do not separate cleanly, centrifuge the samples for a few minutes. (MAKE SURE THE CENTRIFUGE IS BALANCED!) Use a pipet to remove the organic layer containing the caffeine and transfer it to an Erlenmeyer flask. Note: dichloromethane is more dense than water, so the organic layer is the bottom layer.
5. Carry out the extraction two more times and combine the CH_2Cl_2 from all three extractions in a small erlenmeyer flask.
6. Dry the organic layer by adding calcium chloride until the liquid is no longer cloudy.
7. Pipet the solution into a 50 mL round-bottom flask (with 19/22 joint) and concentrate the sample to about 0.5 mL under reduced pressure on the rotary evaporator.

Purification by Column Chromatography

8. Preparation of column

Fill the column about 1/2 full with alumina. If available, add a thin layer of sea sand to the top of the column (this step is not crucial). Fill the column with CH_2Cl_2 and let it drain to the top of the sand, while tapping gently to release air bubbles. The solvent level should be just even with the top of the upper layer of sand - if it is too low there will be air bubbles in the column, and if it is too high then your caffeine sample will diffuse up into the solvent rather than go onto the column.

9. Dissolve the caffeine sample in a minimum volume of CH_2Cl_2, SAVE **one drop** in a clean vial for a TLC sample, and add all of the remainder to the top of the column. Allow the liquid to drain to the top of the sand. Rinse the flask with an additional 0.5 mL of dichloromethane and add that to the column, then allow it to drain to the top of the sand.
10. Fill the column with 5% ethyl acetate/CH_2Cl_2. Allow it to drain to the top of the sand, while collecting the eluent into a clean container (small beaker or erlenmeyer).

(Note: you can make the column drain faster by CAREFULLY squeezing air through a pipet bulb placed on the top of the column). Next, fill the column with 10% ethyl acetate/CH_2Cl_2, allow it to drain into a separate container. Repeat with 20%, 40%, and 60% ethyl acetate/CH_2Cl_2. Each container is one "fraction" from the column.

11. Check the purity of the fractions by TLC: prepare a TLC plate with lanes for each fraction and one lane for your crude caffeine. Develop the plate (as you did in the TLC lab) with 5% methanol/ethyl acetate, visualize with UV and identify the fractions containing the caffeine.
12. Combine the fraction(s) containing caffeine and remove all of the solvent under reduced pressure on the rotavap (tare your flask first!). Determine the weight of caffeine isolated and calculate the mass percent caffeine in tea.

Lab Report:

Your report for this experiment should include:

I. Introduction: a summary of the concepts of extraction and column chromatography and the purpose of the lab

II. Table: structure and properties of caffeine

III. Procedure and Observations (parts I, II and the Procedure part of III should be completed before lab). Observations should include any deviations from procedure and relevant observations-this is what you will use to write up your results. The observations are recorded during lab and don't need to be complete sentences. This may include drawings of TLC plates, although this can be by calculations.

IV. Calculations: Rf calculations and percent recovery.

V. Results and Conclusions: This is basically a summary of your observations and should be written after the lab, in complete sentences. This should include Rf value for caffeine, as well as column results (which fractions contained caffeine), and the percent recovery of caffeine from the tea.

APPENDIX E

Traditional Spectroscopy Lab

Infrared Spectroscopy and NMR to Identify an Unknown

Techiques: IR spectroscopy, NMR spectroscopy

Purpose: To identify a simple unknown compound using ^{1}H and ^{13}C NMR, and IR. First, use IR to identify which type of functional group is present in both a solid and a liquid unknown. Then, obtain specific information on number and type of unique carbons (^{13}C NMR) and connectivity and types of protons (^{1}H NMR) to assign a full structure to the liquid unknown.

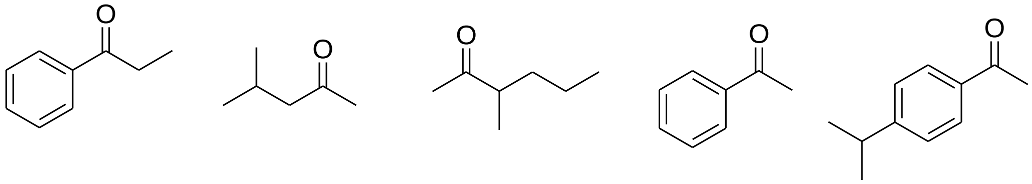
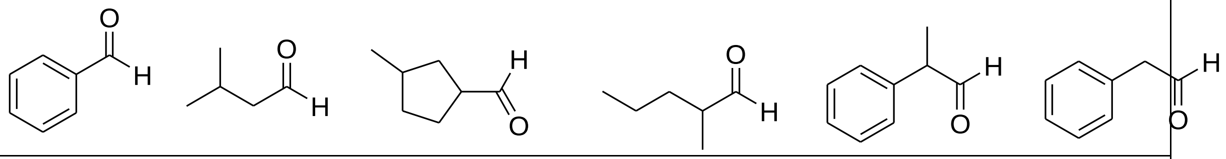
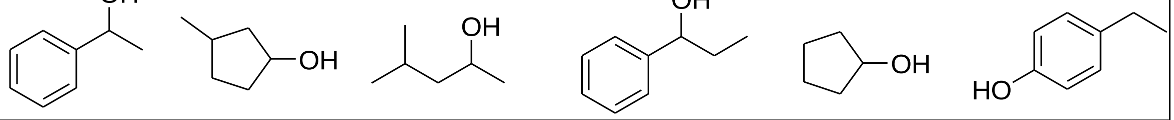
Infrared spectroscopy is one of the fastest methods for determining information about molecular structure on a wide variety of samples. It is especially useful for identifying functional groups present in a molecule. However, the pattern of absorptions in each spectrum is as unique as a fingerprint for a given compound, so it can also be used as an indication of whether a reaction formed the desired product. Infrared data is measured in units of frequency $\underline{\nu} = \nu/c$, conventionally given in units of cm^{-1} (although physicists use μm). We will be using Fourier-transform instruments, which collect data from 400-650 cm^{-1} all at once, then transform it to a y scale expressed in % transmittance (0-100) or absorbance (0-1). Next semester, you will be responsible for measuring IR spectra of many of your products to turn in with your laboratory reports.

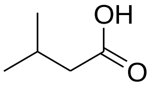
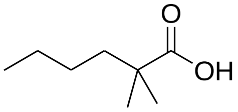
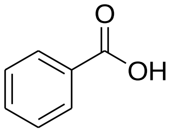
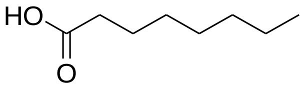
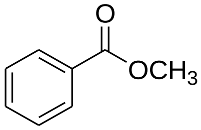
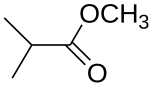
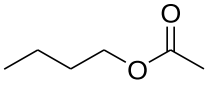
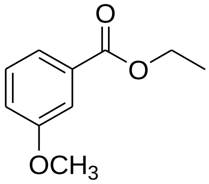
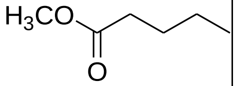
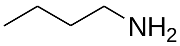
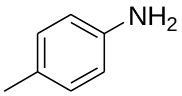
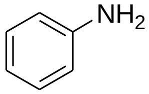
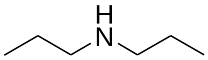
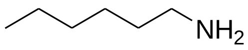
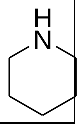
IR Interpretation. The identity of a functional group and its environment within the molecule determine the position and intensity of absorption bands. Generally, there are 4 major regions of the spectrum to look for clues (Table 2-7 in Solomons and Fryle).

Sample Preparation. One of the great things about IR is that it can be measured on samples in nearly every physical state. Samples as diverse as industrial stack gases to thin films of paint on aluminum cans may be analyzed quickly. The two IR's in the organic instrument room are equipped with standard sample compartments for measuring solids and liquids, the normal physical states for our products. *Liquids.* Normally, liquids are measured neat (without solvent) or in solvent between NaCl or AgCl plates, which do not absorb in the IR region of interest. However, salt plates are hygroscopic or light sensitive and fragile.

In our lab, both IR's have a special apparatus called a single-bounce attenuated total reflection apparatus (ATR) to collect data for liquid and some solid samples. The ATR is very convenient because it almost completely cuts out sample preparation. The principle behind this technique is based on the use of mirrors to direct the IR beam into the crystal at an angle that causes it to bounce off onto another mirror that delivers the beam to the detector. The geometry of the apparatus is such that the energy of the IR beam is affected by absorptions in the thin layer just above the crystal face (~1 μm). If the IR beam "sees" a sample of air (as it does in the background), absorbances due to water vapor and perhaps CO_2 (if you breathed on the sample) are present. If something else is in close contact with the crystal, absorbance due to that material will be detected.

Since many of the functional groups that are easily identified by IR will not be discussed thoroughly until CHEM 3020, we will only consider spectra for the following **functional groups** in this lab: *alkanes, aromatics, alkynes, alkenes, alcohols, nitriles, aldehydes, and ketones, amines, and esters*. Your unknown will be one of the structures given below:

KETONES: 
ALDEHYDES: 
ALCOHOLS: 
CARBOXYLIC ACIDS:

   
CARBOXYLIC ESTERS:     
AMINES:      

Procedure: NMR spectroscopy

1. Find your assigned liquid unknown (make sure you use the same sample number for both the IR and NMR!!!). Go to the NMR room (back of DSB 216). In order to save time, NMR samples of the unknowns have already been prepared for you in $CDCl_3$. Add your sample to the queue. The only change in procedure from the elimination laboratory is that you will push the [carbon/DEPT 135 and proton] buttons instead of only [carbon]. Each sample normally takes <10 minutes to run and print.

3. Use Tables 14.5 and 14.5 (Smith) to assign peaks to protons in your simple organic liquid unknown. Recall that $CDCl_3$ has residual protons and these appear at 7.26 ppm in the proton spectrum, along with the TMS reference peak at 0.00 ppm. In ^{13}C NMR, the solvent shows up in a large triplet at 77.00 ppm.

4. You can verify whether you have assigned your unknown correctly by modeling the ^{1}H and ^{13}C spectra using ChemDrawUltra (instructions also provided previously).

IR Instructions

Don't look into the IR beam, since it can damage your eyes.
Taking an IR spectrum

1. Take a background spectrum by selecting the **Background** key with an empty compartment and clean ATR crystal (this will most likely be done by your TA and does not need to be repeated for each sample).

START HERE FOR CHEM 3011 and 3021.

2. Place one drop of liquid on the ATR crystal. (be careful not to breathe into the sample compartment).
3. Collect the spectrum by clicking on the "collect sample" icon.
4. Select the "top" spectrum from the grid at the bottom of the screen.
5. AT THIS POINT, THE NEXT USER CAN CLEAN THE ATR AND PLACE HIS/HER SAMPLE ON THE CRYSTAL
6. Choose **Peak label.**
7. **Print** (twice if needed). The next user is ready to go.

NMR Interpretation Hints

In order to have more time for interpretation, the NMR samples of each liquid unknown are already prepared. Each team will set up ^{1}H and ^{13}C NMR and DEPT 135 experiments. This will take about 10 minutes once the sample is in the NMR. After this, you should begin to interpret the spectra.

All the liquid unknowns have simple structures (and one functional group). The best approach to fully solving the unknown structure is to:

1. Look at the IR for clues about functional groups.
2. Count the number of unique carbons in the ^{13}C NMR spectrum and get an idea about the environment of the carbons (e.g. aromatic, carbonyl, etc.). Remember that the

DEPT spectrum will have NO quaternary carbons, CH/CH_3 will point up and CH_2 will point down. NOTE: the instrument doesn't really "know" which signals are CH/CH_3 and which are CH_2 so it automatically phases the largest signal "up". SOMETIMES the largest signal is actually a CH_2, so the DEPT will be inverted. This usually occurs when there are long alkyl chains. Think about what makes sense...for instance if you see one "down" signal and many "up" signals in the alkyl region, you may have an inverted DEPT.

3. Look at the ^1H NMR spectrum to get an idea about the environment of the protons (from ppm values), connectivity (from multiplicity), and ratios of protons in each signal (from integration) in the molecule (see below for brief descriptions).

There are a few tricky aspects for the beginner in analyzing an unknown.

1. *Symmetrical molecules*. Some molecules will appear to have fewer carbons and protons (usually 1/2) than expected due to symmetry of the molecule. For example, diethyl ether, $CH_3CH_2OCH_2CH_3$, shows 2 carbons in the ^{13}C spectrum and 2 groups of protons in a ratio of 2:3 at 3.5 and 1.1 ppm, respectively. This would first lead one to suspect that the molecule was ethanol, but no alcohol proton is present. This could be confusing without the IR spectrum, which shows that there is an ether C-O stretch and no alcohol O-H stretch.
2. *Extra peaks:* Don't forget that NMR solvents are deuterated. In ^{13}C samples run in $CDCl_3$, deuteriums split the carbon into a large triplet at 77.00 ppm. Residual protons in the NMR solvent show up in ^1H NMR as well. For example, TMS protons appear at 0 ppm, and residual $CHCl_3$ in $CDCl_3$ results in a singlet at 7.26 ppm.
3. *Missing peaks.* Occasionally, peaks will be partially cut off on the high ppm (downfield) side of the spectrum in proton or carbon (ask your TA if you think that it has happened to you). Note that unusually broad peaks can indicate the presence of exchangeable protons such as those in alcohols or carboxylic acids.

Chemical Shifts:

The value on the x axis (chemical shift) for each peak or group of peaks is a good indicator of the proton (or carbon) environment in a molecule. Apply the usual rules to interpreting ^1H and ^{13}C spectra. Remember, protons near two functional groups are affected by both (e.g. in benzyl bromide, $C_6H_5CH_2Br$, the methylene protons appear at 4.10 ppm, much further downfield than expected for either benzylic or bromoalkane protons.

Integration:

Integrals will be reported on your spectrum. You will usually need to scale this so that the smallest signal = 1 proton.

Multiplicity:

If the splitting (s, d, t, m, etc.) is not clear in your ^1H spectrum, **ask your TA to expand the region of interest** in the spectrum so that you can look at it more closely. Multiplicity is useful for determining connectivity (n+1 rule).

Report: Write a normal report, including introduction, procedure, results, and conclusions. The results section should include printouts of: IR spectra, ^1H and ^{13}C NMR spectra of your liquid sample. Include also a ChemDraw Ultra ^1H and ^{13}C model of your liquid unknown. Instead of a discussion section, label the peaks *on the spectra* that support identification of your IR functional group *and* those that support the presence of either an aromatic or aliphatic skeleton. For example, for a peak at 1730 cm^{-1}, write C=O above the peak. **Draw the structure** of the proposed compound **on your NMR spectra** and draw lines from protons in the molecule (drawn on your spectra) to peak or group of peaks in the spectrum. For example, a ^{13}C line at 210 ppm could correspond to a carbonyl carbon (C=O) in a ketone, so draw a line from your proposed ketone structure from the carbonyl carbon to the line at 210 ppm. NOTE: If you just turn in spectra with no analysis by **YOU** (not just ChemDraw Ultra) and no support for your structural assignment, you will **NOT** get a good grade!!

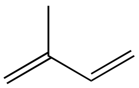
APPENDIX F

Discovery-Based Lab Unit

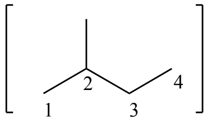
Terpene Isolation Unit

The volatile oils of many plant materials contain terpenes. Terpenes are hydrocarbons that may or may not be oxygenated. They are all formed biosynthetically from isoprene, or 2-methyl-1,3-butadiene, which is one of nature's most common building blocks. The C_5H_8 units are linked head-to-tail and may be linear or cyclic. Many terpenes are chiral, and some have distinctive odors. The most common combinations are monoterpenes, which have two isoprene units, sesquiterpenes, with three units and diterpenes, with four units. Some examples of each, with the isoprene units numbered, are shown below.

Terpenes



isoprene
(2-methyl-1,3-butadiene)



isoprene unit: isoprene units combine in linear or cyclic form; they may contain double bonds or oxygens

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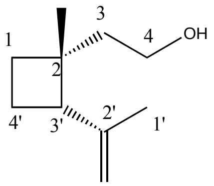
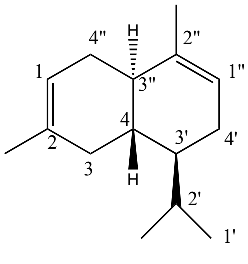
Monoterpene: two isoprene units (C_{10})



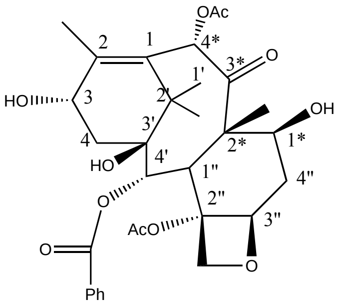
grandisol

Sesquiterpene: three isoprene units (C_{15})



β-cadinene

Diterpene: four isoprene units (C_{20})



Baccatin (precursor to taxol)

In this three-week unit, you will isolate a terpene from a plant source by steam distillation. You will then carry out further purification if necessary. This is to be determined by TLC, and column chromatography if needed. You will then collect IR, NMR and optical rotation of your pure terpene. Assignment of all signals will provide valuable practice in NMR spectroscopy. At the end of the unit, the class will assemble a table including structures, supporting data, and *per cent* recovery.

The materials isolated in this unit will be your starting material for reactions in the next unit, so recovery is important!

Week 1 Pre-lab exercise:

For the chosen plant materials, identify the expected major component(s). Include structure and boiling point. Identify the isoprene units.

Week 1 Steam Distillation of Terpenes from Herbs/Spices

In this experiment, you will use the same steam distillation procedure that is used for the isolation of R-(+)-limonene, although you will use different plant materials. Possible plant materials that will be available include spearmint, caraway, lavender, rosemary and coriander seed. You may also try a different plant or spice, but you will need to have it prepared (ground up) before lab, and also have an idea what to expect as the major component.

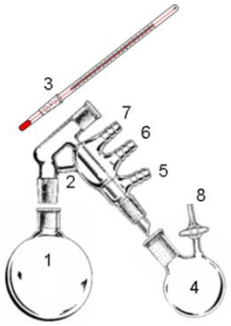
Taken in part from:

http://academic.bowdoin.edu/courses/s05/chem226/laboratory/images/limonene.pdf

Isolation of Limonene by Steam Distillation

This technique basically involves boiling orange peel in water, but to understand why this works we need to delve into distillation theory a bit. Distillation is the process of heating and vaporizing a liquid, and condensing and collecting the vapor. In the distillation of a pure substance the temperature of the system will stay constant (at the boiling point of the liquid) as long as there is both liquid and vapor in the system. **Remember that the boiling point is defined as when the vapor pressure of a liquid equals the applied pressure.** You have already carried out a distillation of a mixture of MISCIBLE liquids.

However, limonene is **not soluble** in water. Therefore when you boil orange peel and water, the limonene droplets are not miscible in the water and the two liquids exert their own vapor pressure independent of each other. These two pressures (from water and limonene) commonly exert pressure against the atmospheric pressure, and when the sum of the two partial pressures equals 760 mm Hg, boiling occurs. So if water boils at 100˚C at 760 mm, and limonene boils at 176˚C at 760 mm Hg, when you are heating a non-miscible mixture of the two, the **combined** pressure exerted by the two liquids will reach 760 mm Hg below the boiling point of either of them! This technique is called steam distillation – used for distilling high boiling materials at a temperature below their boiling points. OF COURSE MUCH WATER DISTILLS THIS WAY TOO, SO YOU STILL HAVE TO SEPARATE THE LIMONENE FROM THE WATER. Since they are not miscible this will be done by liquid-liquid extraction using water and dichloromethane. You will be using a short-path distillation set-up, which will allow for distillation of larger volumes than the one in the yellow kit. A picture is given below:



1	Still pot	2	Short-path distillation head
3	Thermometer	4	Distillate receiver
5	Connection to vacuum or inert gas	6	Coolant inlet
7	Coolant outlet	8	Connection to vacuum or inert gas

Procedure:

1. If you are using seeds, grind them in a mortar/pestle. Leaves can be chopped or put whole into the distillation pot. You will want enough to fill the flask about ½ (approximately 2 cups of leaves).
2. Weigh out plant material.
3. Place the material and 150ml H_2O in a 250ml round-bottom flask, along with a couple of boiling chips.
4. Attach the 24/40 to 14/20 adapter, short-path distillation head and a 100ml flask for collection.
5. Heat to boiling, record temp. and collect as much liquid as possible in 2hrs.
6. Pour the collected water/oil mixture into a separatory funnel. Add 15ml CH_2Cl_2, shake and separate. Collect the bottom (organic) layer. Add a second 15ml to the separatory funnel, shake, separate and combine the two organic layers. Add calcium chloride to dry the organic layer. Transfer to a tared round-bottom flask and evaporate the dichloromethane on the rotavap. Record final weight of oil, calculate % yield of crude oil.
7. TLC: run two TLC plates in different eluting solvents (1:15 ethyl acetate/hexane and 1:3 ethyl acetate hexane). Record in notebook, with Rf values.

Weeks 2 and 3

Purification of Terpenes from Herbs/Spices and Data Collection

There are two main objectives this week. The first is purification, if necessary, of your oil collected last week. Purity should be have been determined by TLC, and if there is more than one major component, you will need to do column chromatography. The second objective is to collect spectroscopic data. You will collect both IR and NMR data. Also, IF your yield of material was < 300mg you may want to distill more material.

This can be set up at the beginning of lab and continue while you work with your oil from last week.

Week 2. PRE-LAB

Build structures of two expected products on ChemDraw, and model the NMR spectra.

Week 2 Procedure:

1. Pack the chromatography column with silica gel (about 2/3 full), and add hexane to wet the silica.
2. Dissolve your crude oil in about 0.5ml CH_2Cl_2. Save one drop for TLC, pipet remaining material onto the column. Rinse the flask with another 0.5 ml CH_2Cl_2 and add to top of column. Drain to top of silica. Fill with hexane and elute 4x with hexane.
3. TLC fractions 1-4. If no compound has come out, you will elute next with ethyl acetate/hexane mixtures, increasing the polarity each time (higher % of ethyl acetate). This will be different for each student.
4. Collect the appropriate fractions, evaporate and record weight.
5. Collect NMR (1H, COSY and 13C if enough compound is present).

Week 3 Procedure:

1. Analyze all NMR data. Propose structures and explain how spectroscopy supports your assignment.

Spectroscopy basics:

IR: Infrared spectroscopy is one of the fastest methods for determining information about molecular structure on a wide variety of samples. It is especially useful for identifying functional groups present in a molecule. However, the pattern of absorptions in each spectrum is as unique as a fingerprint for a given compound, so it can also be used as an indication of whether a reaction formed the desired product. Infrared data is measured in units of frequency $\underline{\nu} = \nu/c$, conventionally given in units of cm^{-1}. We will be using Fourier-transform instruments, which collect data from 4000-650 cm^{-1} all at once, then transform it to a y scale expressed in % transmittance (0-100) or absorbance (0-1).

IR Interpretation. The identity of a functional group and its environment within the molecule determine the position and intensity of absorption bands. Generally, there are 4 major regions of the spectrum to look for clues (Ch. 13 in Smith).

Sample Preparation. One of the great things about IR is that it can be measured on samples in nearly every physical state. Samples as diverse as industrial stack gases to thin films of paint on aluminum cans may be analyzed quickly. The two IR's in the organic instrument room are equipped with standard sample compartments for measuring solids and liquids, the normal physical states for our products. *Liquids.* In our lab, both IR's have a special apparatus called a single-bounce attenuated total reflection apparatus (ATR) to collect data for liquid and some solid samples. The principle behind this technique is based on the use of mirrors to direct the IR beam into the crystal at an angle that causes it to bounce off onto another mirror that delivers the beam to the detector. The geometry of the apparatus is such that the energy of the IR beam is affected by absorptions in the thin layer just above the crystal face (~1 μm). If the IR beam "sees" a sample of air (as it does in the background), absorbances due to water vapor and perhaps CO_2 (if you breathed on the sample) are present. If something else is in close contact with the crystal, absorbance due to that material will be detected.

NMR Interpretation: NMR provides far more information than IR. 13C-NMR, in combination with $DEPT_{135}$ tells you how many unique carbons are in the compound, and of those, how many are C, CH, CH_2 or CH_3. Also, the chemical shift gives valuable information about any functional groups that are present. 1H-NMR tells how many unique hydrogens are in the compound, information about functional groups based on chemical shift, as well as a "map" based on spin-spin splitting. You will need to assign as many signals as you possibly can for your structure.

Extra peaks: Don't forget that NMR solvents are deuterated. In ^{13}C samples run in $CDCl_3$, deuteriums split the carbon into a large triplet at 77.00 ppm. Residual protons in the NMR solvent show up in ^{1}H NMR as well. For example, TMS protons appear at 0 ppm, and residual $CHCl_3$ in $CDCl_3$ results in a singlet at 7.26 ppm.

13C-NMR: Count the number of unique carbons in the ^{13}C NMR spectrum and get an idea about the environment of the carbons (e.g. aromatic, carbonyl, etc.). Remember that the DEPT spectrum will have NO quaternary carbons, CH/CH_3 will point up and CH_2 will point down. NOTE: the instrument doesn't really "know" which signals are CH/CH_3 and which are CH_2 so it automatically phases the largest signal "up". SOMETIMES the largest signal is actually a CH_2, so the DEPT will be inverted. This usually occurs when there are long alkyl chains. Think about what makes sense...for instance if you see one "down" signal and many "up" signals in the alkyl region, you may have an inverted DEPT.

^{1}H NMR: The value on the x axis (chemical shift) for each peak or group of peaks is a good indicator of the proton (or carbon) environment in a molecule. Apply the usual rules to interpreting ^{1}H and ^{13}C spectra. Remember, protons near two functional groups are affected by both (e.g. in benzyl bromide, $C_6H_5CH_2Br$, the methylene protons appear at 4.10 ppm, much further downfield than expected for either benzylic or bromoalkane protons.

Integration:

Integrals will be reported on your spectrum. You will usually need to scale this so that the smallest signal = 1 proton.

Multiplicity:

If the splitting (s, d, t, m, etc.) is not clear in your ^{1}H spectrum, ask your TA to expand the region of interest in the spectrum so that you can look at it more closely. Multiplicity is useful for determining connectivity (n+1 rule).

APPENDIX G

Survey

Survey for Organic Chemistry I labs

Print your name________________________________ Section____________

You do not have to participate in this study to receive the bonus five points, but you must answer ***each*** *question to the best of your ability* ***AND leave nothing blank****. In order to receive credit, I must be able to read your hand writing.*

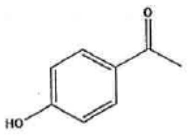
1. Which compound fits the following data?

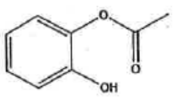
IR: 3400 (broad), 1735, 1610, 1520 cm^{-1}

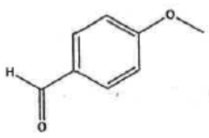
1H-NMR: δ 7.4 (m, 4H), 5.5 (s, 1H), 2.2 (s, 3H)

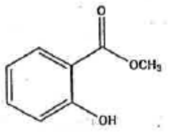
13C-NMR: δ 185, 140, 135, 130, 128, 125, 120, 35

MS: molecular ion at m/z = 152









Explain in detail how you came to choose this answer:

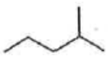
2. For each of compounds, A, B, and C, indicate how many signals would be seen in either the ^1H or ^{13}C-NMR spectrum, whichever is listed. (Just give the number of signals, **NOT** the splitting or chemical shift).

Compound A

1H-NMR

____

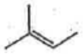
Compound B



1H-NMR

____

Compound C



13C-NMR

____

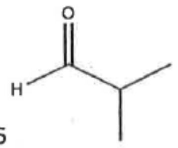
Explain in detail how you came to choose this answer:

A)____

B)____

C)____

3. Which 13C-NMR data matches the following compound?



1- δ 16, 21, 41, 205

2- δ 16, 41, 75

3- δ 16, 41, 205

4- δ 16, 21, 41, 75

Explain in detail how you came to choose this answer:

4. Which 1H-NMR data matches the following compound?

1- δ 1.3 (t, 3H), 1.6 (t, 2H), 3.8 (t, 2H), 2.0 (s, 6H), 2.8 (broad s)

2- δ 1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0, 3.8 (t, 6H), 2.8 (broad s)

3- δ1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0 (s, 3H), 2.1 (s, 3H), 2.8 (broad s)

4- δ 1.3 (t, 3H), 1.6 (m, 2H), 1.8 (t, 2H), 2.0 (s, 6H), 2.8 (broad s)

Explain in detail how you came to choose this answer:

5. Which 13C-NMR data matches cycloheptanol?

1) δ 20, 25, 28, 29, 32, 35, 62
2) δ 20, 25, 32, 64
3) δ 20, 25, 28, 69, 72, 110, 120
4) δ 20, 25, 32, 110

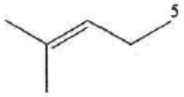
Explain in detail how you came to choose this answer:

6. What would be the splitting pattern for the hydrogen on carbon 4 in a 1H-NMR?

1) doublet
2) triplet
3) quartet
4) multiplet (complex)

Explain in detail how you came to choose this answer:

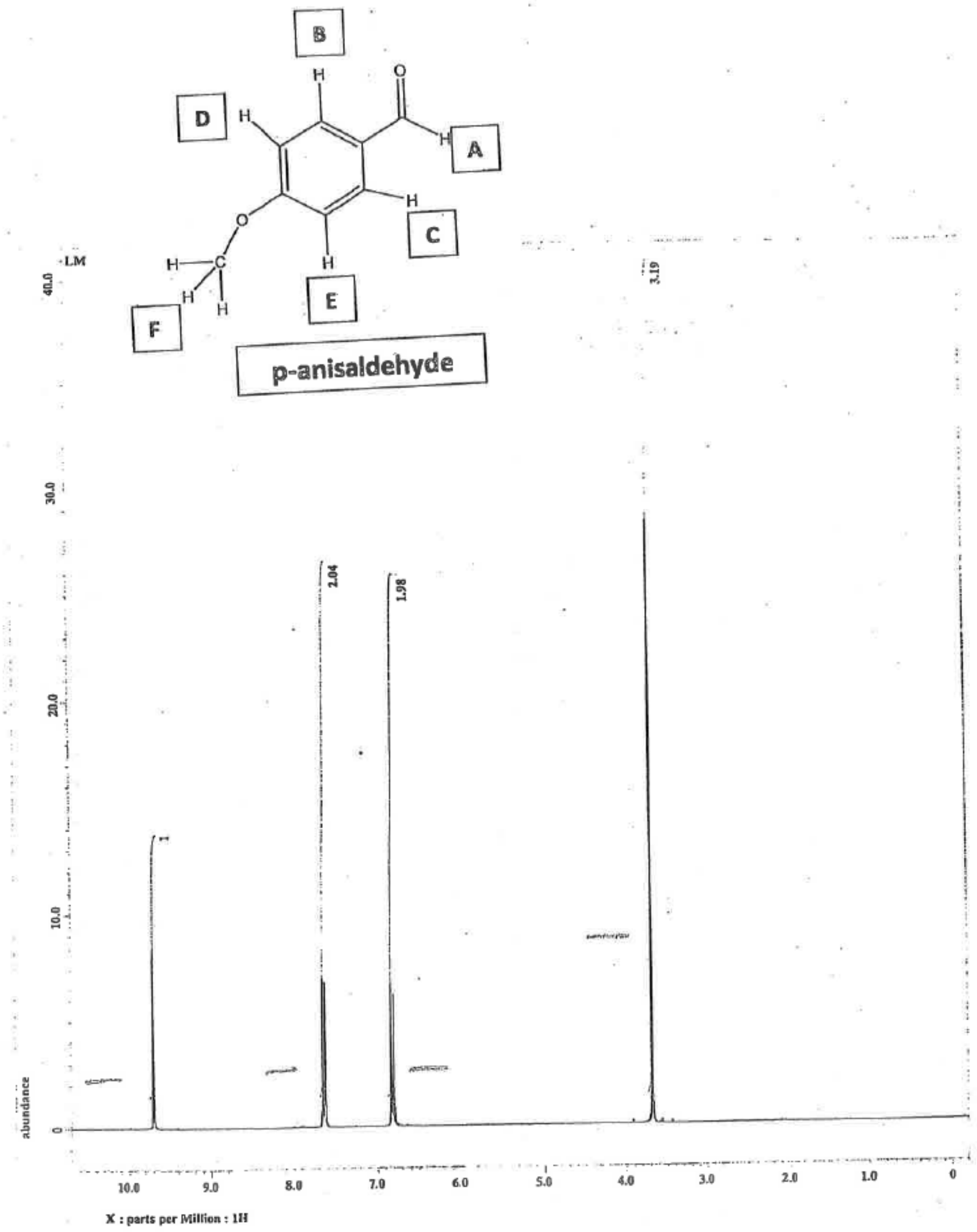
7. What would be the splitting pattern for the hydrogens on carbon 5 in a 1H-NMR?



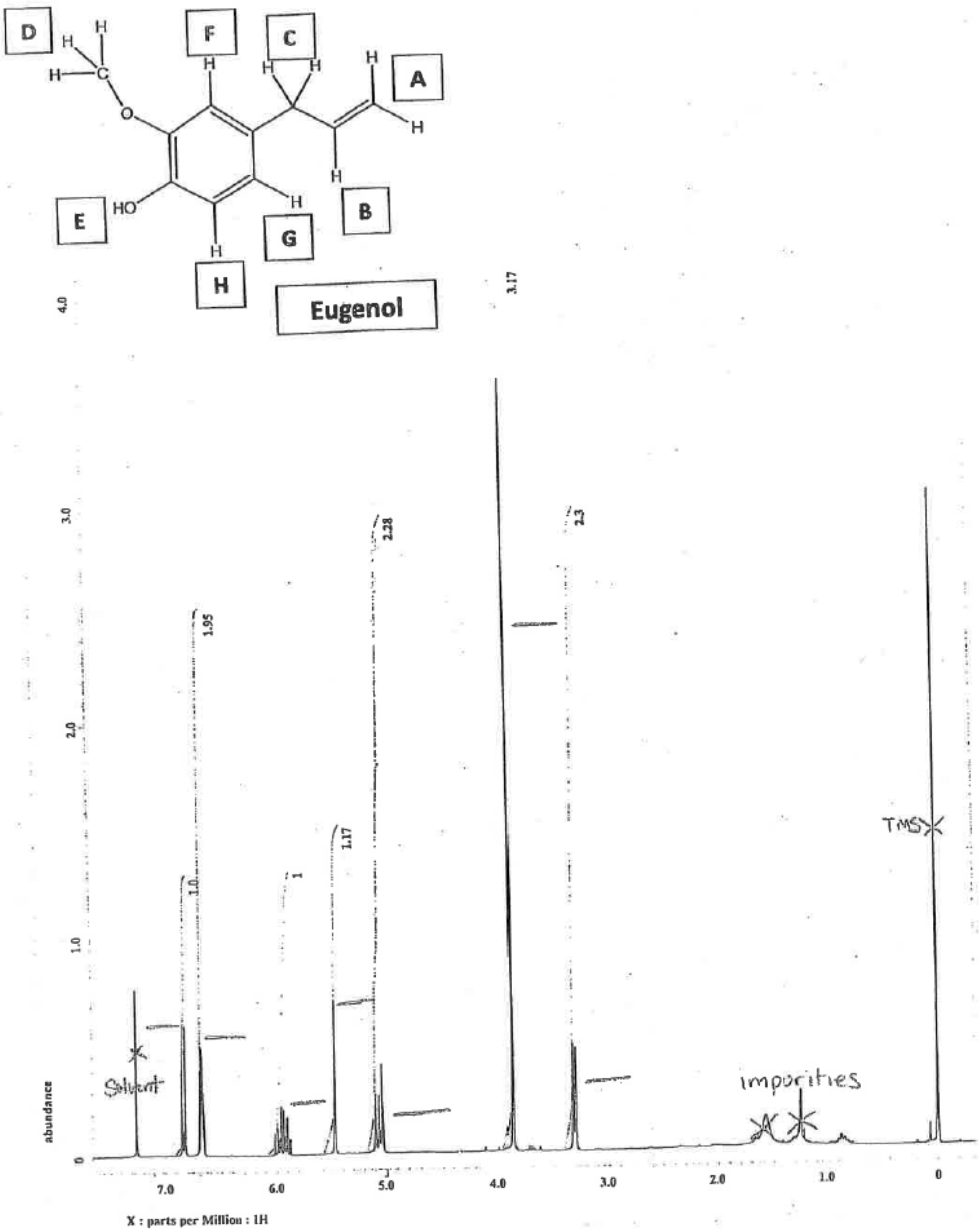
1) doublet
2) triplet
3) quartet
4) multiplet (complex)

Explain in detail how you came to choose this answer:

8. For the 1H-NMR spectrum of the compound drawn below; assign as many individual H's as possible to the signals identified in the spectrum. Put the letter(s) of the identified hydrogen on the line provided next to the signal in the spectrum. (There may be more than one letter for each space)



9. For the 1H-NMR spectrum of the compound drawn below; assign as many individual H's as possible to the signals identified in the spectrum. Put the letter(s) of the identified hydrogen on the line provided next to the signal in the spectrum. (There may be more than one letter for each space)



10. Use the following scale to rank each statement.

A	B	C	D	E
Strongly Disagree (Strongly Dislike)	Disagree (Dislike)	Undecided	Agree (Like)	Strongly Agree (Strongly Like)

____The labs enhanced my ability to interpret ^{1}HNMR.

____The labs enhanced my ability to interpret ^{13}CNMR.

____The labs enhanced my ability to interpret IR.

____ The labs did not help me learn spectroscopy.

____The lab design made interpreting spectra meaningful and gave a sense of purpose.

____How much did you like the overall lab?

____ I found this semester labs to be fun.

____ I found this semester labs to be confusing.

____ I found this semester labs to be frustrating.

____ This semester, lab has increased how much I like science in general.

____ This lab has helped me gain useful knowledge.

____ I have benefited from taking this lab.

____ The labs were easy.

____ The labs were challenging.

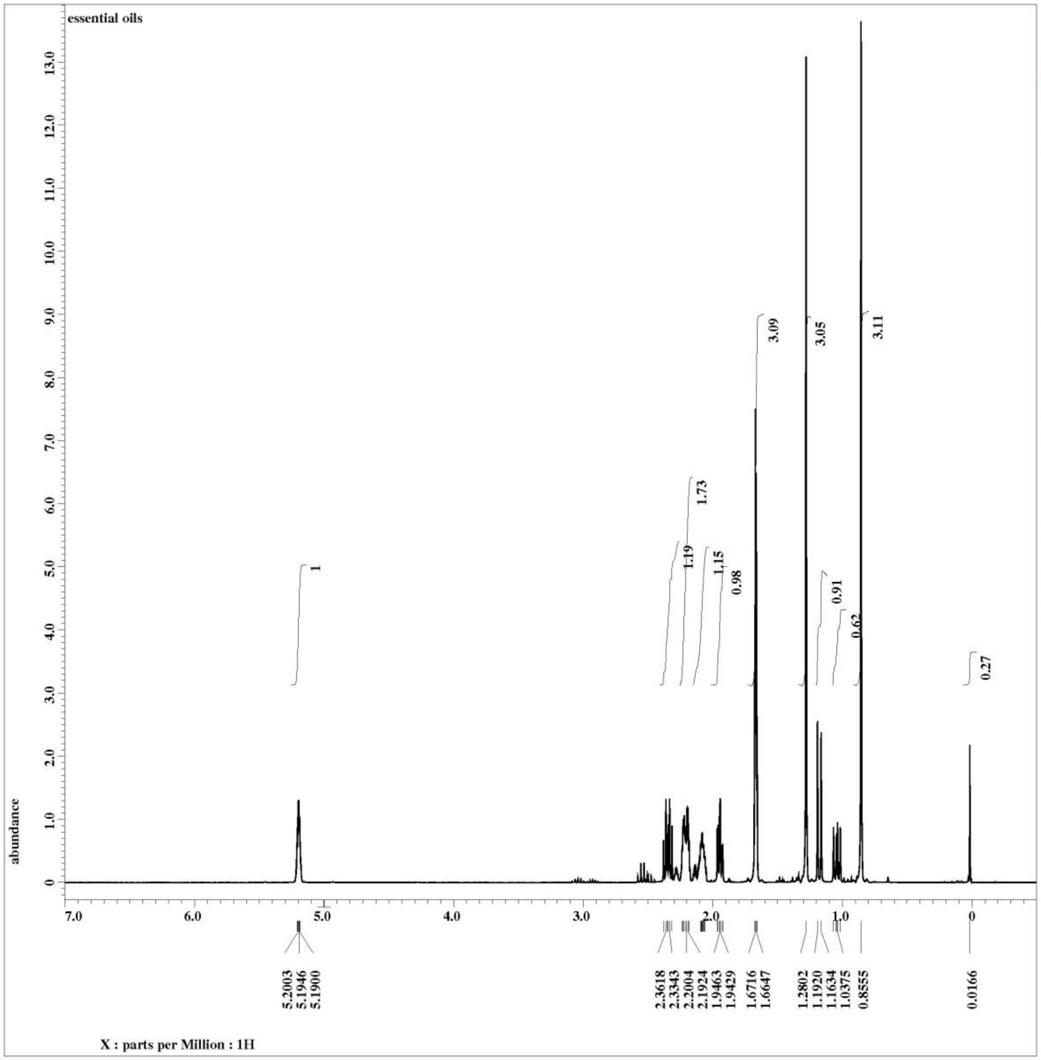
____ I learned interesting new things in lab.

What final grade do you expect to receive for your lab? ____

What final grade do you expect to receive for the course? ____

APPENDIX H

Spectroscopy of Essential Oils



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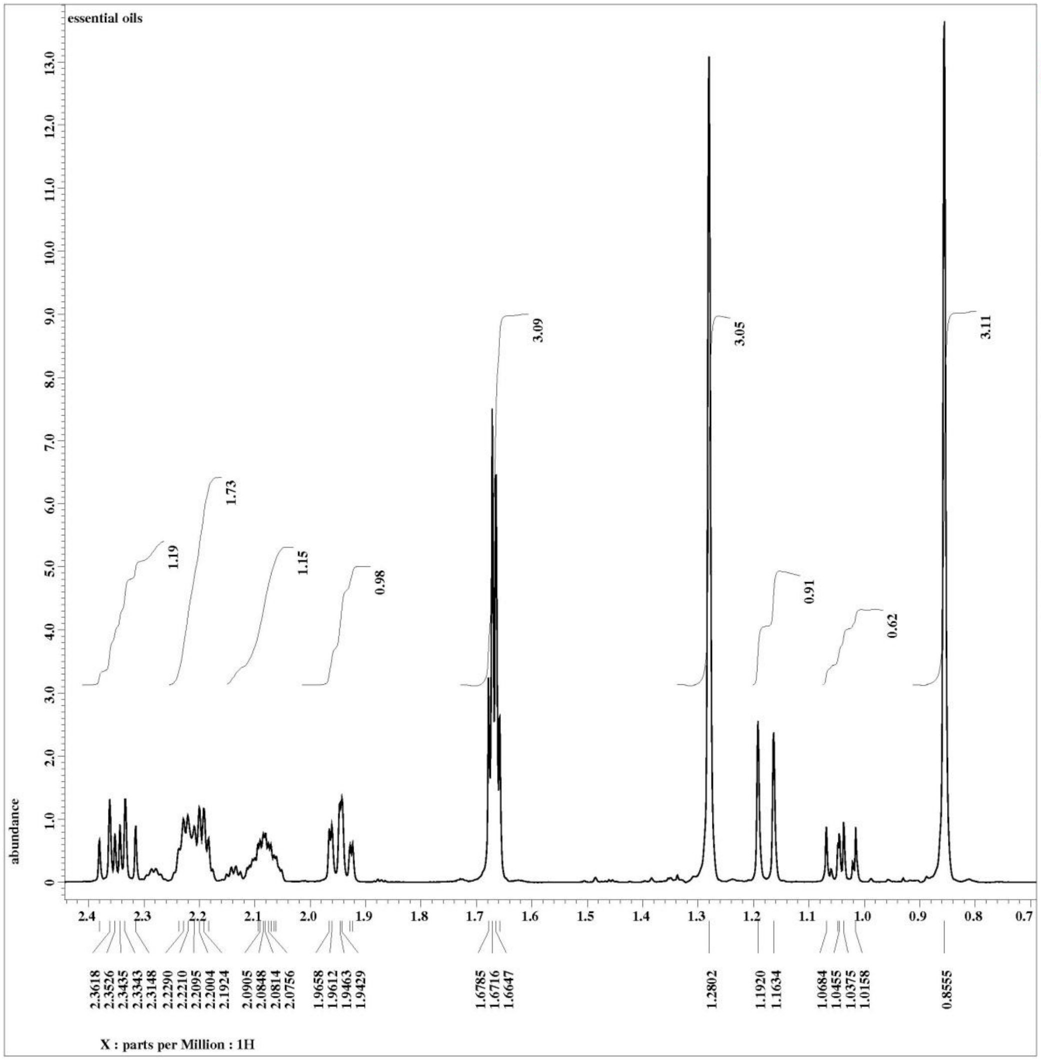

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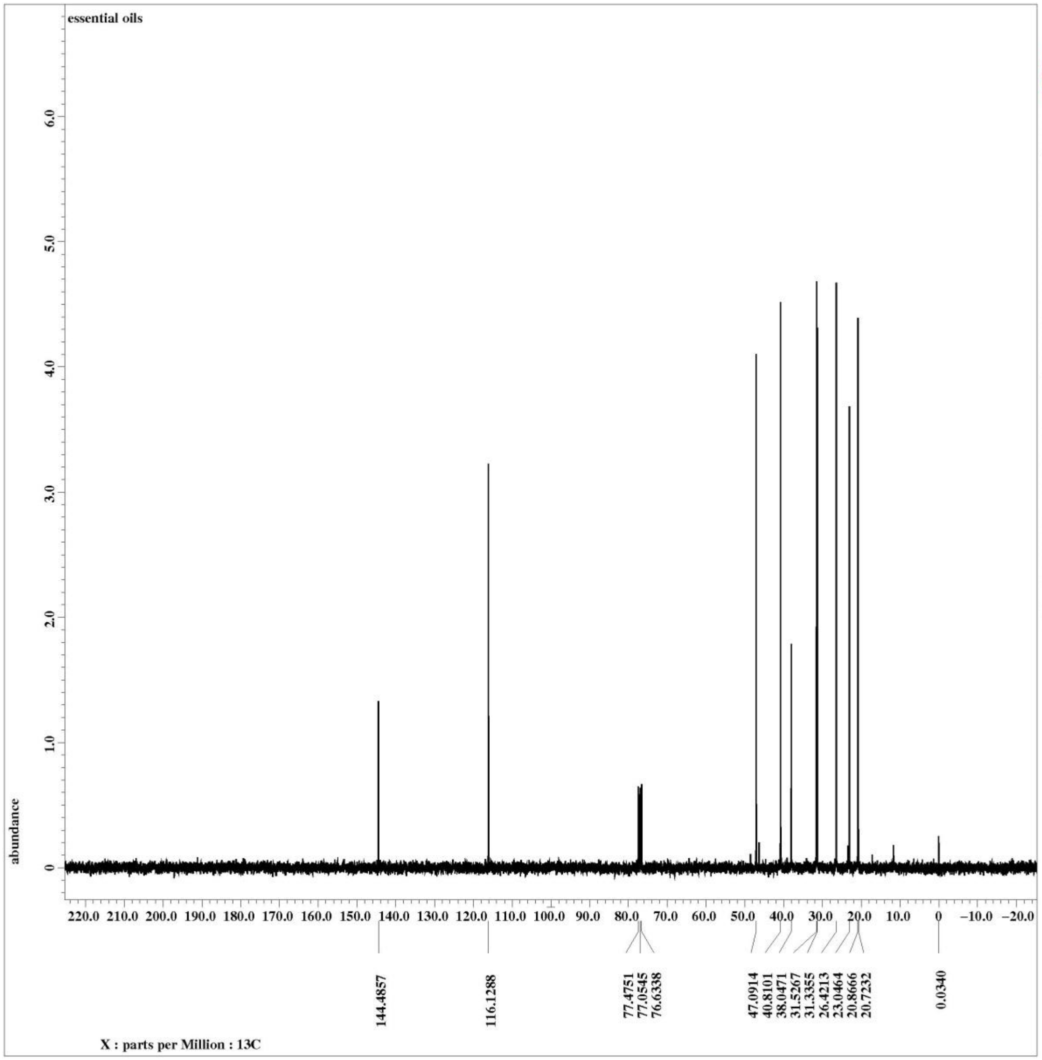

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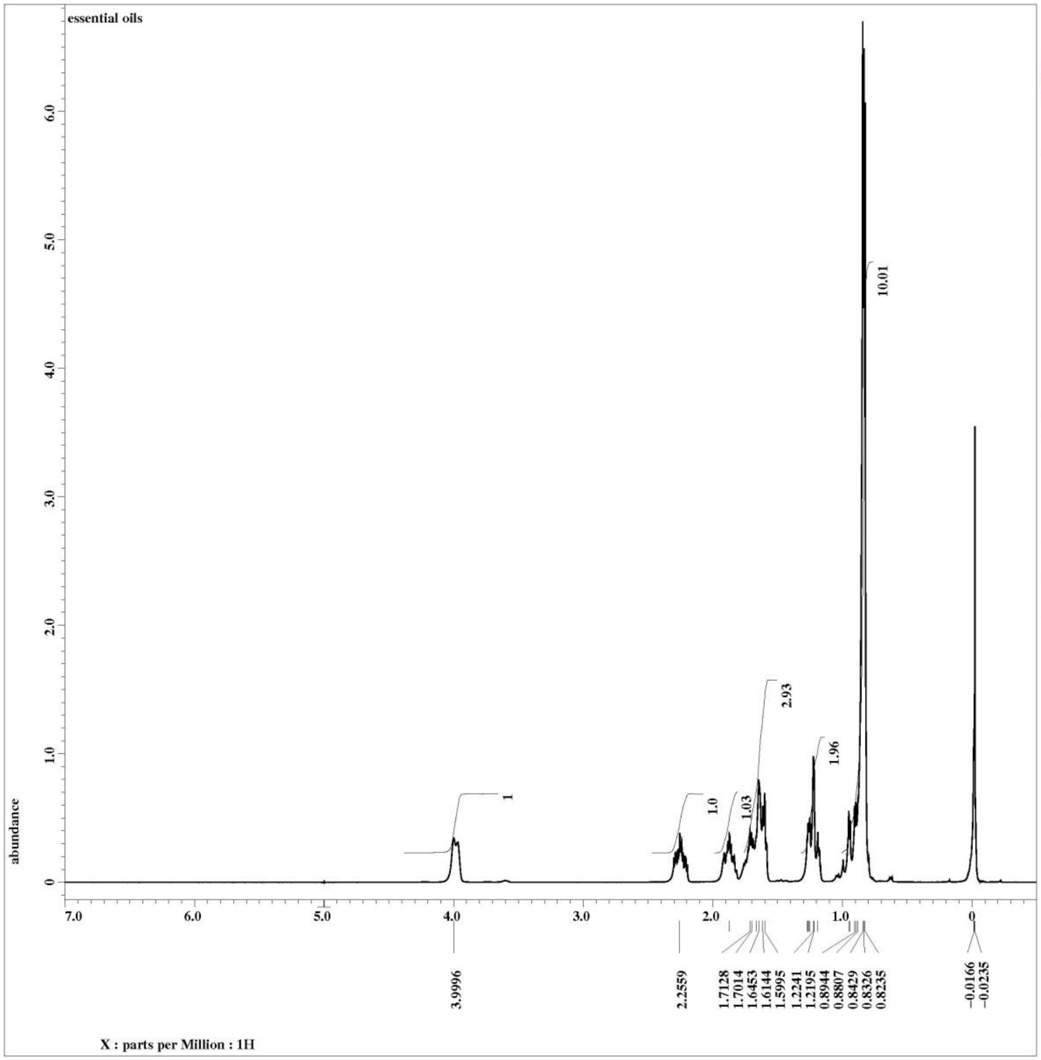

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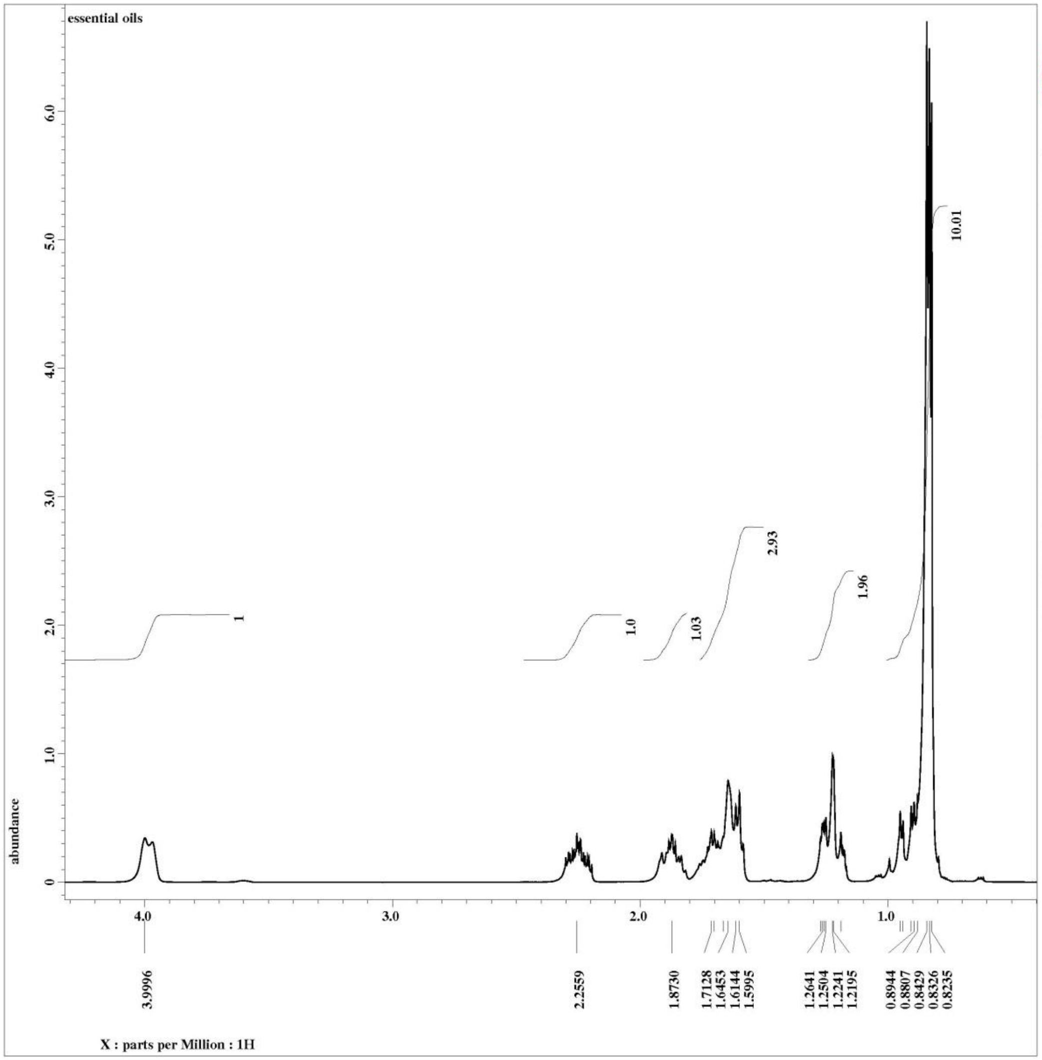

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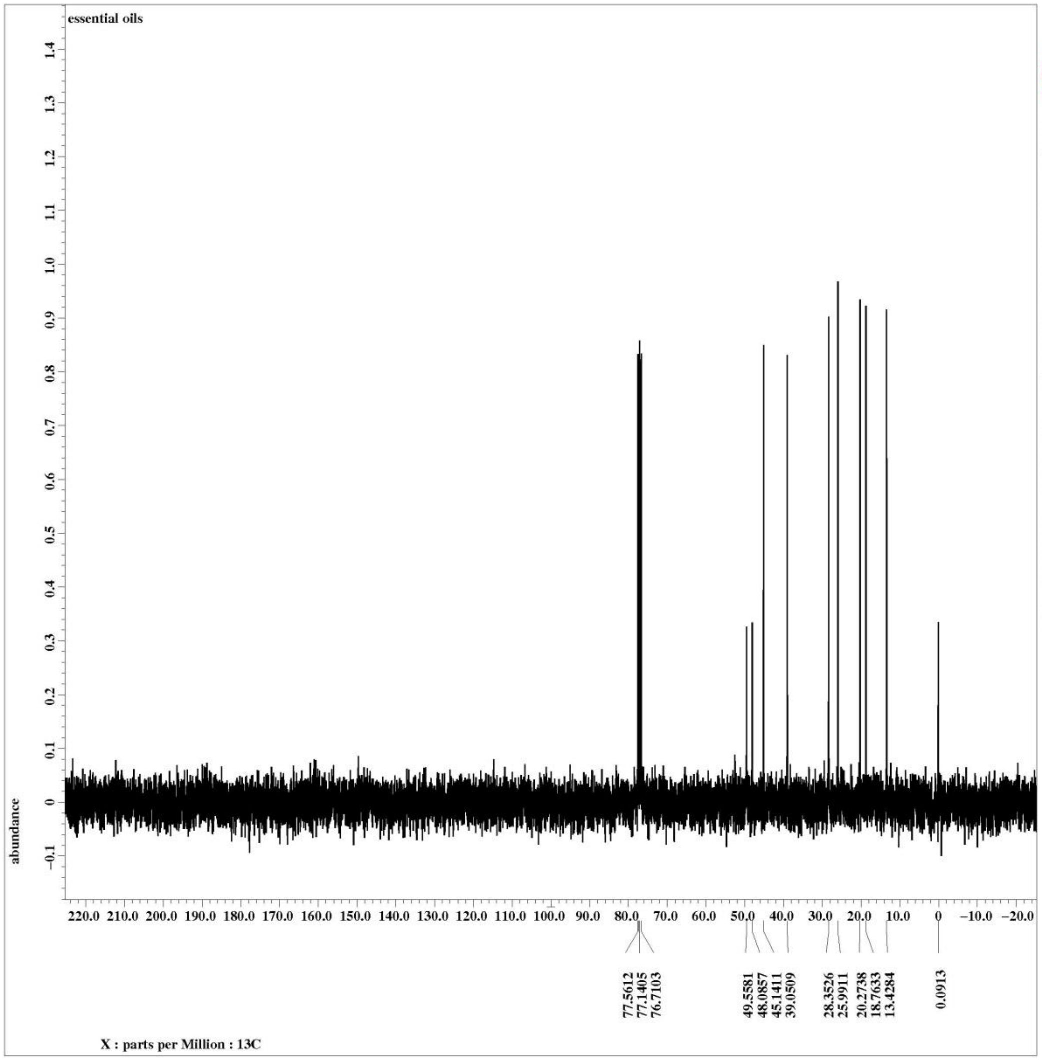
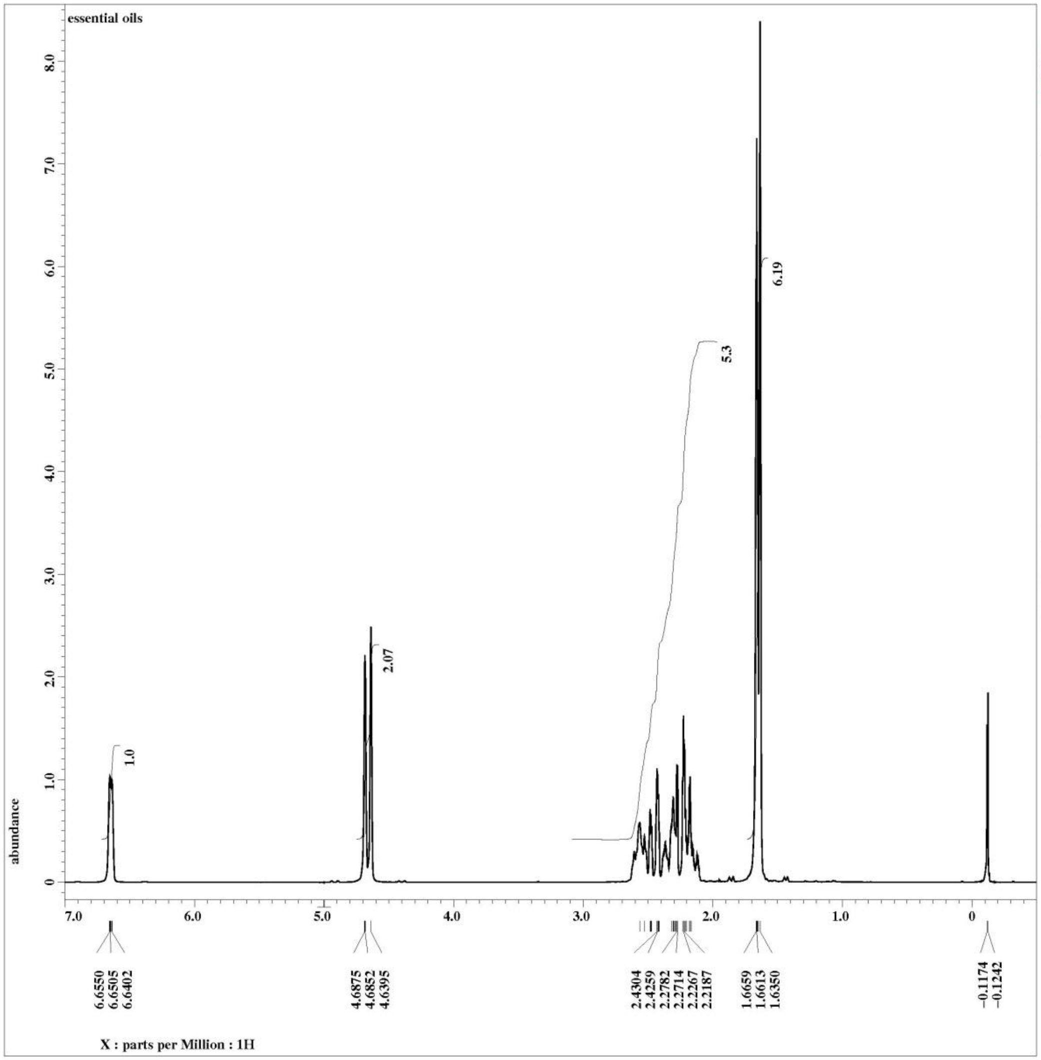
X_atn             = 3[dB]
X_pulse           = 6.281[us]
Irr_mode          = Off
Tri_mode          = Off
Dante_presat      = FALSE
Initial_wait      = 1[s]
Relaxation_delay  = 4[s]
Repetition_time   = 6.90717696[s]
Temp_state        = TEMP OFF
Temp_set          = 25[dC]
Temp_get          = 20[dC]
```



```
Filename          = borneol_CARBON-10.jdf
Sample_id         = borneol
Machine           = mtsu300sp
Creation_time     =  6-OCT-2014 16:13:14
Comment           = essential oils

Field_strength    = 7.0586013[T] (300[MHz
X_acq_duration    = 1.38412032[s]
X_domain          = 13C
X_freq            = 75.56823426[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.72248054[Hz]
X_sweep           = 23.67424242[kHz]
Irr_domain        = 1H
Irr_freq          = 300.52965592[MHz]
Irr_offset        = 5[ppm]
Clipped           = TRUE
Mod_return        = 1
Scans             = 20
Total_scans       = 20

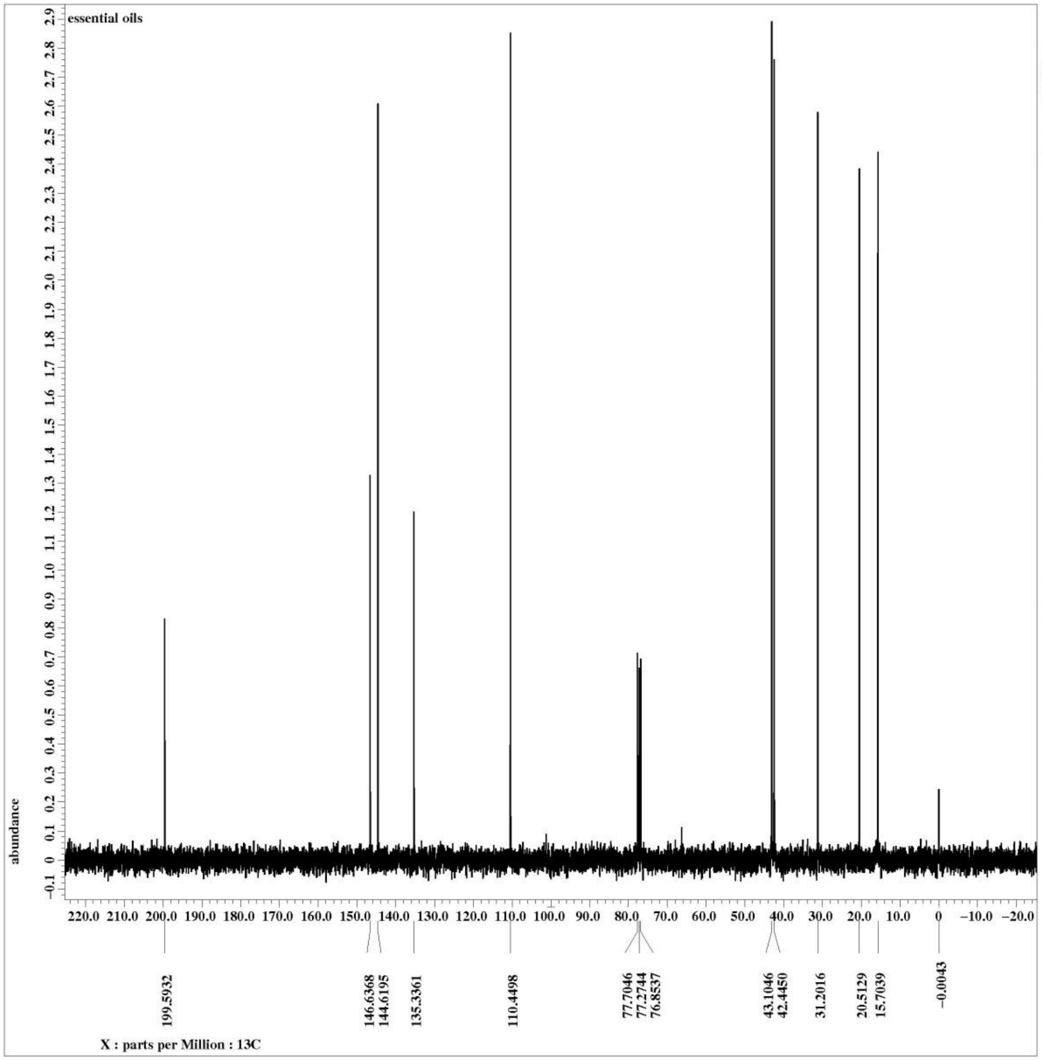
X_90_width        = 9.78[us]
X_acq_time        = 1.38412032[s]
X_angle           = 30[deg]
X_atn             = 6[dB]
X_pulse           = 3.26[us]
Irr_atn_dec       = 24.6[dB]
Irr_atn_noe       = 24.6[dB]
Irr_noise         = WALTZ
Decoupling        = TRUE
Initial_wait      = 1[s]
Noe               = TRUE
Noe_time          = 2[s]
Relaxation_delay  = 2[s]
Repetition_time   = 3.38412032[s]
Temp_state        = TEMP OFF
Temp_set          = 25[dC]
Temp_get          = 20.1[dC]
```



```
Filename           = carvone_PROTON-15.jdf
Sample_id          = carvone
Machine            = mtsu300sp
Creation_time      =  6-OCT-2014 16:50:10
Comment            = essential oils

Field_strength     = 7.0586013[T] (300[MHz
X_acq_duration     = 2.90717696[s]
X_domain           = 1H
X_freq             = 300.52965592[MHz]
X_offset           = 5[ppm]
X_points           = 16384
X_prescans         = 1
X_resolution       = 0.34397631[Hz]
X_sweep            = 5.63570784[kHz]
Irr_domain         = 1H
Irr_freq           = 300.52965592[MHz]
Irr_offset         = 5[ppm]
Tri_domain         = 1H
Tri_freq           = 300.52965592[MHz]
Tri_offset         = 5[ppm]
Clipped            = FALSE
Mod_return         = 1
Scans              = 16
Total_scans        = 16

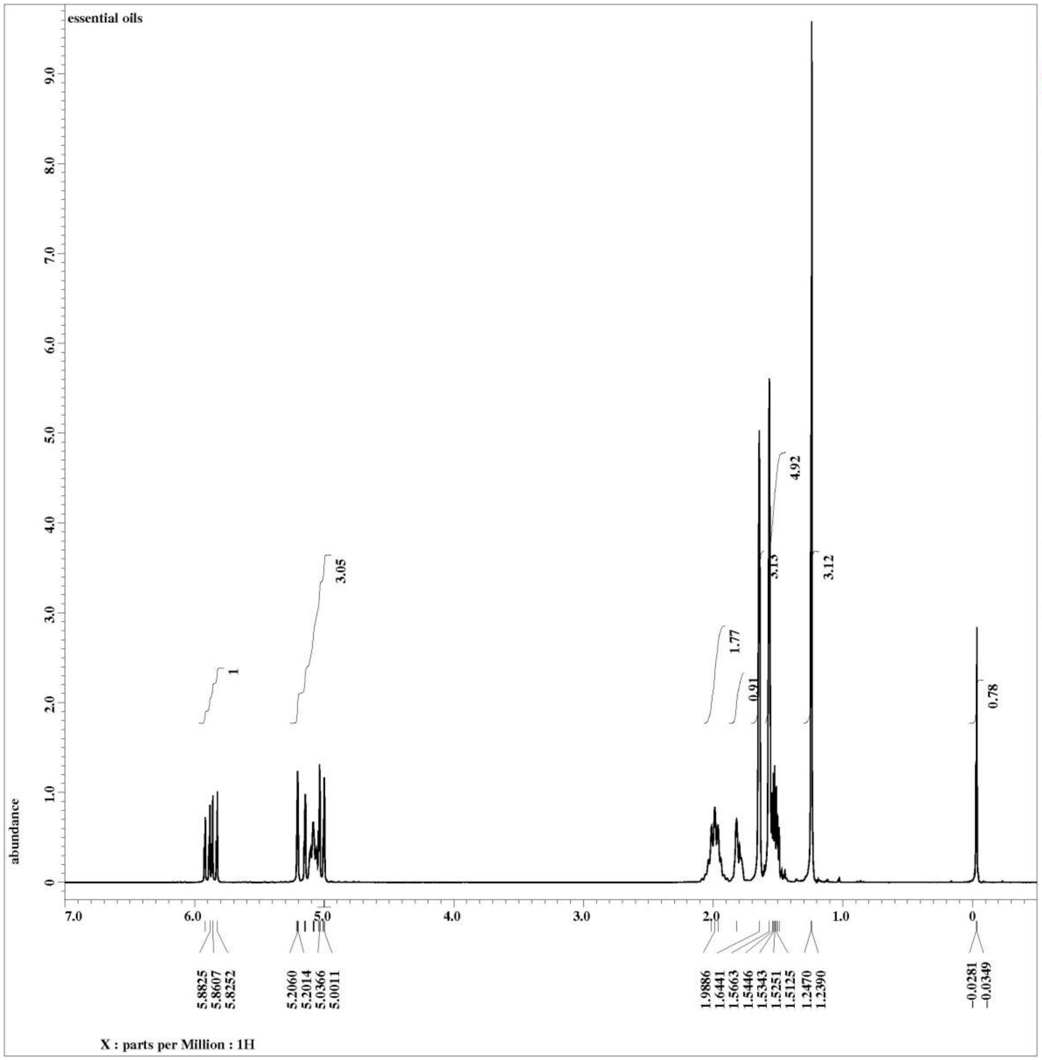
X_90_width         = 12.562[us]
X_acq_time         = 2.90717696[s]
X_angle            = 45[deg]
X_atn              = 3[dB]
X_pulse            = 6.281[us]
Irr_mode           = Off
Tri_mode           = Off
Dante_presat       = FALSE
Initial_wait       = 1[s]
Relaxation_delay   = 4[s]
Repetition_time    = 6.90717696[s]
Temp_state         = TEMP OFF
Temp_set           = 25[dC]
Temp_get           = 20[dC]
```



```
Filename          = carvone_CARBON-9.jdf
Sample_id         = carvone
Machine           = mtsu300sp
Creation_time     =  6-OCT-2014 16:51:49
Comment           = essential oils

Field_strength    = 7.0586013[T] (300[MHz
X_acq_duration    = 1.38412032[s]
X_domain          = 13C
X_freq            = 75.56823426[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.72248054[Hz]
X_sweep           = 23.67424242[kHz]
Irr_domain        = 1H
Irr_freq          = 300.52965592[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 14
Total_scans       = 14

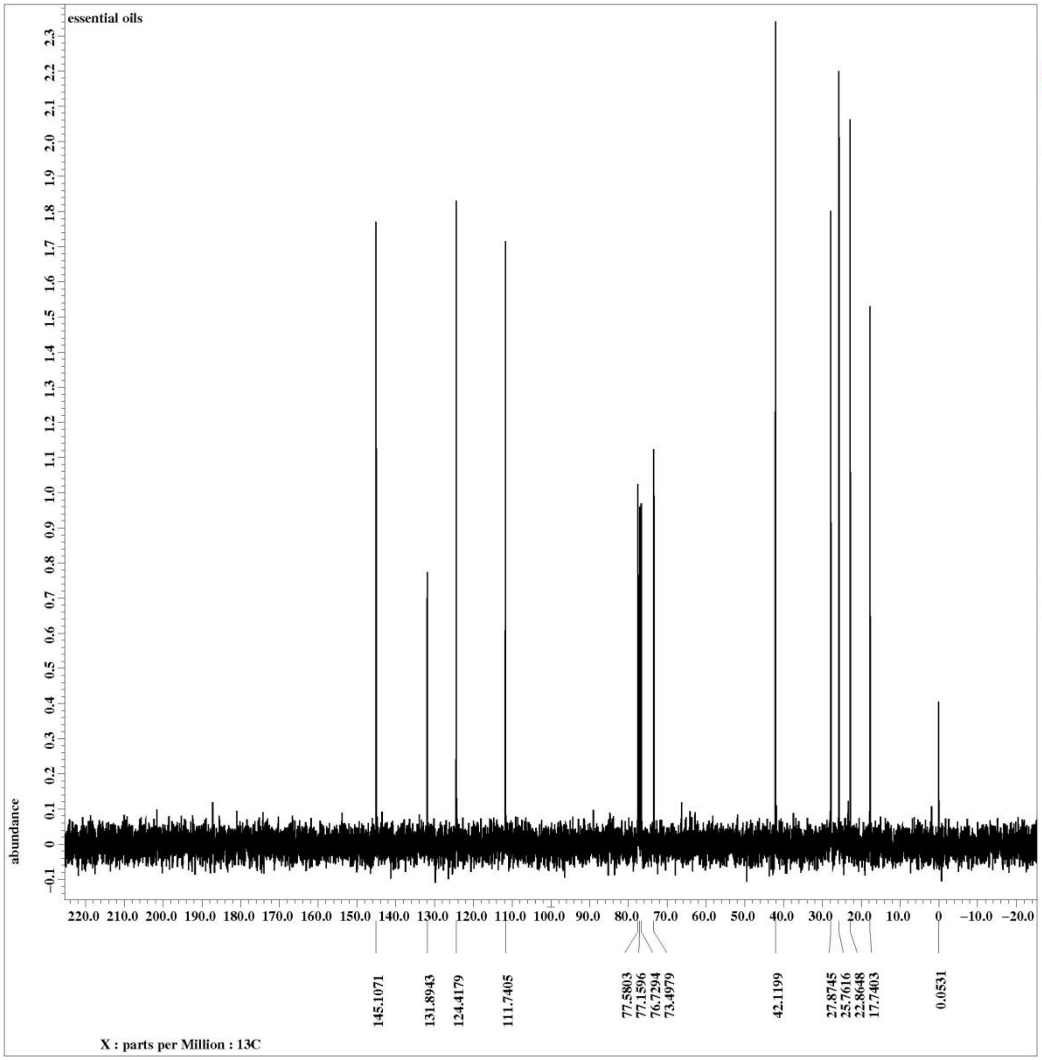
X_90_width        = 9.78[us]
X_acq_time        = 1.38412032[s]
X_angle           = 30[deg]
X_atn             = 6[dB]
X_pulse           = 3.26[us]
Irr_atn_dec       = 24.6[dB]
Irr_atn_noe       = 24.6[dB]
Irr_noise         = WALTZ
Decoupling        = TRUE
Initial_wait      = 1[s]
Noe               = TRUE
Noe_time          = 2[s]
Relaxation_delay  = 2[s]
Repetition_time   = 3.38412032[s]
Temp_state        = TEMP OFF
Temp_set          = 25[dC]
Temp_get          = 20[dC]
```



```
Filename          = linalool_PROTON-15.jd
Sample_id         = linalool
Machine           = mtsu300sp
Creation_time     =  6-OCT-2014 17:28:38
Comment           = essential oils

Field_strength    = 7.0586013[T] (300[MHz
X_acq_duration    = 2.90717696[s]
X_domain          = 1H
X_freq            = 300.52965592[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 1
X_resolution      = 0.34397631[Hz]
X_sweep           = 5.63570784[kHz]
Irr_domain        = 1H
Irr_freq          = 300.52965592[MHz]
Irr_offset        = 5[ppm]
Tri_domain        = 1H
Tri_freq          = 300.52965592[MHz]
Tri_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 16
Total_scans       = 16

X_90_width        = 12.562[us]
X_acq_time        = 2.90717696[s]
X_angle           = 45[deg]
X_atn             = 3[dB]
X_pulse           = 6.281[us]
Irr_mode          = Off
Tri_mode          = Off
Dante_presat      = FALSE
Initial_wait      = 1[s]
Relaxation_delay  = 4[s]
Repetition_time   = 6.90717696[s]
Temp_state        = TEMP OFF
Temp_set          = 25[dC]
Temp_get          = 20[dC]
```



```
Filename         = linalool_CARBON-8.jdf
Sample_id        = linalool
Machine          = mtsu300sp
Creation_time    =  6-OCT-2014 17:30:09
Comment          = essential oils

Field_strength   = 7.0586013[T] (300[MHz
X_acq_duration   = 1.38412032[s]
X_domain         = 13C
X_freq           = 75.56823426[MHz]
X_offset         = 100[ppm]
X_points         = 32768
X_prescans       = 4
X_resolution     = 0.72248054[Hz]
X_sweep          = 23.67424242[kHz]
Irr_domain       = 1H
Irr_freq         = 300.52965592[MHz]
Irr_offset       = 5[ppm]
Clipped          = TRUE
Mod_return       = 1
Scans            = 14
Total_scans      = 14

X_90_width       = 9.78[us]
X_acq_time       = 1.38412032[s]
X_angle          = 30[deg]
X_atn            = 6[dB]
X_pulse          = 3.26[us]
Irr_atn_dec      = 24.6[dB]
Irr_atn_noe      = 24.6[dB]
Irr_noise        = WALTZ
Decoupling       = TRUE
Initial_wait     = 1[s]
Noe              = TRUE
Noe_time         = 2[s]
Relaxation_delay = 2[s]
Repetition_time  = 3.38412032[s]
Temp_state       = TEMP OFF
Temp_set         = 25[dC]
Temp_get         = 20[dC]
```

APPENDIX I

Screen Plot for Factor Analysis of Likert Scale Survey