MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 6(2) pp. 073-079, February, 2018
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Isolation and Purification of 1,3,7-Trimethyl-1H-Purine-2,6(3H,7H)-Dione from tea bags using the green chemistry technology

Abdullahi[1]* S., Maria[2] D., Ndeham[3] R. V., William[4] E. and Maxwell[5] H

[1, 2, 4, 5]Department of Science Laboratory Technology, The Federal Polytechnic, Mubi, PMB 35 Adamawa State.

[3]Basic Sciences Department, Adamawa State College of Agriculture, Ganye- Nigeria

***Corresponding Author's Email: sayeedabdullahi@yahoo.co.uk**
Phone: 08036531780

Abstract

The design of green and sustainable extraction methods of natural products is currently a hot research topic in the multi-discipline areas of chemistry and technology. Herein, attempt was made to apply the six principles of green-extraction, describing a multifaceted strategy to apply this concept at micro level. The mainstay of the working protocol is new and innovative. The concept, principles and examples of green extraction herein discussed, offer an updated glimpse of the huge technological effort that is being made and the diverse applications that are being developed. Caffeine was extracted from the contents of five teabags by the use of solid-liquid and liquid-liquid extraction methods respectively. An acid/base liquid-liquid extraction technique was employed in order to force caffeine into the organic layer. The isolated product (caffeine), has a melting and boiling points of 236 ºC and 364.5 ºC respectively. It gave a percent yield of 6.56% was found to be soluble and slightly soluble in some organic solvents. It was isolated, purified and characterized by some analytical parameters; FTIR spectroscopy, color, melting/boiling points and solubility techniques. The Infrared-spectral data of the isolated caffeine showed absorption bands similar to those obtained in books and literatures. The Infrared-spectrum indicates the absolute purity of the purified caffeine. A pure product from sublimation method gave a mass of 0.0001 g of caffeine (white). This gave a calculated value of 0.30% as the percent recovery of the pure caffeine.

Keywords: Caffeine, green chemistry, solvents, phase and extraction

INTRODUCTION

Caffeine is a chemical compound that is naturally found in plant sources including coffee, cocoa, cola nuts, guarana etc. Chemically, it can be classified as belonging to the heterocyclic group of compounds called the purines. It has a chemical formula; $C_8H_{10}N_4O_2$. Its IUPAC (International Units of Pure and Applied Chemistry) name is 3,7-dihydro-1,3,7-trimethyl-1Hpurine-2,6-dione and a common name of trimethylxanthine. Caffeine can also be classified as an alkaloid since it occurs as a metabolite of nitrogen metabolism (scheme 1). It has a molecular weight of approximately 194.2 g/mol. It is soluble both in water and organic solvents such as alcohol and chloroform. Interestingly, caffeine can be obtained from its natural sources by extraction; however, it can also be synthesized from uric acid as a precursor. In its purified state, it occurs as a white, odourless powder with a bitter taste. Caffeine has a boiling point of 178°C and a melting point of 238°C. It sublimes at its boiling point. It has a density of $1.2g/cm^3$ with a volatility of 0.5% and a vapour pressure of 101kPa at 178°C. It has almost a neutral pH of 6.9 and a water solubility of 2.17%. It was first isolated and purified by the German chemist Friedrich Ferdinand Runge in 1819 (Weinberg and Bealer, 2001) while H. E. Fischer first synthesized caffeine in 1895. Its molecular

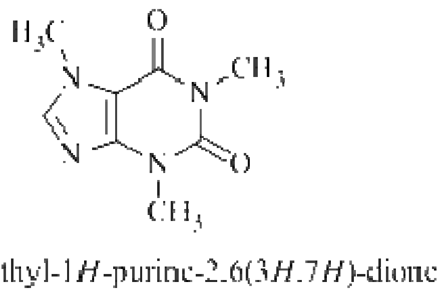
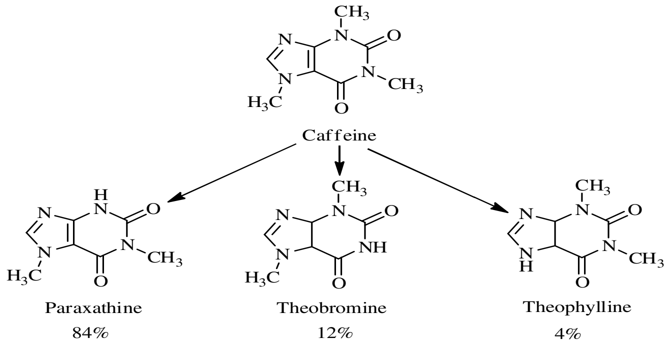


Figure 1. The molecular structure of caffeine

structure is as shown in Figure 1 above.

Caffeine is a planar aromatic molecule which leads to the hypothesis that it could very easily forms π–π complexes with other planar aromatic molecules such as nucleo-bases in DNA and several types of anticancer drugs known to intercalate DNA based on their planar structures (Tragonos *et al.,* 1991 and Davies *et al.,* 2001). Thus caffeine has the ability to form complexes with metal ions from the second series of the periodic table since it contains some nitrogen, oxygen atoms involved in coordinative bonds.

Caffeine can act as antioxidant to prevent diseases. Antioxidants are substances that help protect cells in the body against damage, acting as a defense against oxidative damage. The role of an antioxidant is to help reduce oxidation reactions and thus reduce damage to body tissues. Antioxidants have been linked to a number of potential health benefits, including protection against heart disease and most forms of cancer. Chlorogenic acid, caffeic acid and melanoidins are all the types of antioxidants found in coffee.



Scheme 1: Caffeine Metabolism

The Green Chemistry and its applications

Green chemistry is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. It applies across the life cycle of a chemical product, including its design, manufacture, use and ultimate disposal. Green chemistry is also known as sustainable chemistry. Green Chemistry underlies our commitment to potentially harmful technologies by developing alternative syntheses to prevent environmental pollution.

Green chemistry:

a. Prevents pollution at the molecular level.

b. It's a philosophy that applies to all area of chemistry, not a single discipline of chemistry.

c. Applies innovative scientific solution to real-world environmental problems.

d. Results in source reduction because it prevents the generation of pollution.

e. Reduces the negative impacts of chemical products and processes on human health and the environment.

f. Lessens and sometimes eliminates hazards from existing products and processes.

g. Design chemical products and processes to reduce their intrinsic hazards.

The challenge is to develop in a way that meets the needs of the present generation without compromising the ability of future generations to meet their own needs – in other words, without causing a lot of environmental damage and wasting limited resources. This type of development is called 'sustainable development' and it will be more and more critical as the population of the world increases. One of the ways in which the chemical industry is working towards sustainable development is by using the 'Green Chemistry.' One of the basic ideas of Green Chemistry is to prevent pollution and the production of hazardous materials instead of producing them and then cleaning them up. Attempts have been made in the extraction and purification of caffeine by using reactants/chemicals that are environmentally friendly. It is hoped that the application of the technology will go a long way in the educational development of chemistry in Nigerian schools.

Literature reviews

By employing a proton and carbon nuclear magnetic resonance (NMR) spectroscopy and a spiking method to extract and quantify the chemical components of coffee from roasted coffee bean extract (RCBE), caffeine was confirmed as a coffee component and isolated as a caffeine-chlorogenate complex (Wei *et al*., 2011). Caffeine has also been found to be contained in green tea seeds of *Camellia sinensis* (Hasegawa *et al*., 2011). Using X-Ray crystallographic analysis on equimolecular suspension of (-)-gallocatechin-3-O-gallate (GCg) and caffeine, it was also shown that the resulting complex was capable of showing π-π interactions between the A, B' rings of GCg and the two six-membered rings of caffeine. This threw more light on the stereochemical structure and intermolecular interaction of caffeine (Tsutsumi *et al*., 2011). Studies of the N-heterocyclic carbene Pt(II) complexes from caffeine has further shown that the complex has an opposite stereochemistry and a shorter Pt-C(carbene) bond compared to that of an analogous benzimidazole-derived N,N-heterocyclic carbene (NNHC) Pt complex, suggesting a lower trans influence of pyridyl N, compared to cyclometallated carbon and an increased Pt-NHC π-backbonding in caffeine (Hu *et al*., 2011).

A study employing whole coffee fruit extracts and subsequent characterization of its caffeine components showed the presence of caffeoylquinic acids, feruloylquinic acids, dicaffeoylquinic acids, caffeoylferuloylquinic acids etc. along with a methyl ester of 5-caffeoylquinic acid. These extracts also demonstrated some antioxidant activity (Mullen *et al.,* 2011). A pH dependent Raman spectroscopic study of caffeine showed that the pH value can dramatically affect the surface enhanced Raman spectroscopic (SERS) properties of caffeine, but barely affect the normal Raman spectrum of caffeine aqueous solution. The study concluded that such characteristics can essentially affect the reorientation of caffeine molecule to a Ag colloid surface, but cannot impact the vibration of functional groups and chemical bonds in caffeine molecule (Kang *et al*., 2011). A parallel stacking of caffeine (CAF) a poorly water soluble solute, with riboflavin (RBF) in aqueous solutions showed that such a molecular stacking improves the hydrotropic solubilization of CAF, possibly, by the phenomenon of molecular hydrotropy whereby the presence of a large amount of a solute perhaps improves the solubility of a component (Cui, 2010). The study demonstrates that CAF and RBF undergo molecular parallel stacking in the aqueous solution and that such a stacking are found to be both structural and dynamic under a given condition suggesting that perhaps the self-stacking of CAF is the primary effect, and incorporation of RBF is the secondary effect.

In a study to compare the solubilities of caffeine in supercritical carbon dioxide at different temperatures and over a pressure range with xanthine compounds of similar chemical structures (e.g. theophylline and theobromine), it was observed that although, the chemical structures of the xanthines are very similar, their solubilities in supercritical carbon dioxide vary substantially. The solubilities of caffeine in CO_2 are one order of magnitude higher than those of theophylline and two orders of magnitude higher than those of theobromine (Johannsen and Brunner, 1994).

MATERIAL AND METHODS

All reagents were purchased commercially and used without further purification. The materials and reagents used in this research work include the following; 125cm^3 Erlenmeyer flask, 50cm^3 graduated measuring cylinder, spatula, funnel, 250cm^3 beaker, spectrophotometer, micro-capillary tubes, retort stand, tea bags, propan-2-ol, calcium carbonate ($CaCO_3$), sodium chloride ($NaCl_2$), $CaSO_4$, standard caffeine (0.2g)

Instrumental analysis

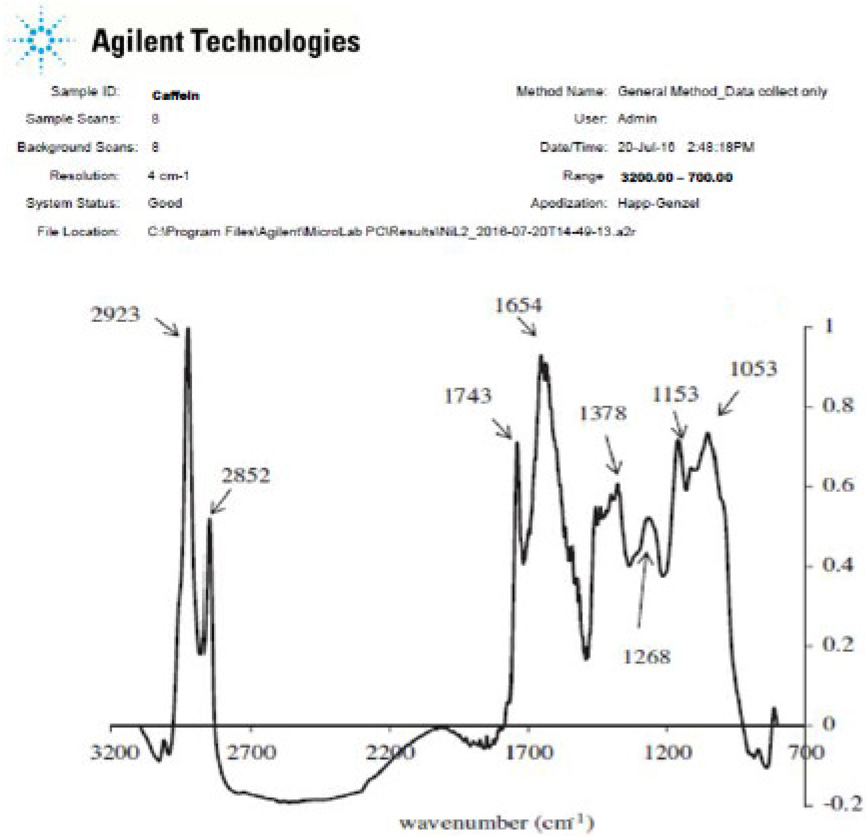
All weighing were carried out on an electric metlar balance model (H3OAR), melting point temperatures were measured using Stuart SMP-10 (Digital) melting point apparatus and were uncorrected.IR spectral were recorded using FTIR 8400S (Fourier Transformed Infrared Shimadzu Spectrophotometer) in KBr at the range of (700-3200) cm^{-1} range.

Isolation and purification Methods

Caffeine was extracted from teabags using methods similar to (Williamson and Katherine 2011; Fieser and Williamson, 1992) but with some modifications. The content of 5 tea bags was carefully weighed and poured into a 150 cm^3 beaker. About 2.50 grams of $CaCO_3$ (Calcium carbonate) was added followed by 100cm^3 water. The mixture was heated to boil for 10 minutes before it was allowed to cool down to room temperature. A 5cm^3 Buchner funnel was inserted into a 125 cm^3 filter flask with a piece of Whatman filter paper, the mixture was filtered. Approximately 20g NaCl (sodium chloride) and 1g calcium hydroxide, $Ca(OH)_2$ was added to the extract, continuously stirred and heated to near boiling for 15 minutes. The hot solution was filtered so as to remove any undissolved solids using a Buckner funnel before it was transferred to 15 cm^3 centrifuge tubes, and 2cm^3 of propan-2-ol was added to the solution. The solution formed was stirred for about a minute and centrifuged at medium speed. Upon completion of centrifugation, 2 layers of material became apparent (a brown layer containing tannins and other materials, and a brown-green layer at the top containing the caffeine dissolved in the solvent). A micro-pipette was used to extract the brown-green organic layer and collected in a small beaker. Another 2cm^3 of propan-2-ol was added to the

Table 1. Physical properties of the caffeine

Compound	Formular	Colour	Yield(%)	M.pt(°C)	B.pt(°C)
Caffeine	$C_8H_{10}N_4O_2$	White	6.56	236	364.5



Agilent Technologies

Sample ID: Caffein
Sample Scans: 8
Background Scans: 8
Resolution: 4 cm-1
System Status: Good
File Location: C:\Program Files\Agilent\MicroLab PC\Results\NiL2_2016-07-20T14-49-13.a2r

Method Name: General Method_Data collect only
User: Admin
Date/Time: 20-Jul-16 2:48:18PM
Range 3200.00 – 700.00
Apodization: Happ-Genzel

Figure 2. FTIR spectra result for the purified caffeine

solid mixture left in the tube, stirred, centrifuged and the portion of the solution containing the caffeine was extracted with a micro-pipette again. The process was repeated a total of 4 times. After the 3rd centrifugation, in order to have a distinct separation of layers, a small amount of sodium chloride (NaCl) was added to aid the process. This concluded the final set of preparation, centrifugation and extraction of caffeine. Anhydrous calcium sulfate (a drying agent) was added to the extract and allowed to stay for about 5 minutes with occasional stirring until the mixture became clear. A micro-funnel was prepared by tightly packing small piece of cotton wool at the tip of a pipette clamped to a vertical bar in the hood such that it was about half way into a 25 cm^3 Erlenmeyer flask. The caffeine extract was transferred to the micropipette via a syringe and allowed to pass into the clean 25 cm^3 Erlenmeyer flask. The remaining calcium sulfate in the centrifuge tube was rinsed with about 5 cm^3 propan-2-ol and poured through the micro-funnel. The Erlenmeyer flask containing the filtered solution was placed on a hot plate (set at a relatively low heating level), allowing for the propan-2-ol to evaporate. The flask was removed when a dry dark green residue was formed at the bottom of the flask and all the propan-2-ol seemed to have evaporated. The flask was allowed to cool to room temperature before it was weighed.

The crude extract was poured into a clean, dry Petri dish. The dish was placed on a hot plate and covered with a stack of three disks of filter paper (such as Whatmann 1). A 250cm^3 beaker containing 30-50 cm^3 of water was placed on the disks to help keep the filter paper in place and provides cooling. The heating control on the hot-plate was turned to a low-medium setting. After few minutes of heating, white vapors of caffeine inside the Petri dish was observed when looking from the side of the dish. The sublimation process was continued

Table 2. Infrared Spectral Data of the caffeine

Compound	C-H	C=C	C=O	C-N	C=N
Caffeine	2923	1654	1743	1268	1654

Table 3. Weight of Crude caffeine

Items	Weight (g)
Tea	10.6200
Empty flask	106.7930
Flask + crude caffeine	107.4900
Crude caffeine	0.6970

for some 5 minutes more before the heat source was finally turned off. The closed system was cooled down to room temperature before taking the beaker off the setup. The water in the beaker was carefully poured into the sink and the purified caffeine scraped from the filter paper onto a piece of pre-weighed weighing paper (aluminum-foil paper). The purified caffeine was weighed and the % recovery of the sublimation process determined. The success of extraction involving a natural product is often expressed as percentage recovery, Abdul Mumin *et al.,* 2006

. % recovery = $\frac{\text{Grams of caffeine recovered}}{\text{Grams of tea leaves}}$

RESULTS AND DISCUSSIONS

Caffeine was extracted from five teabags by the use of solid-liquid and liquid-liquid extractions methods respectively. The product (caffeine), which gave a percent yield of 6.56%, was isolated as white crystalline substance with a melting/boiling points of 236ºC and 364.5ºC respectively (Table 1). It was purified by sublimation and characterized by some analytical parameters; color, melting/boiling points and solubility tests. A pure product from the sublimation of the crude caffeine (Table 3) gave a mass of 0.0021 g (Table 4); this represents a percent recovery of 0.30% for the purified sample. The substance obtained was found to be soluble and slightly soluble in some organic solvents (Table 5). The Infrared-spectral data of the isolated caffeine (Table 2) showed absorption bands similar to those obtained in books and literatures (Misty, 2009; AbdulMumin *et al.,* 2006 and Sablinskas *et al.,* 2003). The Infrared-spectrum indicates the absolute purity of the purified caffeine. It is important to note that the reaction cannot go to completion, so 100% yield was not possible. A loss of product could have occurred due to emulsions and due to not thoroughly "washing" with propan-2-ol to extract as much caffeine as possible. There were a lot of transfers throughout the procedure, which presented many opportunities to lose product. It is also possible that the concentration of caffeine was not high enough because too much water was added.

While extracting caffeine, water was kept at a high temperature in order to increase solubility of caffeine in water to about 670 mg/ml at 100°C. The solution was cooled to a lower temperature in order to; impact the solubility once more and to minimize the attraction to the aqueous layer while in the centrifuging tubes, to prevent propan-2-ol from evaporating out of the solution. Calcium carbonate (a base) was added to the extraction medium to ensure that the acidic components in the tea bags remain water soluble, place the caffeine in a more basic environment so that it has a higher affinity for propan-2-ol and to cause the tannins to form phenolic salts in the aqueous solution. Because tannins are acidic and can be converted to phenolic salts by deprotonation of the –OH group when a base is added, it is possible to separate the tannins from caffeine (Dullo, 2008). A drying agent was added to the organic layer because propan-2-ol dissolved not only the caffeine, but water as well. The drying agent,

Table 4. Weight of purified caffeine

Item	Weight (g)
Weight of filter paper	0.793
Weight of petri dish	49.909
Weight of filter paper + petri dish + caffeine	50.703
Weight of pure caffeine	0.0021

Table 5a. Solubility of caffeine in water and some common organic solvents

Solvents	Solubility
Dimethylbenzene	S S
N-butane	S S
Propan-1-ol	S
Propanone (acetone)	S S
Ethanol	S S

Table 5 (cont). Solubility of caffeine in water and some common organic solvents

Methanol	S
Carbon tetrachloride	S
Methanol	S S
Acetic acid (glacial)	S
Water	S S

Key: S = Soluble; SS = Slightly Soluble

$CaSO_4$ was added to remove excess water so that a pure sample of caffeine could be obtained after the solvent evaporated at room temperature (Williamson and Katherine 2011). The pellets stopped clumping together when excess water was removed. It is also very rapid, effective, and ideal for micro-scale works such as the case at hand. In order to remove the propan-2-ol, the beaker was placed in a hot water bath so that the solvent could evaporate and leave a pure sample.

CONCLUSION

Caffeine has been isolated, purified and characterized by some analytical parameters; colour, FTIR spectroscopy, melting/boiling point temperatures and solubility tests in different organic solvents. From the results of the analysis conducted on the substance and from available books and literatures, it could be established that the substance isolated using the Green Chemistry Technique was caffeine.

REFERENCES

AbdulMumin M, Kazi FA, Zainal AM, Zakir HM (2006). Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography, *Malay. J. Chem.* 8(1), 045 – 051.

Cui Y (2010). Parallel stacking of caffeine with riboflavin in aqueous solutions: the potential mechanism for hydrotropic solubilization ofriboflavin. *Int. J. Pharm.* 397, 36-43.

Davies DB, Veselkov DA, Djimant LN, Veselkov AN (2001). Hetero association of caffeine and aromatic drugs and their competitive binding with aDNA oligomer, *Eur. Biophys J.* 30, 354–366

Dullo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J (1999). Efficacy of green tea extracts rich in catechinpolyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Ame. J. Clin. Nutr.* 70(6), 1040-5

Fieser, Land WilliamsonK. (1992). Organic Experiments, 7th Ed. D. C. Heath and Company Lexington, Massachusetts-Toronto. 125, Spring Street. Lexington, MA 02173. Pp. 99 -116

Hasegawa T, Akutsu K, Kishi Y, Nakamura K (2011). Constituents of the green tea seeds of *Camellia sinensis*. *Nat Prod Commun.*6,371-4.

Hu JJ, Bai SQ, Yeh HH, Young DJ, Chi Y, Hors TS (2011). N heterocycliccarbene Pt(II) complexes from caffeine: synthesis, structures and photoluminescent properties. *Dalton Trans.*40, 4402-6.

Johannsen M, Brunner G (1994). Solubilities of the xanthines caffeine, theophylline and theobromine in supercritical carbon dioxide.*Fluid Phase Equilibria.*95, 215-226.

Kang J, Gu H, Zhong L, Hu, Y, Liu F (2011). The pH dependent Raman spectroscopic study of caffeine. *Spectrochim Acta Mol. BiomolSpectroSc.* 78, 757-62.

Mullen W, Nemzer B, Ou B, Stalmach A, Hunter J, Clifford MN, Combet E (2011). The antioxidant and chlorogenic acid profiles of whole coffee fruits are influenced by the extraction procedures. *J. Agric. Food Chem,* 59, 3754-62.

Sablinskas V, Steiner G, Hof M (2003). Applications. In G. Gauglitzand T. Vo-Dinh (Eds) Handbook of spectroscopy. Weinheim, Germany: Wiley-VCH.Pp. 89–168.

Tragonos F, Kapuscinski J, Darzynkiewicz Z (1991). Caffeine modulates the effects of DNA-intercalating drugs in vitro: A flow cytometric and spectrophotometric analysis of caffeine interaction with novantrone, doxorubicin, ellipticine, and the doxo-rubicin analogue AD198, *Cancer Res*, 51(3), 682-3689

Tsutsumi H, Sato T, Ishizu T (2011). Stereo-chemical structure and intermolecular interaction of complexes of (-)-Gallocatechin-3-O-gallate and caffeine. *ChemPharm Bull (Tokyo)* 59, 100-5.

Wei F, Furihata K, Hu F, Miyakawa T, Tanokura M (2011). Two Dimensional (1)H-(13)C Nuclear Magnetic Resonance (NMR)-Based Comprehensive Analysis of Roasted Coffee Bean Extract. *J. Agric. Food Chem.* 17, 9065-73.

Weinberg BA, Bealer BK (2001). The world of caffeine, New York and London: Routledge. ISBN 0-415-92722-6.

Williamson K, Katherine Masters (2011). Macro-scale and Micro-scale Organic Experiments, 6th ed.; Brooks/Cole, Pp. 155-163.