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Laboratories and Demonstrations

Qualitative Analysis of Herbs by Gas Chromatography/Mass Spectrometry (GC/MS). An Undergraduate Instrumental Analysis Laboratory Exercise

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… a simple laboratory exercise is described here that focuses on the analysis of real-world herbal extracts.

A laboratory exercise to be used in an undergraduate instrumental methods course is presented for the qualitative analysis of interesting real-world herbal extracts. The objective of this experiment is to familiarize students with a modern experimental method gas chromatography/mass spectrometry (GC/MS).

Introduction

Analytical laboratory sequences at most universities have traditionally placed equal emphasis on both wet chemistry and instrumental methods. Classic titrimetric methods are convenient to run in large laboratory sections where inexpensive burets are the only necessary instrumentation. Students often run a number of similar titrations throughout the course in order to develop good technique, and they are usually graded on their results. Due to the repetitive nature of

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titrations, students often lose interest in the laboratory work and in analytical chemistry. In addition, these methods often produce large volumes of waste, especially in a large classroom application. In contrast to teaching laboratories, industrial and research laboratories over the years have expanding interests in instrumental methods and ever-decreasing interests in traditional volumetric methods. These latter methods have been automated or replaced by more-sophisticated instrumental methods. Thus, there is a real need for students to be more knowledgeable about the vast and growing number of instrumental methods currently being used in industrial and academic research settings.

We present an interesting laboratory exercise that introduces students to an instrumental method that is rapidly becoming a routine method of analysis in both industrial and academic research settings—gas chromatography with a mass spectrometer detector (GC/MS). These instruments are now commonly found in modern chemistry teaching laboratories, yet there are relatively few published laboratory experiments illustrating this important technique[1–4]. The purpose of this article is to provide educators with an additional interesting undergraduate exercise for illustrating the principles of gas chromatography and mass spectrometry. With proper organization, it is not unreasonable for this experiment to be adapted for a reasonably sized laboratory section (15–20 students). Since detailed discussions of GC/MS are found throughout the literature, this technique will not be discussed here. Readers are referred to various textbooks for discussions of relevant theory and principles of GC/MS [5, 6] or off the internet at www.scimedia.com/chem-ed/ms/analytic/ac-meths.htm. Instead, a simple laboratory exercise is described here that focuses on the analysis of real-world herbal extracts. This experiment provides students with a stimulating project to illustrate the theory and practice of GC/MS.

Plants in general have long been a source of biologically active compounds; herbs in particular through extracts and tinctures have been used widely as alternatives to pharmaceuticals. Civilizations have been turning plant extracts into medicines for thousands of years [7], long before the determination of chemical composition of these extracts was possible. For example, boldo (*Peumus boldus*), to which the author (ETS) was introduced to during a trip to Chile, was recently found wrapped in seaweed along with human remains that date back over 10,000 years [8]. Ancient Americans likely used this plant for stomach ailments in the same way that its herbal tea is currently used. Today, a large portion of prescription drugs is still derived from plants.

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Many common herbs are readily available for students to analyze by GC/MS. We have found that students are very interested in this topic, and they have brought in their own herbs for analysis as a part of this exercise. Literally hundreds of compounds have been isolated from a single herb [9] and therefore these samples provide a rich source for qualitative analysis. For example, antimicrobial, antiseptic, antispasmodic, sedative, and diuretic compounds have been isolated from hops. In addition, many compounds derived from herbs are familiar to students. For example, peppermints, which contain a number of compounds that stimulate digestion, are typically made available to restaurant patrons after a meal. Some examples of herbs (which can be examined in this experiment), their therapeutic uses, and the principal constituents found in their essential oils are listed in Table 1 (the term essential oil is derived from the facts that plant distillates contain compounds with an *essence*, and they have an oily consistency). Although not presented here, we also examined extracts of many common herbs (basil, bay, marjoram, sage, etc.) with excellent results; students are encouraged to bring in their own herbs for analysis. As a part of this laboratory exercise, they are required to identify constituents in the essential oils, and they are also required to research these constituents (e.g., structure, medicinal application, etc.). Students are directed to the *Merck Index* [10] and the *Illustrated Encyclopedia of Essential Oils* [9] as an initial starting point for their research. Nearly all of the compounds in the essential oils we prepared have been found in the *Merck Index*. This "discovery" approach adds an additional dimension to this interesting project.

Experimental

Herbs were obtained from various sources, including local nutrition, grocery, and herb stores. Other herbs were collected from the countryside in Virginia and northern Chile. We found that fresh herbs work much better than dried herbs, but all yield nice results. All the equipment required for this laboratory is listed in Table 2.

About 3–4 g of any chopped herb and 25 mL of reagent alcohol were placed in a round-bottom flask. A West condenser packed with glass beads was placed in a vertical position above and directly connected to the round-bottom flask. The mixture was percolated for 30 minutes at a gentle boil with refluxing. Since a number of components of interest have low boiling points, it is important to ensure that as much of the sample as possible is condensing. The cooled solution was decanted from the round-bottom flask, and was extracted with 5 mL dichloromethane in a 250-mL

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TABLE 1. Selected herbal extracts.

Herb	Genus/Species	Principal Constituents*	Action
Eucalyptus	*Eucalyptus globulus*	Cineole	Expectorant
Saltwort	*Chenopodium abrosiodes*	Ascaridole	Anthelmintic
Sassafras	*Sassafras albidum*	Safrole	Pediculicide
Spearmint	*Menthal spicata*	Menthol, menthone	Gastric sedative

* Structures of these molecules are illustrated in Figure 1–4.

TABLE 2. Equipment used to prepare essential oil.

Equipment
Standard round-bottom boiling flask (100 mL, 19/22 joint)
West condenser (19/22 joint) filled with glass beads
Heating mantle and variable transformer
25 mL reagent alcohol
5 mL dichloromethane
1 g anhydrous magnesium sulfate
250-mL separatory funnel
13-mm disposable syringe filter and 10-mL syringe

separatory funnel. If an emulsion formed, 2 mL of saturated NaCl solution was added in order to separate the mixture into two distinct phases. The organic layer was then removed and dried with approximately 1 g anhydrous magnesium sulfate. This mixture was then filtered with the aid of a disposable 13-mm-diameter syringe filter (0.25-μm pore size) to remove particulate matter.

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We tried a variety of distillation arrangements to prepare essential oils and we found the above method required the least time and equipment, and yielded an extract rich in compounds. Essential oils of herbs have traditionally been obtained through distillation with water, ethyl acetate, diethyl ether, and ethanol. We tried only water and reagent alcohol distillations, and the latter yielded superior results. Reagent alcohol also poses less of a waste disposal problem than alternative solvents.

Analysis of the essential oils was carried out utilizing a Hewlett Packard HP5890 GC fitted with a 5% poly(phenylmethylsiloxane) capillary column (30 m × 0.25 mm) and a 5972 MSD quadrupole detector. A 2-μl sample was injected under split mode. Experimental conditions were: initial temperature, 70 °C; initial time, 4 min; final temperature, 300 °C; temperature ramp, 10 °C/min to 140 °C and 20 °C/min to 300 °C; and gas flow, 1 mL/min. If necessary, temperature ramping conditions were altered to optimize resolution. The mass spectral data were compared to data in the HP library [11].

Results

Blue Gum Eucalyptus (Eucalyptus Globulus Var. Globulus)

A representative chromatogram of an extract of fresh eucalyptus leaves is shown in Figure 1A. The largest peak, which eluted after 7 min, was identified as cineole, also known as eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane; MW = 154.24), and its structure is shown in the upper right hand corner of this figure. This compound has therapeutic activity as an inhalational expectorant [10]. The mass spectrum of this chromatographic peak and a mass spectrum of pure cineole from the HP library are shown in Figures 1B and 1C, respectively. Other minor compounds identified from the mass spectra include pinene (insecticide), camphene, and caryophyllene (perfume).

Saltwort (Chenopodium Abrosioides)

A representative chromatogram of an extract of *Chenopodium abrosioides* is shown in Figure 2A. Ascaridole (1-methyl-4-(methylethyl)-2,3-dioxabicyclo[2.2.2]oct-5-ene; MW = 168.23), which eluted after about 12 min, has previously been reported to constitute up to 60–80% of the essential oil of saltwort [9]. Ascaridole is an anthelmintic (antiparasitic) that has been used in the treatment of roundworm [10]). Although detected, it did not appear as the major constituent of our extract probably due to the manner in which the extract was prepared. The experimentally determined

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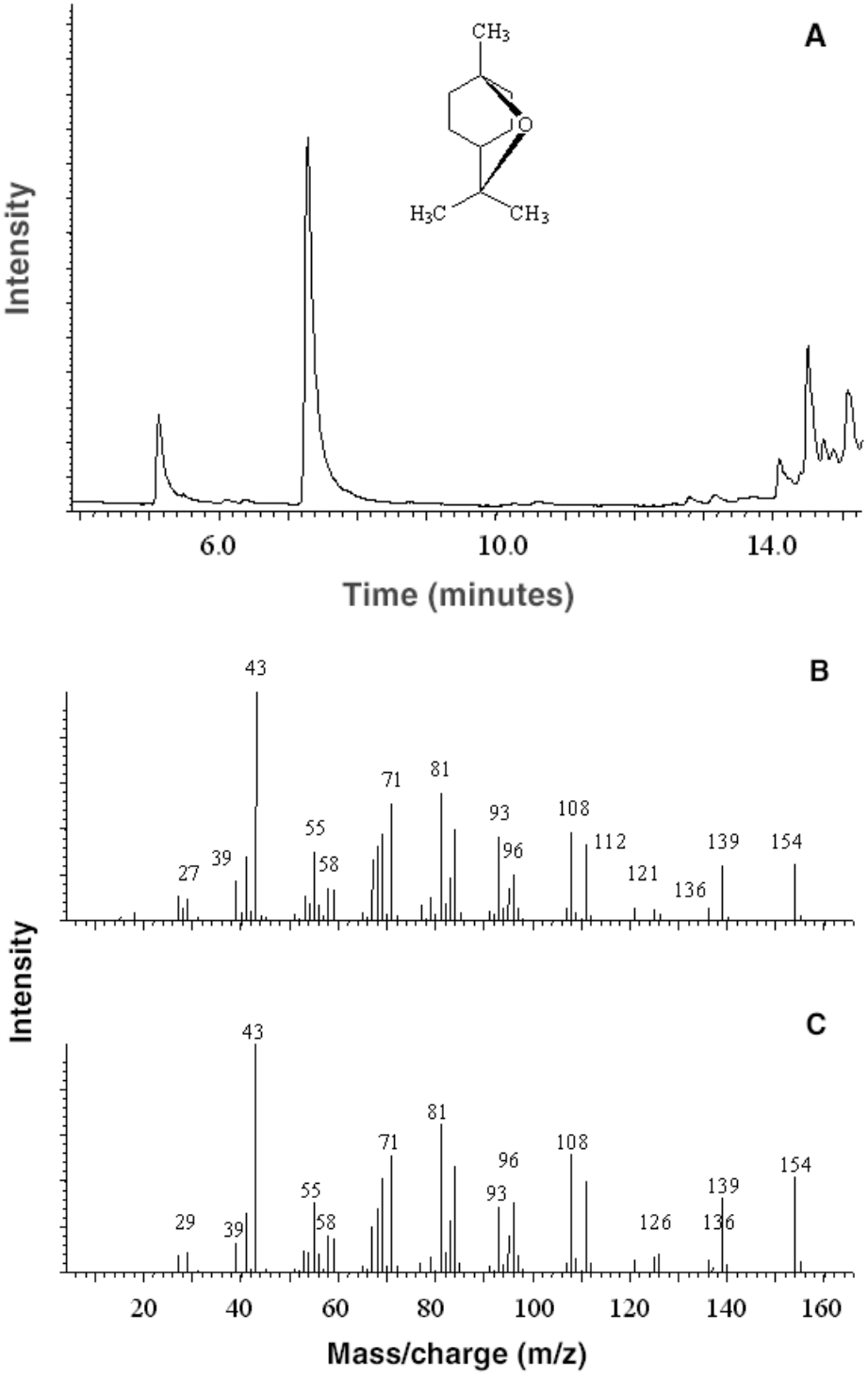

FIGURE 1. (A) CHROMATOGRAM OF AN EXTRACT OF BLUE GUM EUCALYPTUS (*EUCALYPTUS GLOBULUS*) FOR EXPERIMENTAL CONDITIONS DESCRIBED IN THE TEXT; THE INSET IS THE STRUCTURE OF CINEOLE (EUCALYPTOL). (B) MASS SPECTRUM OF THE CHROMATOGRAPHIC FRACTION THAT ELUTED AFTER 7 MINUTES AS SHOWN IN A. (C) MASS SPECTRUM OF PURE CINEOLE OBTAINED FROM THE HP LIBRARY [11].

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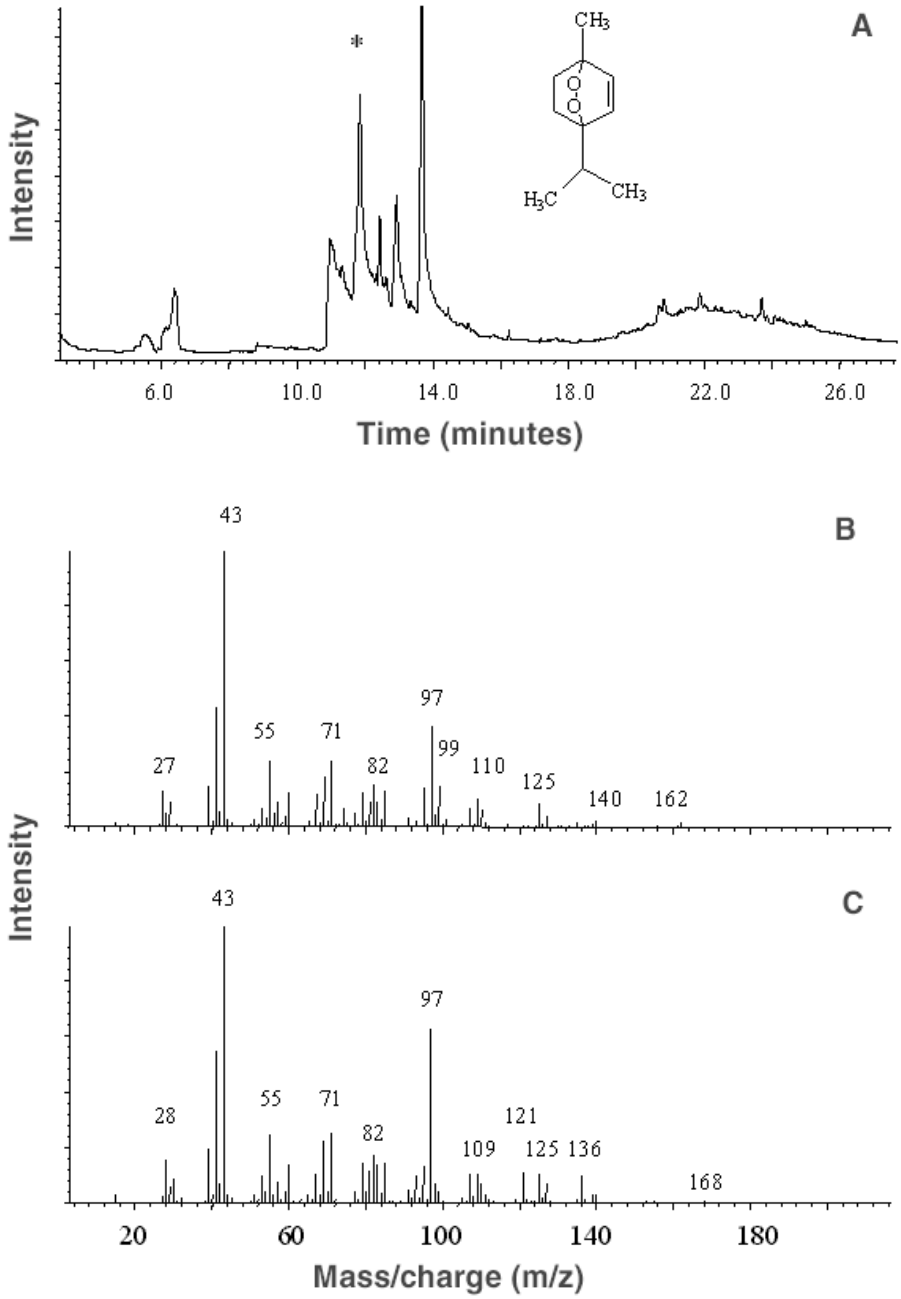

FIGURE 2. (A) CHROMATOGRAM OF AN EXTRACT OF SALTWORT (CHENOPODIUM ABROSIOIDES) FOR EXPERIMENTAL CONDITIONS DESCRIBED IN THE TEXT; THE INSET IS THE STRUCTURE OF ASCARIDOLE. (B) MASS SPECTRUM OF THE CHROMATOGRAPHIC FRACTION THAT ELUTED AFTER 12 MINUTES, AS SHOWN BY THE ASTERISK IN A. (C) MASS SPECTRUM OF PURE ASCARIDOLE OBTAINED FROM THE HP LIBRARY [11].

mass spectrum of the peak that eluted after 12 minutes in Figure 2A and the mass spectrum of pure ascaridole as extracted from the HP library are depicted in Figures 2B and 2C, respectively.

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Sassafras (Sassafras Albidum)

A representative chromatogram of *Sassafras albidum* extract is shown in Figure 3A. The largest peak, which eluted after approximately 11 min, was identified as safrole (5-(2-propenyl)-1,3 benzodioxole; MW = 162.18) by mass spectrometry. Safrole is a pediculicide that is used in the treatment of lice [10]. Other minor compounds identified in sassafras include caryophyllene and cineole. The mass spectrum of the largest chromatographic peak, and a mass spectrum of safrole obtained from the HP library are shown in Figures 3B and 3C, respectively.

Spearmint (Mentha Spicata)

Both a fresh spearmint extract and an extract prepared from pure peppermint tea purchased at a nutritional food store yielded similar chromatograms. A representative chromatogram of *Mentha spicata* extract is shown in Figure 4A. The largest peak, which eluted after approximately 8 min, was identified as menthol by mass spectrometry. Other minor compounds identified in spearmint include methyl acetate (perfume), menthone (perfume), caryophyllene, cineole, and pulegone (antifungal). The mass spectrum of the largest chromatographic peak, and a mass spectrum of pure menthol (5-methyl-2-(1-methylethyl)cyclohexanol; MW = 156.26) obtained from the HP library are shown in Figures 4B and 4C, respectively. Menthol has been used externally as a mild local anesthetic and antiseptic, and it has been used internally as a carminative and gastric sedative [10].

Discussion

The primary purpose of this article is to introduce instructors to an interesting GC/MS laboratory exercise. Although not discussed here, much of the instruction for this experiment is in the operation and principles of a GC/MS instrument. Many fundamental gas chromatography and mass spectrometry problems can be contrived for this experiment based on the theory presented in the laboratory lectures, or questions can be readily adapted from existing questions in a textbook on chemical instrumentation [5, 6]. Some suggested questions are provided as an Appendix (33es1897.pdf). Answers to these questions are available to professors from the authors upon request.

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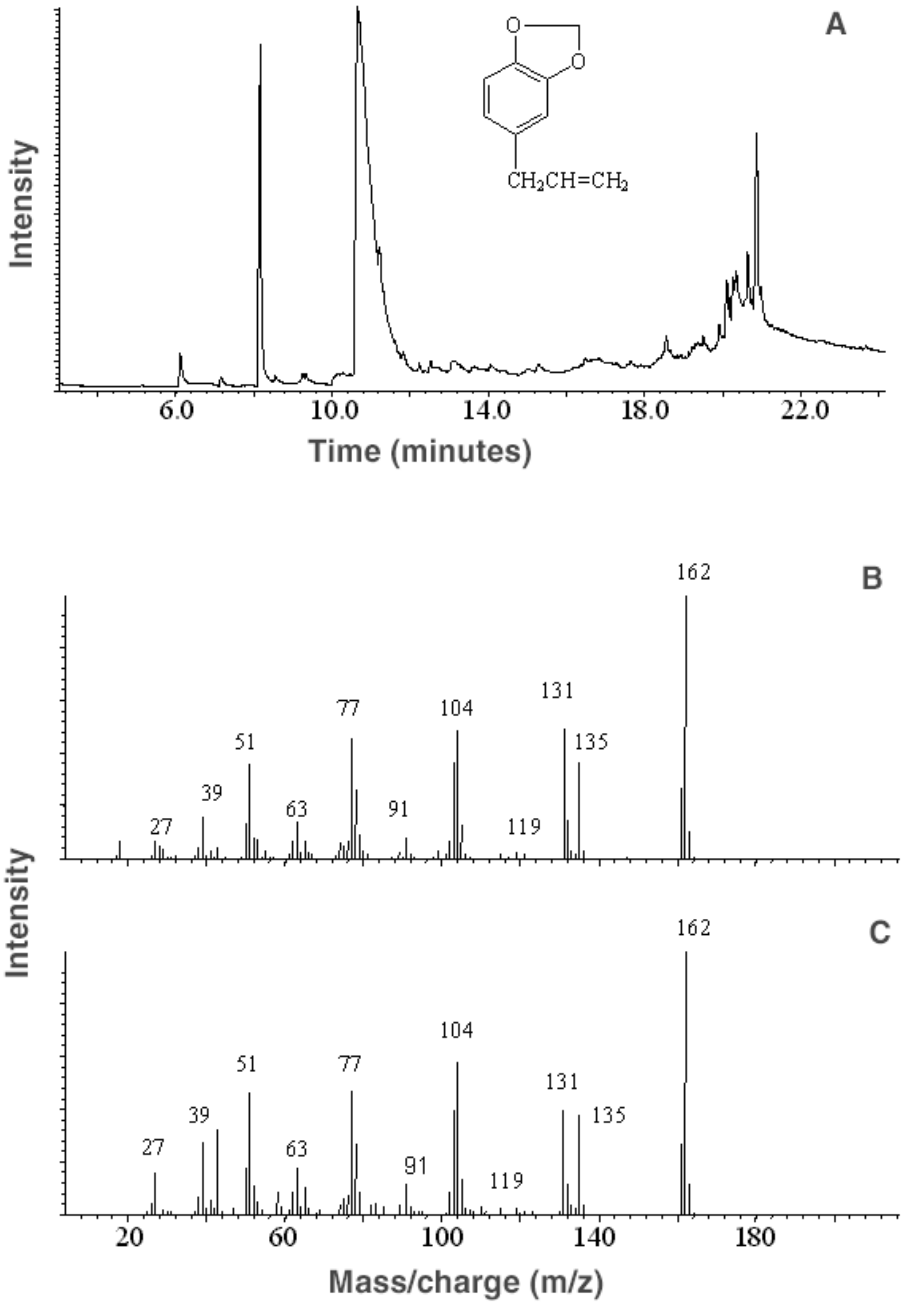

FIGURE 3. (A) CHROMATOGRAM OF AN EXTRACT OF SASSAFRAS (*SASSAFRAS ALBIDUM*) FOR EXPERIMENTAL CONDITIONS DESCRIBED IN THE TEXT; THE INSET IS THE STRUCTURE OF SAFROLE. (B) MASS SPECTRUM OF THE CHROMATOGRAPHIC FRACTION THAT ELUTED AFTER 11 MINUTES, AS SHOWN IN A. (C) MASS SPECTRUM OF PURE SAFROLE OBTAINED FROM THE HP LIBRARY [11].

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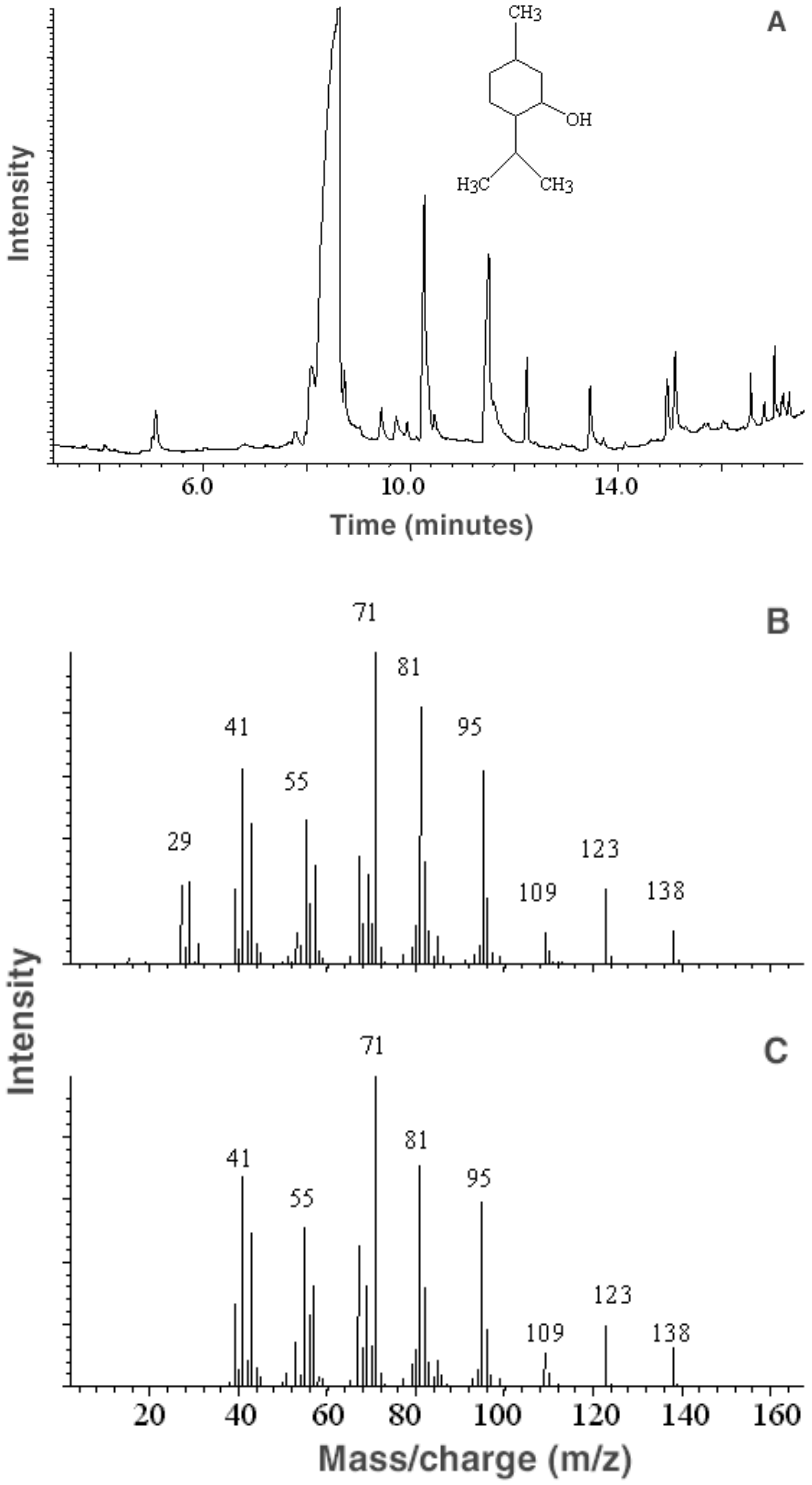

FIGURE 4. (A) CHROMATOGRAM OF AN EXTRACT OF SPEARMINT (*MENTHA SPICATA*) EXTRACT FOR EXPERIMENTAL CONDITIONS DESCRIBED IN THE TEXT; THE INSET IS THE STRUCTURE OF MENTHOL. (B) MASS SPECTRUM OF THE CHROMATOGRAPHIC FRACTION THAT ELUTED AFTER 8 MINUTES, AS SHOWN IN A. (C) MASS SPECTRUM OF PURE MENTHOL OBTAINED FROM THE HP LIBRARY [11].

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We typically used two 4-hour laboratory periods to complete this lab. It takes less than 2 hours to prepare the essential oil, 20 minutes to obtain each chromatogram, and a few hours to analyze the mass spectra. It is possible to further abbreviate this lab into a single 4-hour period if the essential oil is prepared in advance for the students. Advantages to this exercise include that it does not require a lot of time or expense (excluding the cost of the instrument), it does not generate significant amounts of waste material, and the subject is interesting.

Some lecture time is spent on discussing quantitative analysis of essential oils or other natural products by GC/MS. Problems with reproducibility are discussed. For example, how might the results depend on various herbal species, plant-growth conditions, and method of preparation of essential oil? A number of questions may be formulated concerning the partitioning of compounds in the distillation process. An assortment of problems can also be assigned to the students based on the specific mass spectral data they collect (see Appendix 33es1897.pdf).

Due to its familiar characteristic odor and availability of the plant, the eucalyptus sample is perfect to use as an illustration. The students can smell the eucalyptus, and then they are shown the chromatogram and representative mass spectra. The mass spectrum of the largest chromatographic peak is matched with the mass spectrum of a pure compound from the HP library, and the mass spectrum is qualitatively interpreted. From our experience, students enjoy this laboratory exercise very much. They bring in a number of interesting samples to analyze, and they identify and investigate the uses of the compounds in their sample. Nearly all of the compounds in our essential oils have been found in the *Merck Index* [10]. It is an interesting way to introduce students to GC/MS, and this laboratory exercise smells good!

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