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Laboratory Experiment

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Determination of Plant Volatiles Using Solid Phase Microextraction GC−MS

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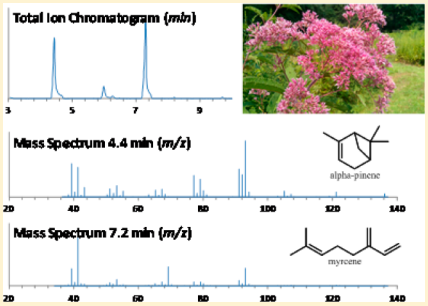
Supporting Information

ABSTRACT: This experiment combines analytical techniques of solid phase microextraction and gas chromatography−mass spectrometry with easily relatable and accessible plant volatile chemistry (floral and vegetative scents of local/available plants). The biosynthesis and structure of these chemicals are of interest in the areas of organic chemistry, biochemistry, and molecular biology. This laboratory exercise is well-suited for a broad range of cross-disciplinary topics in chemistry and biology courses. The methods described here could be used to expose undergraduate chemistry students to broad aspects of biological volatile chemistry in plants, and alternatively, to expose undergraduate biology students to analytical chemistry techniques useful in the study of plant biology and plant−insect interactions.



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In this experiment, solid phase microextraction (SPME) is used with GC−MS to identify the volatile compounds released from plant tissue. This allows students to identify different components of floral and vegetative scents while learning about SPME and GC−MS techniques.

With solid phase microextraction, a small silica fiber is coated with a gas chromatography stationary phase. This fiber is exposed to a mixture and the analytes adsorb onto the surface of the fiber. The sample can be a solution or the headspace vapor above a sample. After the extraction step, the SPME fiber is inserted into the injection port of a gas chromatograph. The high temperature of the injection port volatilizes the analytes, which are then separated by gas chromatography and identified using mass spectrometry.

Since SPME was first introduced in the 1980s, methods have been developed for many different analytes.[1] SPME is student-friendly, and numerous reports in this Journal have used the technique to analyze complex mixtures in a variety of different teaching applications. These applications include a number of quantitative determinations, including caffeine in beverages,[2] cinnamaldehyde in cinnamon,[3] acrolein at parts per million concentrations in water,[4] nicotine in urine and sputum,[5] cocaine on money,[6] and bromoform in swimming pool water.[7] Other experiments are qualitative and include identification of flavor and fragrance components in perfume[8] and in chewing gum and shampoo.[9]

Solid phase microextraction methods have been used extensively in research involving both vegetative and floral volatiles from plants.[10−13] Flowers and wounded vegetative tissue often emit complex blends of volatile organic compounds that may function to attract or deter other organisms in the environment such as insect pollinators or herbivores.[14] These volatile blends can be easily concentrated within low-volatile plastic bags placed around live plant tissues outdoors or in the lab. A SPME fiber is then exposed to the airspace within the bag for sample collection. The majority of floral and vegetative volatiles fall within several well-studied chemical classes including terpenoids, aliphatics, and benzenoids[15] and can be easily identified using standard mass spectral libraries. Furthermore, certain compounds such as linalool and ocimene are extremely common across numerous plant taxa,[15] allowing students to generate similar identifiable results from a broad number of available plants. Finally, several free online databases provide detailed information on individual compounds including molecular structure, biosynthesis, roles as semiochemicals between organisms,[16] and a vocabulary for characterizing common human perceptions of individual scent components.[17]

The experiment presented here was employed in an Instrumental Analysis course taken by chemistry majors during their third year of college. Because SPME is rapid and requires

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minimal sample workup, it makes analysis of complex mixtures much easier to complete during a single laboratory period. Depending upon the course objectives, students can compare different plant materials, the effect of different stationary phases for the extraction fiber, or different chromatographic settings. With this technique, it is possible to analyze a wide range of different samples in a relatively short time.

EXPERIMENT

For this class, each student collected a sample of a different plant material, either flowers or vegetation, from plants on campus. Sample collection, fiber loading, and GC−MS analysis of the samples were done using previously optimized experimental conditions.[18] The seven students in the class each signed up for a 1 h time slot to use the GC−MS. Because the weekly lab time is only 3 h, some students came at other times to run their sample.

The volatile profile of cut plant material changes over time as plant tissue senesces and dies; therefore, students collected fresh plant material shortly before analysis of the volatile sample. Cut plant specimens were brought into the lab and enclosed in a low volatile plastic Reynolds Oven Bag. Oven bags typically have a large volume, but an impulse sealer (American International Electric, City of Industry, CA, 91745) was used to create multiple smaller bags (approximately 4 cm × 6 cm) from a single large oven bag. The smaller bag was sealed around the plant material with a twist tie, and volatiles from the plant tissue were allowed to accumulate within the airspace of the bag for 15 min to equilibrate. Students then inserted a SPME fiber with a 65 μM DVB/PDMS coating into the bag and exposed the fiber for 15 min. The equilibration time, the exposure time, and the SPME fiber can be varied as part of the experimental design. While the fiber was being exposed to the sample, the instructor reviewed the GC−MS parameters and setup.

A Hewlett-Packard 5989B Mass Spectrometer with a 5890 Series II GC was used for analysis. The instrument was controlled using Chemstation G1701 BA Version B.01.00. The GC−MS parameters are included in the Supporting Information. The mass spectra were analyzed using the NIST mass spectral search program V 2.0. Most plant volatile samples yield five or more large chromatographic peaks with numerous smaller peaks. Most large peaks were easily identified in the NIST mass spectral library with good (90% or better) matches.

After identifying the volatile components present in their sample, each student selected several individual compounds for a literature search leading to a short presentation of their experimental results. Students were told to present information on several different scent components using information from Flavornet,[19] the Good Scents Company,[17] and Pherobase.[16] Students were expected to include: CAS RN, retention time, molecular structure, a description of the scent based on human olfactory perception, common plant sources of the compound, and any known biological function.

HAZARDS

There are no special hazards associated with this experiment.

RESULTS

Students identified a wide range of different compounds in this experiment. A subset of identified compounds is listed in Table 1. Identified compounds included monoterpenes, sesquiterpenes, aliphatic compounds, and benzenoid compounds. These compound classifications are based on broadly known biosynthetic pathways, as classified by Knudsen et al.[15]

Table 1. Common Plant Volatiles Identified during This Lab

Compound	Kovats Index[a]	Biosynthetic Class[a]	Formula	Descriptive Adjectives[b]
β-myrcene[c]	1145	monoterpene hydrocarbon	$C_{10}H_{16}$	balsamic, musty, spice, soapy, peppery
(*Z*)-β-ocimene	1245	monoterpene hydrocarbon	$C_{10}H_{16}$	citrus, herb, flower, sweet
α-pinene[c]	1032	monoterpene hydrocarbon	$C_{10}H_{16}$	pine, turpentine, woody, sweet
limonene[c]	1178	monoterpene hydrocarbon	$C_{10}H_{16}$	lemon, orange, minty
linalool[c]	1537	monoterpene alcohol	$C_{10}H_{18}O$	flower, lavender, lemon, sweet
α-terpineol	1688	monoterpene alcohol	$C_{10}H_{18}O$	oil, anise, mint, floral
acetoin	1287	short chain aliphatic	$C_4H_8O_2$	butter, cream, fatty, wet
3-methyl-1-butanol	1205	short chain aliphatic	$C_5H_{12}O$	whiskey, malt, burnt, onion, cheese, balsamic
2-phenylethanol[c]	1925	benzenoid	$C_8H_{10}O$	honey, spice, rose, lilac
benzaldehyde[c]	1495	benzenoid	C_7H_6O	almond, burnt sugar
benzyl acetate	1510	benzenoid	$C_9H_{10}O_2$	fresh, boiled vegetable, burnt, floral, sweet
germacrene D	1705	sesquiterpene hydrocarbon	$C_{15}H_{24}$	woody, spice, oily
caryophyllene[c]	1594	sesquiterpene hydrocarbon	$C_{15}H_{24}$	woody, spice, sweet
1-hexanol	1360	*LOX* pathway product	$C_6H_{14}O$	resin, floral, green, herbal
(*E*)-3-hexen-1-ol	1391	*LOX* pathway product	$C_6H_{12}O$	grasslike, earthy, fresh
(*E*)-3-hexenyl acetate	1327	*LOX* pathway product	$C_8H_{14}O_2$	green, banana, fruity, sharp

[a]Kovats Indices for carbowax column (C20M), from Flavornet.[19] [b]Descriptive adjectives were compiled from adjectives suggested on the databases The Good Scents Company,[19] Flavornet., and Pherobase.[16] [c]Floral compound found in more than 50% of seed plants sampled.[15]

Terpenoid biosynthesis is well understood in plants.[15,20] Monoterpenes are common in both floral and vegetative scents, whereas sesquiterpenes are more commonly associated with purely vegetative scents. Monoterpenes and sequiterpenes are produced via different biosynthetic pathways, but both classes of compounds are constructed by five-carbon isoprene units, and their shared common structure produces characteristic mass fragments and easily recognizable mass spectra. For example, compounds of both classes frequently have strong ion fragments at m/z 93, m/z 121, and m/z 136. Sesquiterpenes additionally tend to have fragments at m/z 161 and m/z 204.

Benzenoid biosynthesis is also relatively well documented in plants.[15,21] The most common benzenoid volatiles are produced via the Shikimate pathway. These compounds are common in floral fragrance, and although their mass spectra are less consistent than the terpenes, they frequently have an m/z 91 fragment ion.[22]

Other plant volatiles include short-chain aliphatics, produced via fatty acid biosynthetic pathways (e.g., acetoin or 3-methyl-1-butanol), C6 compounds produced via the lipoxygenase pathway (e.g., 3-hexen-1-ol or 3-hexenyl acetate), or nitrogen-

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or sulfur-containing volatiles (e.g., indole or dimethyl disulfide) produced via amino acid metabolism.[15]

DISCUSSION

The seven students in this class each analyzed different plant samples. The experiment was run in the spring when many plants were coming into bloom. The plant materials included a variety of flowers and fresh cut vegetation. Students detected a wide range of different volatile compounds from both the floral and vegetative plant specimens. Most samples were dominated by five or more large chromatogram peaks and numerous smaller peaks.

Students encountered several challenges in obtaining useful results. The first challenge was sample collection and preservation. Because the volatile profile of plant material changes after it is cut, students had to coordinate sample collection and scheduling of the instrument. The most significant challenge was getting good library matches from the NIST database. For many samples, students had to use background subtraction, signal averaging, and extracted ion chromatograms to identify accurate retention times and generate a clean mass spectrum for the library search. After obtaining high quality mass spectra, many of the components yielded clear matches in the mass spectral library. Several components, however, did yield ambiguous matches and students were unable to distinguish between several possible structures. Even in these cases, the mass spectra provided clues as to the compound class of the component. As a result, students learned about the strengths and weaknesses of this technique.

This experiment culminated in a 15 min oral presentation by each student, summarizing sampling methodology, GC−MS data analyses, compound identification, and background information on identified components (including common sources, ecological relevance, structure, and perceptual qualities). An unexpected outcome from these presentations was that the students found that many of the compounds were observed in multiple samples. The students did not realize this until they gave their presentations and recognized compounds that they had identified in their plant materials during other student presentations. This provided an opportunity to discuss the biological significance of these compounds. Some of this information came from the suggested databases[16,17,19] used by students when preparing their in-class presentations. In addition, a botanist colleague familiar with this methodology (K. Goodrich) assisted with the experimental protocol, sat in on the student presentations, and provided an interdisciplinary guest lecture on plant chemical communication and biosynthesis related to the common compounds identified by the students. This interdisciplinary piece added a great value to the presentations.

Another benefit of the presentations is that each student approached the presentation differently. During the discussions, students were able to identify effective aspects of each presentation and compare the use of different reference sources. In particular, the contrast between the Flavornet database,[19] the Good Scents Company,[17] and Pherobase[16] helped students understand the importance of interpreting and integrating the sources used for background information. Because different students in the class approached the same material in different ways, the overlap helped the students learn about effective presentation and careful review of the literature.

Based on these results and outcomes, the experiment met the traditional learning objectives for an instrumental analysis course: teaching extraction techniques, gas chromatography, mass spectrometry, background signals, field blanks, and data processing. Student scores were uniformly high on the presentations, with two most recent students receiving the highest score of the semester on the discussion for this experiment. End-of-course evaluations consistently demonstrate student learning and engagement in this course, but these evaluations do not address specific experiments.

CONCLUSIONS

The laboratory design used in this experiment can be easily modified to meet different course objectives. In this class, the experimental technique was previously identified, students started with experimental conditions that were previously optimized and students each collected and analyzed samples independently. In this design, students did not see the connections in the compounds identified until the final presentations.

The experiment could be easily modified so that students receive significantly less guidance on the procedure with a guided inquiry format used to determine the sampling technique and details of the experimental procedure. The variety of tasks is well suited to students working as a team to collect samples, optimize the experiment, and collect data. In addition, the sampling technique, the selection of the SPME stationary phase, the fiber equilibration, GC column selection, and the GC temperature parameters could be optimized by the students. There are a very wide range of experimental questions that can be addressed using this technique and the Supporting Information includes suggestions and references to facilitate these changes. Larger classes might require modification of the chromatography conditions to reduce the time required for each analysis, having students work in groups, or additional time scheduled outside of lab for students to run samples.

ASSOCIATED CONTENT

Supporting Information

Student Instructions; grading and presentation rubrics. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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