Ferrocene-Based Electroactive Derivatizing Reagents for the Rapid Selective Screening of Alcohols and Phenols in Natural Product Mixtures Using Electrospray−Tandem Mass Spectrometry

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The alcohols and phenols of oil of cloves, lemon oil, rose absolut, and oil of peppermint were derivatized with ferroceneoyl azide to generate their ferroceneoyl carbamates. These derivatives are selectively detected at the attomole level, in nanomolar concentrations by electrospray−tandem mass spectrometry (ES−MS/MS) without the need for sample cleanup. The ES−MS/MS analyses of the four essential oils revealed all the expected alcohols, and, in the case of lemon oil, it detected α-terpineol as a trace component that was not readily observed by GC−MS. The ES−MS/MS analyses complements the more conventional GC−MS analysis. The ES−MS method has the advantage of speed, selectivity, and sensitivity over GC−MS for detection of a targeted alcohol of a specific mass or structural type. The ES−MS method does not require a chromatographic separation of the components to accomplish its task. In contrast, GC−MS remains the preferred method for the determination of the total constituents of an oil. The ES−MS method may produce artifact ions, especially if the sample is wet and an excess of the ferroceneoyl azide is used; however, the artifacts did not interfere with the analyses.

The determination of the composition of natural product extracts is an arduous task because of the complexity of the mixtures. Targeted compounds are often present at low concentration, which further complicates their analysis. The development of new methodologies that simplify and accelerate the screening of mixtures for the presence of targeted components for subsequent study increases research productivity.[1] In the present work, the potential of the selective derivatization of alcohols in conjunction with electrospray tandem mass spectrometry (ES−MS/MS) for the rapid selective detection of targeted alcohols from intact, nonpolar, natural product extracts has been examined. This combination is projected to provide selectivity and sensitivity both through the derivatization process and through ES−MS/MS detection. Furthermore, the method is projected to be rapid because the procedure does not require sample workup or separation prior to analysis.

In ES−MS, the analyte in ionic form is transferred from solution into the gas phase by means of an electric field at the tip of a capillary through which the solution of the analyte is passed. The ions then enter the mass spectrometer, where they are separated according to their mass-to-charge ratio (m/z) and detected.[2] With ES−MS, the analytes must be ionic by the time they exit the capillary or they will not be detected.[3] Usually, the pseudomolecular ions, for example, $(M + H)^+$ or $(M + cation)^+$ in positive ion mode or $(M − H)^−$ or $(M + anion)^−$ in negative ion mode, are the major ions from ES responsive analytes that are observed in the ES mass spectrum. Such pseudomolecular ions are the product of Brønsted acid/base or Lewis acid/base chemical processes and as such are formed as a function of analyte functional group chemistry and solution chemistry (e.g., pH). Molecular cation radicals, $M^{•+}$, or anion radicals, $M^{•−}$, may also be observed for analytes with the appropriate redox character under certain ES−MS

operating conditions.[4] To generate the $M^{•+}$ or $M^{•−}$ ion, either the molecule must undergo some form of chemical charge-transfer process,[4,5] or it must be ionized electrolytically in the ES capillary.[6,7] Regardless of the type of molecular ion observed for an analyte, little or no fragmentation is promoted by the ES ionization process.

Polar natural products are ideal candidates for ES−MS analysis because they are either ionic or can be readily ionized by modification of their functional groups through simple solution chemistry manipulations. However, ES−MS cannot be applied directly to most of the smaller, less polar organic compounds found in nonpolar plant extracts. A focus of our research has been the selective "activation" of targeted compound types in such mixtures for ES−MS analysis by means of chemical derivatization.[5,8,9] Because the solvents and most compounds in the nonpolar plant extracts do not directly generate ions in ES−MS, this derivatization approach has the potential both to improve the detectability of particular analytes and to add a high degree of selectivity to an analysis. When combined with tandem mass spectrometry detection (i.e., ES−MS/MS) even greater detection specificity, as well as structural information, may be realized. As applied also to FABMS or secondary ion mass spectrometry (SIMS), two other techniques that also require "preformed ions", this same type of derivatization approach is sometimes called "reverse derivatization".[10] This term arose from the fact that the derivatization typically converts the analyte into a more polar or ionic species, in contrast to the classic derivatization strategies for gas chromatography−mass spectrometry (GC−MS) in which the analyte is made less polar to facilitate volatilization.

The chemistry used in "reverse derivatization" is often based on classical functional group tests that are still used in undergraduate organic chemistry laboratory courses. For example, alcohols are converted into the ionic *N*-methyl-pyridinium ethers by reaction with *N*-methyl-2-fluoro-pyridinium tosylate.[11] Simple aldehydes and ketones may be activated for negative ion ES−MS analysis by deriva-

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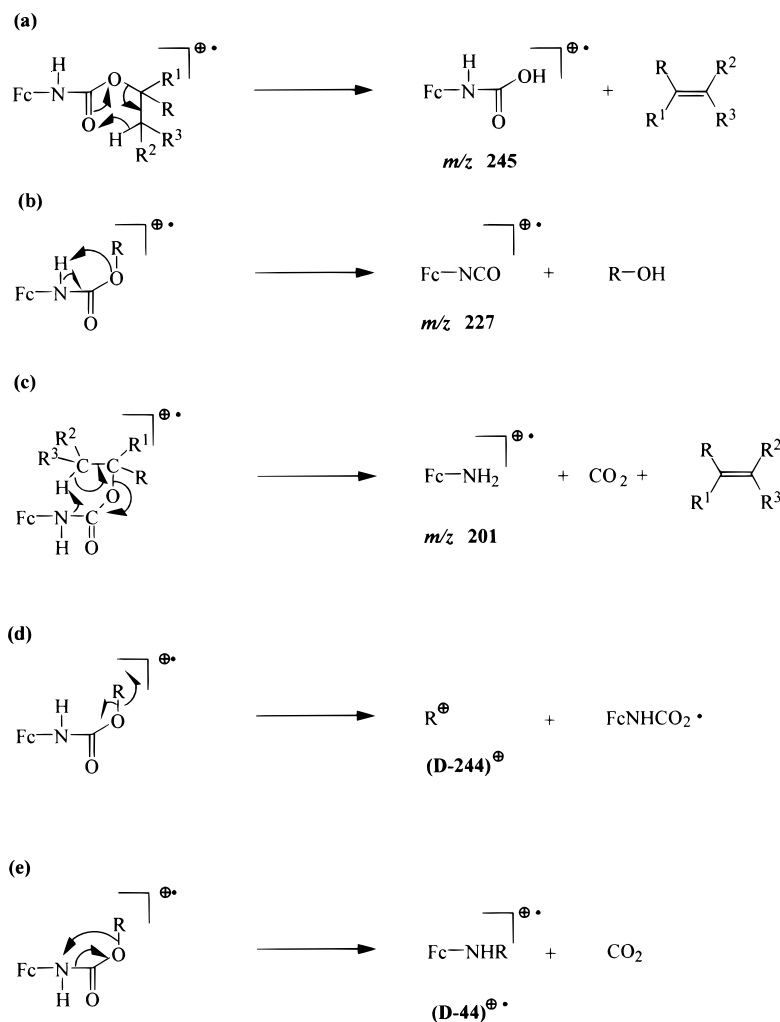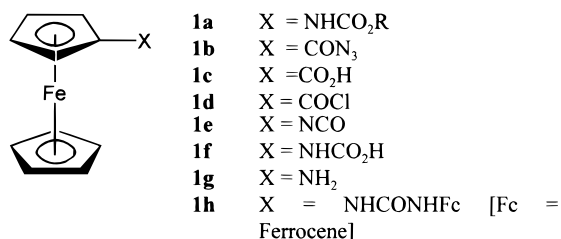† Florida International University.
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tization with 2,4-dinitrophenylhydrazine.[12] The 2,4-dinitrophenylhydrazone is deprotonated on treatment with base, and the anion is detected by ES−MS.[13] In addition to these types of derivatives, we have been exploring the formation of ferrocene-based "electrochemically ionizable" derivatives as an alternative derivatization procedure for enhanced ES−MS and ES−MS/MS analysis of alcohols.[9] These ferrocene-based derivatives are neutral compounds designed to take advantage of the electrolysis process inherent in the operation of the ES ion source for subsequent ionization.[6,7] A range of ferrocene-based derivatizing reagents was developed originally for the selective electrochemical detection of compounds bearing specific functional groups (see Van Berkel et al.[9] and citations therein). These derivatives oxidize at a low positive potential, forming the stable ferrocenium cation, which has proven to be readily detectable by ES−MS.[6,9,14]

In this paper, we describe the selective formation of ferroceneoyl carbamate (**1a**) derivatives of the alcohols in several plant essential oils (selected as the model natural product matrices for this study) combined with selective ES−MS/MS detection for potential use in screening applications. This study proves the principle of the derivatization/detection strategy in screening applications and lays the foundation for subsequent work to target molecules bearing other functional groups. The complementary nature of this approach to the more traditional GC−MS method is also discussed.

Results and Discussion

The Derivatization Strategy. Primary, secondary, and tertiary alcohols react with ferrocene isocyanate, which is generated in situ by the Curtius rearrangement of ferroceneoyl azide, to form the electroactive ferroceneoyl carbamate derivatives (eq 1). These derivatives were selected

$$\text{Fc−CON}_3 \xrightarrow{\text{Heat}} \text{Fc−NCO} \xrightarrow{\text{ROH}} \text{Fc−NHCO}_2\text{R} \qquad (1)$$

as the focus of the current investigation for three reasons.

First, the method can be used to derivatize almost any alcohol. In our laboratories, to date, more than 70 alcohol (primary, secondary, tertiary, allylic, and benzylic) and phenol standards have been prepared and analyzed efficiently by ES−MS. The product (daughter) ion spectra of the molecular ions of each of the derivatized standards have been obtained.[9,16] These studies provide a valuable database for the current study.

Second, the ferrocene component of the ferroceneoyl carbamate products can be detected at very low, nanomolar concentrations by ES−MS. In terms of absolute quantities, the ferroceneoyl carbamates were detectable at the attomole level.[9] The carbamate substituent lowered the oxidation potential below that of ferrocene itself because of the electron-releasing effect of the nitrogen atom that is directly bonded to the ferrocene ring. Thus, the sensitivity

of the derivatized analytes was enhanced further. In contrast, derivatized analytes in which the ferrocene ring is substituted with electron-withdrawing substituents are much less sensitive toward ES−MS analysis because the oxidation potential of the ferrocene unit is increased. For instance, ferroceneoyl chloride (**1d**) proved to be a rather ineffective derivatizing reagent for alcohols. Not only was the reagent not very reactive with alcohols, but also the presence of the electron-withdrawing ester substituent on the ferrocene ring inhibited detection in ES−MS. These results were in keeping with the previous study of Shimada et al.,[17] who used amperometric detection in their analyses of alcohol derivatives of ferroceneoyl chloride and ferroceneoyl azide.

Third, we planned to use ferroceneoyl azide as the starting material for the preparation of a suite of other ferrocene-based derivatizing agents that will be used for the selective detection of other functionalities. Thus, it was important to establish whether this detection strategy would afford the selectivity and sensitivity needed for the analysis of natural products.

Efficiency of the Derivatization Process. The derivatization of alcohol standards using ferroceneoyl azide was shown to be highly efficient. The synthesis of standards on a large scale (ca. 30 mg) usually proceeded in good yields (75−85% yield of isolated product).[9] There were no obvious differences in yields observed in the preparation of primary, secondary, and tertiary alcohols. In fact, the largest problem lay in the isolation; efficiently crystallizing the products proved to be difficult as they were very soluble in most common organic solvents. The derivatization could also be carried out effectively using very low concentrations of alcohol standards (100 nM solutions).

Although our previous studies had demonstrated that the ferroceneoyl carbamate derivatives could be detected at very low concentrations,[9] it was important to establish that the ferroceneoyl azide was capable of efficiently derivatizing the alcohols in a complex matrix, such as an essential oil. Merely detecting the components did not provide proof that the reagent had derivatized all the alcohols in the mixture. Therefore, a sample of peppermint oil, which contains one abundant alcohol, menthol (**2**, 56% w/w), together with trace amounts (<1% w/w) of isomeric alcohols, was analyzed by GC−MS before and after derivatization with an excess of ferroceneoyl azide. The GC−MS analysis revealed that less than 0.1% of menthol remained when the oil was treated with a ca. 5-fold excess of ferroceneoyl azide.

Byproducts of the Reaction. For the analyses of natural product mixtures it would be necessary to use an excess of the derivatization reagent. Furthermore, organic extracts of the natural products are likely to contain some water. Therefore, it was necessary to determine the byproducts resulting from the decomposition of the derivatization reagent itself, and the products of the reaction with water.

Scheme 1. Proposed Origin of the Artifact Ions from Derivatization of Alcohols with Ferroceneoyl Azide

$$\text{Fc−CON}_3 \xrightarrow{\text{Heat}} \underset{(\text{m/z 227})}{\text{Fc−NCO}} \xrightarrow{\text{H}_2\text{O}} \underset{(\text{m/z 245})}{\text{Fc−NHCO}_2\text{H}} \xrightarrow{-\text{CO}_2} \underset{(\text{m/z 201})}{\text{Fc−NH}_2}$$

$$\text{Fc−NH}_2 \;+\; \text{Fc−NCO} \longrightarrow \begin{matrix} \text{FcNH} \\ \diagdown \\ \diagup \\ \text{FcNH} \end{matrix}\!=\!\text{O}$$

$$(\text{m/z 428, M}^{\oplus\bullet}/\text{ m/z 214}^{2\oplus})$$

(a)

(b)

(c)

(d)

(e)

Figure 1. Proposed fragmentation pathways to form (a) m/z 245, (b) 227 (c) 201, (d) (D-244)$^+$, and (e) (D-44)$^+$.

Unreacted ferroceneoyl azide and the ferrocene isocyanate (**1e**), which is formed in situ, will generate the molecular ions at m/z 255 and 227, respectively. The reaction of ferroceneoyl azide with water gives rise to three products. Initially the ferrocene isocyanate will react with water to form the thermally labile ferrocene carbamic acid (**1f**), which gives rise to ions at m/z 245. On heating, **1f** was decarboxylated to generate the amine **1g**, detected at m/z 201. The ferrocene amine can react with ferrocene isocyanate to form the third decomposition product, the ferroceneoyl urethane **1h**, which is usually the major side product. In ES−MS, this urethane generates both a singly charged molecular ion m/z 428 and the doubly charged molecular ion at m/z 214. The reaction pathway to the byproducts is summarized in Scheme 1.

1a	X = NHCO$_2$R
1b	X = CON$_3$
1c	X = CO$_2$H
1d	X = COCl
1e	X = NCO
1f	X = NHCO$_2$H
1g	X = NH$_2$
1h	X = NHCONHFc [Fc = Ferrocene]

ES−MS of Ferrocene Derivatives of Alcohols: Minimal Impact of Reaction Byproducts.

As expected, the molecular ions of ferrocene derivatives of alcohols, D$^{\bullet+}$, are the major ions observed in conventional ES−MS. As symbolized here, D is the mass of the ferrocene carbamate derivative (**1a**) and is equal to (M + 227), where M is the mass of the original analyte. The ES−MS are characterized by a distinctive isotopic pattern in the molecular ion region, which can assist in their identification. The isotope cluster is due to the presence of the iron atom. The ^{54}Fe isotopomer of the derivatives appears as a small peak, ca. 5.8% of the relative abundance of the ^{56}Fe isotopomer, which reflects the relative abundances of the two isotopes. However, at low levels of the targeted derivative or in the presence of chemical noise, this isotopic fingerprint might not be apparent.

More selective detection and some structural information may be obtained by collision-induced dissociation (CID) of the molecular ion in a tandem mass spectrometry experiment.[18] The product ion spectra of the ferroceneoyl carbamate derivatives of alcohols and phenols are somewhat sensitive to the nature of the analyte.[9,16] Thus, phenols produce the m/z 227 ion as virtually the only product ion, whereas most primary, secondary, and tertiary alcohols usually generate three product ions at m/z 201, 227, and 245. The proposed fragmentation pathways to these ions are shown in Figure 1.

Tandem mass spectrometry precursor ion scans, monitoring for one or more of the characteristic fragment ions from the derivatives, allow one to screen rapidly all the ions observed in the spectrum to determine if they are

indeed molecular ions of the derivatized alcohols or phenols. For example, a precursor ion scan for m/z 245 identifies the presence of an alcohol, while the precursor ion scan for m/z 227, in conjunction with the observed absence of an ion at the same m/z from the m/z 245 precursor ion scan, identifies the presence of a derivatized phenol. In general, phenols do not generate the m/z 245 fragment ion. These types of tandem mass spectrometry detection schemes also eliminate most of the problems owing to the presence of the byproduct ions in the ES−MS. None of the byproducts of the reaction fragments to m/z 245. However, the ferroceneoyl urethane byproduct ion at m/z 428 and some other minor byproducts (known and unknown) across the m/z scale generate fragments at m/z 227. This fact somewhat limits the utility of the m/z 227 precursor ion scan for the detection of phenolic derivatives.

Although the precursor ion scans are the most valuable means of confirming the presence of ferroceneoyl carbamate derivatives in the ES−MS, neutral loss scans can also be very useful in identifying the derivatives. The neutral loss mass spectra for the $(D-244)^+$ and $(D-44)^{•+}$ ions are particularly valuable for the identification of simple sterols.[9]

Selection of the Essential Oils. Essential oils are ideal matrixes to test the efficacy of the derivatization strategy for natural product analysis. The oils are often highly complicated, nonpolar mixtures of organic compounds, the vast majority of which cannot be detected directly by ES−MS. Therefore, these oils provide a significant test of the combined derivatization, ES−MS/MS approach to screening for the presence of alcohols without separation or cleanup of the sample, pre- or post-derivatization. All the constituents of an essential oil are sufficiently volatile to be analyzed by GC−MS. Therefore, the results of the ES−MS/MS analysis could be validated by comparison with literature reports[19] or with in-house GC−MS analysis of the oil. A suite of four essential oils was selected for analysis to test various facets of the method. Each of the oils was derivatized and analyzed by ES−MS and ES−MS/MS without purification.

Analysis of Clove Oil: Validation of the Concept of Electroactive Derivatization Method. GC−MS analysis revealed that the oil of cloves sample employed in this study was composed primarily of the phenol eugenol (**3**, 80% w/w), together with small amounts of the isomeric phenol isoeugenol (**4**, 2% w/w) and caryophyllene (8.6%). The ES−MS analysis of the crude reaction mixture of derivatized clove oil produced the expected result as shown by the spectrum in Figure 2a. Essentially one component, the molecular cation radical of the derivatized eugenol and isoeugenol, m/z 391, was observed. Note that the distinctive Fe isotope pattern in the molecular ion region of the derivative is apparent in this spectrum. The singly and doubly charged ions, m/z 428 and m/z 214, respectively, of the ferroceneoyl urethane (**1h**) were also observed, along with an unidentified byproduct at m/z 577. Two additional ions were observed at m/z 398 and 414, which correspond to the $(D + Li)^+$ and $(D + Na)^+$ pseudomolecular ions of the eugenol derivative, respectively. In our studies on ferroceneoyl carbamates of alcohols and phenols such cationized species were almost never observed. The presence of a methoxyl group ortho to the phenolic hydroxyl group makes eugenol (**3**) susceptible to complexing with alkali metal ions. Thus, the presence of cationized pseudomolecular ions of ferroceneoyl carbamates is a good indication of the presence of a heteroatomic species on the carbon adjacent to the hydroxyl group.
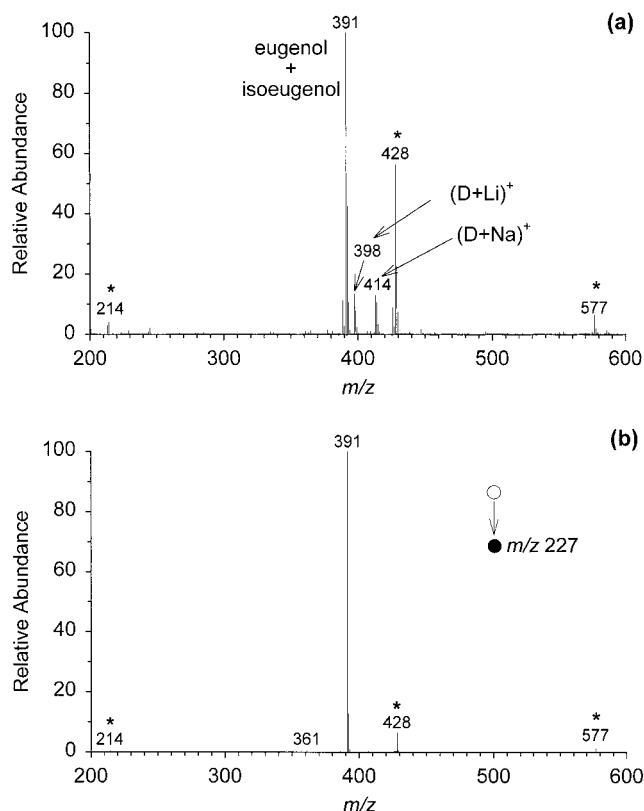
Figure 2. (a) ES−MS of derivatized clove oil and (b) the m/z 227 precursor ion scan spectrum from the same sample (* indicates a byproduct of the derivatization).

In the ES−MS/MS analysis of this derivatized mixture, the ions at m/z 391 were not detected in the m/z 245 precursor ion scan. However, the observation of these ions in the m/z 227 precursor ion scan, shown in Figure 2b, confirmed that they were derived from a derivatized phenol. Furthermore, the reaction byproducts are not a severe impediment to this analysis.

In general, these data indicate that the chemical derivatization coupled with ES−MS/MS analysis is an appropriate screening tool for selective detection of alcohols, as even more examples below will show. Given the slow scan conditions under which these spectra were taken, each spectrum shown took approximately 5−7 min to acquire, which is considerably shorter than a typical GC−MS run (> 30 min). One anticipates that quality spectral acquisition would not require more than half this time and could be reduced even further using mass spectrometers with higher duty cycles, such as ion traps and time-of-flight systems.

Analysis of Peppermint Oil: Demonstration of the Selectivity of the ES−MS Derivatization Method. GC−MS analysis of the peppermint oil sample revealed one abundant alcohol, menthol (**2**, 56% w/w), and five major nonalcoholic components, menthone (24% w/w), menthofuran and an isomer of menthone that coeluted (11% w/w), and menthyl acetate (4% w/w), which together composed more than 95% (w/w) of the oil. Many minor components were detected, including the menthol isomers isomenthol (**5**) and neomenthol (**6**). The menthol concentration for the oil used in this study was rather higher than in some previously reported GC−MS analyses of peppermint oils from different sources.[19−21] The ES−MS of the crude derivatized peppermint oil, shown in Figure 3a, indicated ions at m/z 383, the molecular ion of derivatized menthol, but in this case the reaction byproduct at m/z 428 was the
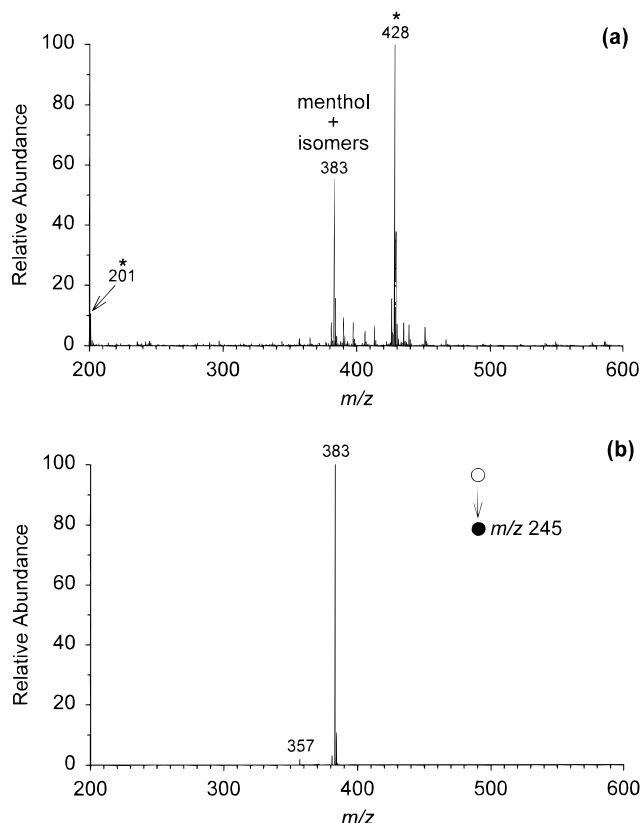
Figure 3. (a) ES−MS of derivatized peppermint oil and (b) the *m/z* 245 precursor ion scan spectrum from the same sample (* indicates a byproduct of the derivatization).
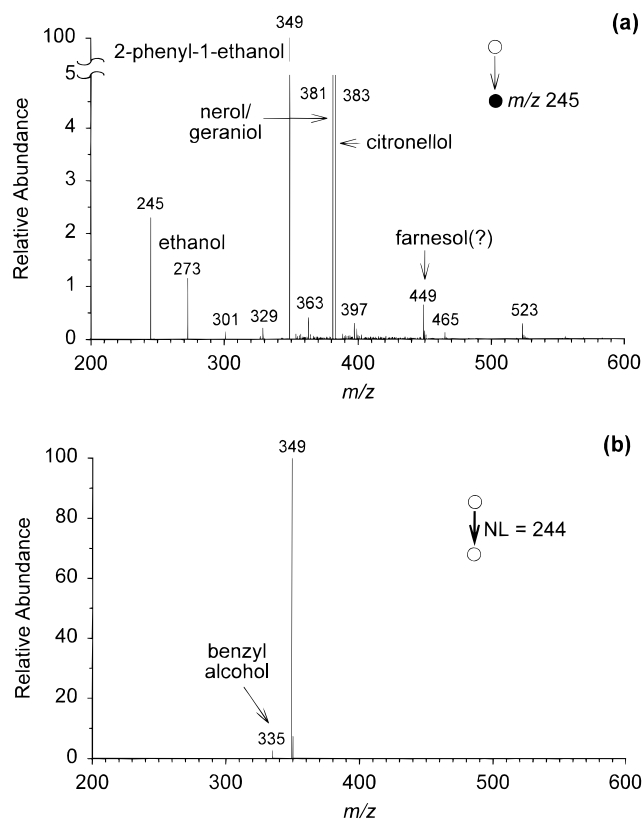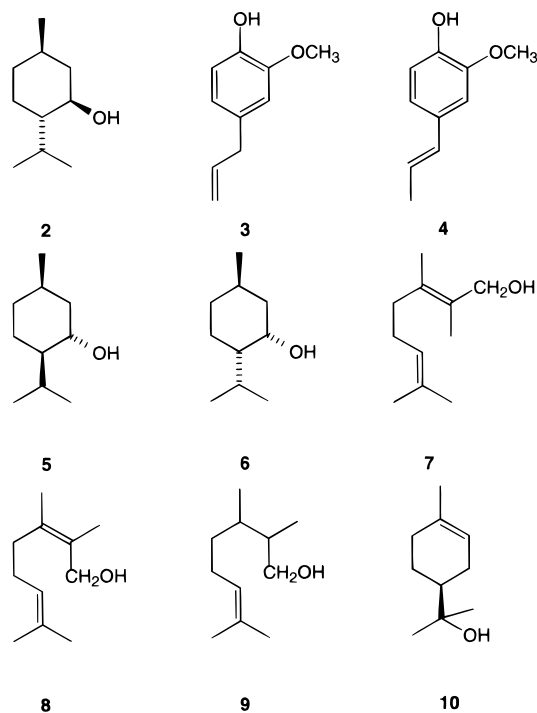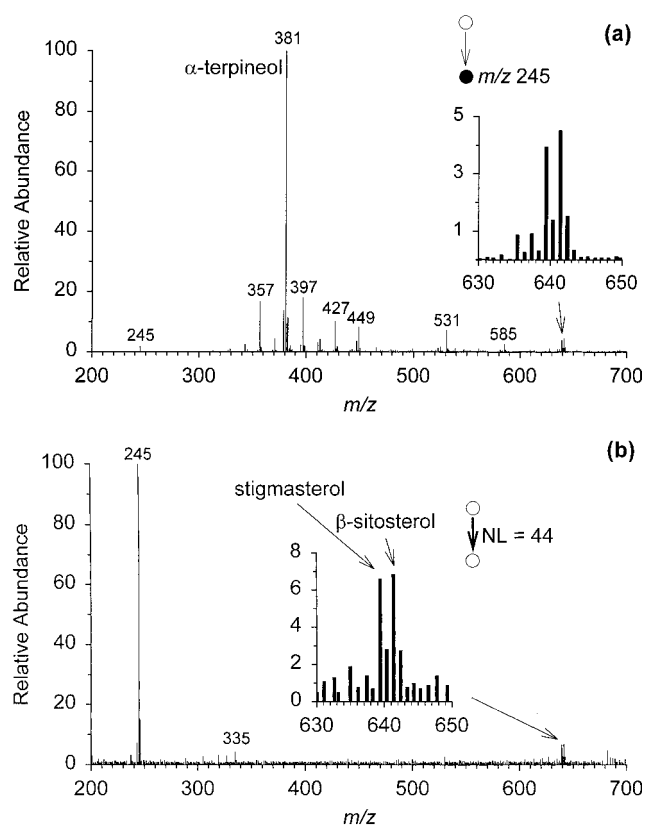

Figure 4. (a) The *m/z* 245 precursor ion scan spectrum of derivatized rose absolut and (b) the neutral loss scan spectrum for (D-244)⁺ from the same sample.

base peak in the spectrum. In addition, there were numerous peaks of much less abundance in the spectrum that could not be assigned with certainty.

The *m/z* 245 precursor ion spectrum of the sample, which is shown in Figure 3b, clarified the origin of the *m/z* 383 peak and the origin of the other peaks. This tandem mass spectrum showed only one major peak, *m/z* 383, which corresponds to derivatized menthol and the minor isomeric isomenthol and neomenthol. It would be necessary to use a separation technique, off-line or on-line, with ES−MS/MS in order to individually identify these isomers. The other peaks that were observed in the mass spectrum are not detected in this specific MS/MS scan, indicating they were not alcohol derivatives from the sample.

Analysis of Rose Absolut: Demonstration of the Ability to Detect Both Abundant and Trace Amounts of Alcohols in a Complex Mixture. Rose absolut, which is an ethanolic extract of *Rosa centifolia*, was used as an example of an oil that contained a variety of alcohols. In the preparation of rose absolut, the ethanol is evaporated after the extraction is complete, leaving the oil. The major alcohol components of rose absolut are 2-phenyl-1-ethanol (73% w/w), the isomeric geraniol and nerol (**7**, **8**, 4% w/w), and citronellol (**9**, 9% w/w), together with lesser amounts of benzyl alcohol (1% w/w). The *m/z* 245 precursor ion scan of the derivatized rose oil (Figure 4a) revealed ions corresponding to the molecular ions of the four derivatized abundant alcohols. In addition, ions corresponding to the molecular ions of derivatized ethanol were also observed. In our GC−MS analysis, it was not possible to detect ethanol in the rose absolut because it eluted with the solvent front.

In addition to this precursor ion scan, a neutral loss scan for (D-244)⁺ was obtained, which confirmed the presence of 2-phenyl-1-ethanol (*m/z* 349) and benzyl alcohol (*m/z* 335)

(Figure 4b). Typically, this neutral loss is observed for simple sterols, such as cholesterol, and aryl alcohols.[16] A plausible mechanism for the fragmentation is shown in Scheme 1.

Analysis of Lemon Oil: Demonstration of the Value of Precursor Ion Scans For Detection of Trace Quantities of Derivatized Alcohols. Lemon oil is composed almost exclusively of a mixture of alkenes. The GC−MS
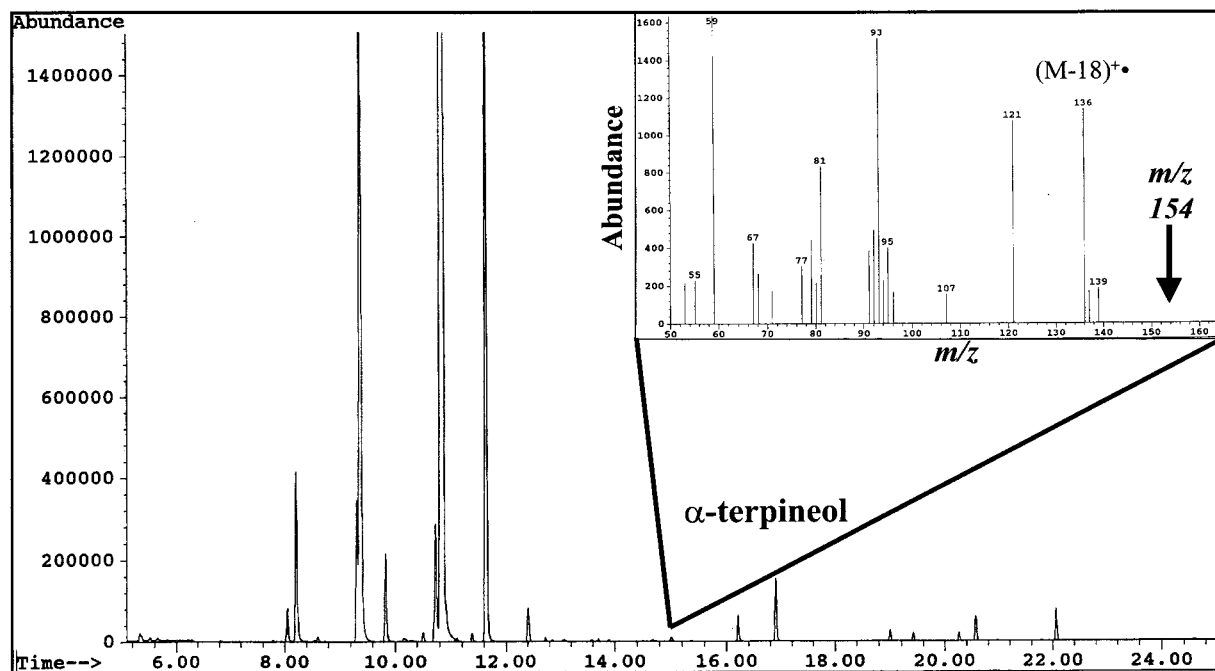
Figure 5. GC−MS chromatogram of lemon oil. Insets show the NIST electron ionization mass spectrum of α-terpineol.

Figure 6. (a) The *m/z* 245 precursor ion scan spectrum of derivatized lemon oil and (b) the neutral loss scan spectrum for (D-44)⁺ from the same sample.

analysis revealed only one minor alcohol component, α-terpineol (**10**, 0.09% w/w) (Figure 5). Thus, this oil presents a severe challenge for the derivatization technique. The ES−MS analysis of the derivatized oil revealed no peaks that could be attributed conclusively to alcohols. However, the precursor ion scan for the *m/z* 245 ion (Figure 6a) proved more fruitful. The base peak in this spectrum at *m/z* 381 corresponds to the mass of the molecular ions of derivatized α-terpineol. Other ions were observed at *m/z* 357, 397, 427,

and 449, which were of uncertain origin. Intriguingly, an ion cluster of very low relative abundance was observed centered at *m/z* 639 and 641. These two ions correspond to the molecular ions of the derivatized phytosterols: stigmasterol and β-sitosterol, respectively. These ions were also apparent for the (D-44)⁺ neutral loss scan (Figure 6b), which is a characteristic fragment of such phytosterols.[9] The compounds were not artifacts or contaminants, because they were absent in blanks. Furthermore, they appeared in the spectra of two different samples of lemon oil.

The Complementary Roles of ES−MS/MS Analyses of Derivatized Alcohols and GC−MS Analyses of Natural Product Mixtures. The GC−MS and ES−MS/MS analyses of essential oils are complementary rather than competitive techniques. The ES−MS/MS analysis of ferroceneoyl carbamate derivatives of alcohols is an excellent tool for the selective and sensitive detection of targeted compound types. The absolute detection levels we have obtained with this method (ca. 100 amol)[9] are superior to the typical detection levels of GC−MS (i.e., 1 pmol).[22] The selectivity and specificity of the ES−MS/MS method is illustrated by the detection of trace quantities of alcohols, such as ethanol in rose oil and α-terpineol in lemon oil. Also, the ES−MS spectrum of the ferrocene derivatives always produce the cation radical as virtually the only ion for a component, thereby providing unambiguously the molecular weight of the alcohol. In GC−MS, the molecular ion of alcohols is often absent from the electron ionization mass spectrum. However, if chemical ionization mass spectrometry is used in place of electron ionization mass spectrometry, the molecular ions usually are obtained.

The combined derivatization, ES−MS/MS analysis method as demonstrated here should prove especially useful as a screening tool for targeted compounds in total organic extracts of natural products. Such preliminary screens (<5 min per sample) can be used to identify those extracts that contain compounds of interest for further, more time-consuming analyses such as GC−MS (>30 min per sample). Moreover, to detect the minor components of an essential oil by GC−MS, it is often necessary to overload the GC column with respect to the major components. Such

processes may somewhat reduce the resolution of the column and will significantly reduce its useful lifetime. Reducing the number of samples necessary to analyze through a rapid screening procedure minimizes the burden on the GC−MS instrumentation.

Nonetheless, GC−MS remains the best method to determine the entire constitution of volatile mixtures. It is also possible to characterize isomeric compounds more precisely than is possible for ES−MS because the electron ionization mass spectra show more structurally significant fragment ions. Similarly, GC−MS will detect hydrocarbons, which are not readily amenable to ES−MS analysis at this time. Thus, a combination of the two methods provides the researcher with great flexibility in the analysis of complex volatile mixtures.

Derivatization of alcohols in essential oils forming electroactive ferroceneoyl carbamates is an efficient process. ES−MS/MS provides a rapid, selective, and sensitive method for the detection of the derivatization products without the need for reaction cleanup. Of the four essential oils examined here, the ES−MS/MS analysis revealed all the expected alcohols, and, in the case of the lemon oil, detected α-terpineol as a trace component that was not readily observed in our GC−MS analyses. The method may produce artifact ions, especially if an excess of derivatizing agents is used, or when the sample is wet; however, the artifacts have not to date interfered with an analysis.

The ES−MS/MS analyses of electroactive derivatives of essential oil alcohols complements the more conventional GC−MS analysis. The ES−MS method has the advantage of speed, selectivity, and sensitivity over GC−MS for detection of a targeted alcohol of a specific mass or structural type. The ES−MS method does not require a chromatographic separation of the components to accomplish its task. In contrast, GC−MS remains the preferred method for the determination of the total constituents of an oil.

There are four distinct themes beyond the one presented here that will be pursued. The first is the evaluation of the approach when coupled with on-line HPLC. Second, the benefits and pitfalls of derivatizations of molecules bearing more than one functionality will be studied. Third, new derivatizing reagents based on the ferrocene carbamate system that will selectively derivatize other functionalities will be tested. And fourth, new forms of selective detection of the ferrocene-based derivative will be pursued, which may be employed in field work. For example, preliminary studies indicate that the ferrocene carbamate derivatives are readily detected on TLC plates because the yellow ferrocene band derivatives turn blue-green on spraying with an oxidizer (e.g., iodine).[16]

Experimental Section

Instrumentation. All ES−MS experiments were performed on either a PE SCIEX API 165 single quadrupole or an API 365 triple quadrupole mass spectrometer (Concord, Ontario, Canada) outfitted with a TurboIonSpray source. A 30-cm long, Teflon encapsulated fused-silica (TEFS) transfer tube (75 μm i.d. fused-silica encapsulated in 1/16 in. o.d. Teflon, CETAC Technologies, Inc., Omaha, NE) connected a 3.5-cm-long stainless steel ES emitter (400 μm o.d., 100 μm i.d.) to the stainless steel 254 μm i.d. bore-through bulkhead grounding port built into the source. The emitter, held at ca. 5.0 kV, was placed 1.5−2.5 cm from the curtain gas plate aperture (1.0 kV) and angled to spray across the aperture. Nitrogen was used for sample nebulization. No "Turbo gas" was used in these experiments. Sample was introduced to the instrument using a syringe pump to deliver solution loaded into 1.0-mL plastic

syringes at a flow rate of ca. 2.5 μL/min. Full-scan mass spectra were acquired using a 0.1-m/z step size and 10-ms dwell time and were typically the average of 10 scans. Precursor ion and neutral loss spectra were the average of 10 scans obtained using a 0.1-m/z step size and 10-ms dwell time with a constant 35 eV laboratory frame of reference collision energy and a collision gas (N_2) thickness of ca. 1.7×10^{15} cm^{-2}.

GC Analyses. Essential oil analyses were carried out on a Hewlett-Packard (Palo Alto, CA) 6890 gas chromatograph coupled to a HP 5973 mass selective detector. HP Chem Station Rev. B. 01.00 software was used for instrument control and data analysis. A 30 m × 0.25 mm HP-5 MS capillary column with a film thickness of 0.25 μm was used. The instrument was set to an initial temperature of 40 °C, held for 1 min, ramped at 8 °C to 300 °C, and held there for 6.5 min. The temperature of the injection port was 250 °C in the splitless mode. The helium flow rate was set at 1 mL/min. The transfer line temperature of the Hewlett-Packard 5973 mass spectrometer was set at 280 °C. The scan range of the mass spectrum was 50−550 Da, and the solvent (chloroform) delay was 5 min.

Semiquantitative GC−MS analyses of the essential oils were carried out by adding nonane or hexadecane to the oils as internal standards (10−25 μg/mL). Assuming a response factor of all compounds in each oil and the internal standards equal to 1.0,[15] the amount of any particular component in the oil was estimated by taking the ratio of the analyte peak area to the area of the internal standard and multiplying this factor by the concentration of the internal standard added. The weight percentage of individual components in the oil was then determined by dividing the concentration of the individual component by the concentration of the oil injected and multiplying this result by 100.

Chemicals. All chemicals were obtained in the purest form possible and used without further purification. All solvents used in this study were HPLC grade unless otherwise specified. Ferroceneoyl azide (**1b**) was prepared from ferrocene carboxylic acid (**1c**) as described previously.[9]

Essential Oils. Lemon oil (*Citrus limonum*, NOW Natural Foods,[7] Glendale Heights, IL), peppermint oil (*Mentha piperita*, NOW Natural Foods[7]), oil of cloves (*Eugenia caryophyllata*, NOW Natural Foods[7]), and Rose Oil Absolut (Cabbage Rose, *Rosa centifolia*, Starwest Botanicals Inc.,[7] Rancho Cordova, CA) were purchased and used without purification.

Derivatization Procedures. *Ferroceneoyl Carbamate Derivatives of Essential Oils.* Ferroceneoyl azide (12.5 mg, ca. 50 μmol) and the appropriate essential oil (ca. 10 μL) were dissolved in dry toluene (0.5 mL) and heated at 90 °C for about 20 min. The solutions darkened from orange-red to brown during the course of the heating. An aliquot of the solution was analyzed directly by ES−MS following dilution [(ca. 50 μM with respect to the ferroceneoyl azide) in CH$_3$CN−CH$_2$Cl$_2$ (1:1 v/v)] containing 100 μM lithium trifluoromethylsulfonate. The derivatization of lemon oil was carried out using 10 times as much oil, because the oil was found to contain less than 0.1% (w/w) alcohols.

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