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Chemical Derivatization for Electrospray Ionization Mass Spectrometry. 1. Alkyl Halides, Alcohols, Phenols, Thiols, and Amines

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Derivatization strategies and specific derivatization reactions for conversion of simple alkyl halides, alcohols, phenols, thiols, and amines to ionic or solution-ionizable derivatives, that is "electrospray active" (ES-active) forms of the analyte, are presented. Use of these reactions allows detection of analytes among those listed that are not normally amenable to analysis by electrospray ionization mass spectrometry (ES-MS). In addition, these reactions provide for analysis specificity and flexibility through functional group specific derivatization and through the formation of derivatives that can be detected in positive ion or in negative ion mode. For a few of the functional groups, amphoteric derivatives are formed that can be analyzed in either positive or negative ion modes. General synthetic strategies for transformation of members of these five compound classes to ES-active species are presented along with illustrative examples of suitable derivatives. Selected derivatives were prepared using model compounds and the ES mass spectra obtained for these derivatives are discussed. The analytical utility of derivatization for ES-MS analysis is illustrated in three experiments: (1) specific detection of the major secondary alcohol in oil of peppermint, (2) selective detection of phenols within a synthetic mixture of phenols, and (3) identification of the medicinal amines within a commercially available cold medication as primary, secondary or tertiary.

The technique of electrospray ionization mass spectrometry (ES-MS) is becoming an increasingly valuable tool for the analysis of a wide range of compounds types in solution (see refs 1–5 for reviews). For example, ES-MS is enjoying widespread use in biopolymer analysis in large part because these analytes (e.g., peptides, proteins, and oligonucleotides) are typically observed as multiply charged pseudomolecular ions, which afford mass measurements using mass analyzers with modest m/z range (i.e., $m/z \leq 4000$). ES-MS is also being applied to the analysis of many types of lower molecular weight organic and biological molecules and inorganic species in solution. However, not all types of analytes are applicable to analysis by ES-MS. The mechanism by which analyte species in solution are transferred via ES to the gas phase as ions is under debate.[6–10] Nonetheless, the best detectability with ES-MS has been achieved in the analysis of analytes either that are ionic in solution or that can be readily ionized in solution via Brønsted or Lewis acid/base chemistry. Neutral, nonpolar molecules that cannot be easily ionized in solution by acid/base chemistry are not typically amenable to the technique.[11,12]

While limiting the compound types amenable to the technique, the selectivity of ES for "preformed ions" minimizes chemical noise. Most organic solvents and nonpolar neutral components present in a sample containing solution-ionizable analytes are not detected. Nevertheless, to increase the analytical utility of ES-MS, we have been investigating methods to expand the range of compound types amenable to the technique and methods to alter the ES behavior/response of specific analytes.[11–18] As part of this work we have been carrying out a comprehensive investigation of chemical methods for conversion of monofunctional organic molecules to "electrospray-active" (ES-active) derivatives, i.e., ionic or solution-ionizable derivatives. Derivatization, along with manipulation of solution chemistry to ionize or neutralize particular analytes, has the potential both to improve the

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detectability of particular analytes in ES-MS and to add a degree of analyte selectivity to an analysis. This derivatization approach is similar to that previously demonstrated for fast-atom bombardment (FAB) and secondary ion mass spectrometry (SIMS).[19-28] Recent reports, both from our group[11,13-18] and from several others,[29-34] have provided an indication of the analytical potential of derivatization for ES-MS analyses.

Three general chemical approaches, collectively referred to here as chemical derivatization methods, are available to effect the solution-phase ionization of analytes, thus making them ES-active derivatives. These three approaches are as follows: (1) ionization by means of Brønsted or Lewis acid/base chemistry, (2) ionization by electron-transfer processes, and (3) chemical transformation of a particular functionality of the analyte into a moiety ionizable in solution using either approach 1 or 2. The nature of the analysis and the chemical characteristics of the analytes of interest dictate the general chemical approach taken, as well as the general synthetic strategy employed when chemical transformation of a functional group is necessary to generate an appropriate derivative. The current work presents derivatization strategies and selected derivatization reactions for conversion of simple alkyl halides, alcohols, phenols, thiols, and amines to ES-active species. These particular compound classes are combined for discussion because the derivatization strategies employed for their transformation to ES-active forms are much the same owing to their similar structural characteristics, i.e., a heteroatom bonded directly either to an sp^3 carbon or onto an aromatic ring. These derivatizations provide for analysis specificity and flexibility through functional group specific derivatization reactions and through the formation of derivatives that can be detected in positive ion mode, negative ion mode, or both (i.e., amphoteric derivatives). The majority of the derivatization methodologies employed are based on either traditional qualitative organic analysis or well-characterized derivatization reactions developed for the enhancement of other analytical methods of detection.[35-39] Therefore, the derivatizations are typically fast, simple, and well documented in the literature, utilize commercially available (or easily synthesized) reagents, and should be applicable for trace or minor component analysis. Application of some of these derivatizations for specific ES-MS detection of analytes in standard and "real-world" mixtures of commercial, environmental, and biomedical interest is demonstrated.

EXPERIMENTAL SECTION

Instrumentation. All ES-MS experiments were carried out using a modified version of a Finnigan-MAT ion trap mass spectrometer (ITMS) adapted to sample from ambient air. Detailed descriptions of this ES-ITMS instrument and its mode of operation have been provided previously.[40-42] The solutions containing the analyte were delivered by continuous infusion (5 μL/min) to a pneumatically assisted ES source as described previously.[43]

Samples. All solvents used in this study were HPLC-grade. All analytes and reagents were obtained from commercial suppliers and used without further purification unless otherwise specified. For ES-MS, solutions were prepared for analysis from the unpurified derivatization products at a concentration of $\sim$50 μM in methanol, dichloromethane, or a mixture of these two solvents. Protonation of analytes for detection in positive ion mode was typically accomplished by addition of 0.1% trifluoroacetic acid by volume to the analyte solution. Deprotonation for detection in negative ion mode was typically accomplished by adding either ammonium hydroxide, sodium hydroxide, or sodium bicarbonate to the analyte solution at the $\sim$1.0 mM level.

Derivatives of Alkyl Halides. *S*-Dodecylisothiouronium bromide (**1**) was prepared by treatment of 1-bromododecane and thiourea in ethanol. On treatment of the isothiouronium salt with sodium hydroxide, sodium dodecyl sulfide (**2**) was formed. The preparations were carried out by the method of Urquhart et al.[44] Trimethyldodecylammonium iodide was prepared from iodomethane and *N,N*-dimethyldodecylamine using the method of Vogel.[39a] Pentanoic acid was prepared from butylmagnesium bromide, which was prepared in situ from 1-bromobutane and magnesium, and carbon dioxide, using the method of Gilman and Kirby.[45]

Derivatives of Alcohols. The *N*-methylpyridyl cholesterol ether (**4**) was prepared by a variation of the method of

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Mukiyama et al.[46] Cholesterol (**3**; 130 mg, 0.33 mmol) was placed in a reactor vial containing a magnetic stirrer and dichloromethane (1.5 mL) and 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (94 mg, 0.33 mmol) were added, forming a slightly cloudy, colorless mixture. On addition of triethylamine (36 mg, 0.35 mmol), the mixture turned canary yellow and then became clear within 30 min. Total reaction time was 1 h.

3-Nitro-2-[(1-methylpropanoxy)carbonyl]benzoic acid (**5**) was prepared by the reaction of 3-nitrophthalic anhydride and 2-butanol, as described by Vogel.[39b] 4-(Methoxycarbonyl)-3-pyridinecarboxylic acid (**7**) and 3-(methoxycarbonyl)-4-pyridinecarboxylic acid (**8**) were prepared by the reaction of 3,4-pyridinedicarboxylic anhydride (**6**; 400 mg, 2.7 mmol) with methanol (0.5 mL) in a similar fashion.

4-(*tert*-Butanoxycarbonyl)-3-pyridinecarboxylic acid and 3-(*tert*-butanoxycarbonyl)-4-pyridinecarboxylic acid were prepared by a variation of the method of Davies et al.[47] *tert*-Butyl alcohol (296 mg, 4 mmol) was treated with triethylamine (454 mg, 4 mmol) and 3,4-pyridinedicarboxylic anhydride (596 mg, 4.5 mmol) at 90 °C for 8 h.

Dodecanoic acid was prepared from the reaction of 1-dodecanol and potassium permanganate as described by Vogel.[39c]

Derivatives of Phenols. (*p*-Chlorophenyl)-1-(dimethylamino)naphthalene-5-sulfonate (**11**) was prepared by treatment of *p*-chlorophenol (**10**) with 1-(dimethylamino)-naphthalene-5-sulfonyl chloride (dansyl chloride, **9**) as described by Frei-Hausler et al.[48] Potassium *p*-phenylphenoxide was prepared by dissolution of *p*-phenylphenol (**12**) in a 1.0 mM solution of potassium hydroxide in methanol.

Derivatives of Thiols. Sodium phenyl sulfide was prepared by dissolving benzenethiol in methanol containing ~1.0 mM sodium hydroxide.

Derivatives of Amines. The 2,4-diphenyl-6-ethylpyridinium tetrafluoroborate derivative of butylamine (**18**) was prepared by treating butylamine (8 mg, 1.1 mmol) in methanol (10 mL) with 2,4-diphenyl-6-ethylpyrylium tetrafluoroborate (**17**; 34.8 mg, 1 mmol) and triethylamine (11 mg, 1.1 mmol).[39d] The yellow mixture was sonicated (5 min) until the solid dissolved. Glacial acetic acid (4 mL) was added, and the solution was sonicated for a further 30 min.

A mixture of 3-nitro-2-[(butylamino)carbonyl]benzoic acid (**19**) and 3-nitro-2-[(*N,N*-dibutylamino)carbonyl]benzoic acid (**21**) was prepared from a mixture of butylamine (73 mg, 1.0 mmol) and dibutylamine (129 mg, 1.0 mmol). The amine mixture was treated with 3-nitrophthalic anhydride (386 mg, 2.0 mmol) that was dissolved in dichloromethane (6 mL), then hexane (3 mL) was added, and the clear solution was warmed on a water bath (20 min). The products **19** and **21** precipitated from solution as a white sticky solid.[39e] A portion of the product (58 mg) was heated for 20 min in an oven to 160 °C to form the phthalimide **20** from **19**.

Selective ES-MS Detection of Mixture Components via Derivatization. *A. Detection of Menthol in Oil of Peppermint.* Oil of peppermint (200 mg, Frontier Cooperative Herbs, Norway, IA) was treated with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (300 mg, 1.1 mmol) and triethylamine (110 mg, 1.1 mmol) as described above for the derivatization of cholesterol. The product, a bright yellow liquid, was analyzed by positive ion ES-MS without purification following dilution in dichloromethane.

B. Selective Detection of Phenols by Variation of Solution pH. Stock solutions (50 μM) of phenol, 2,4-dichlorophenol, pentachlorophenol, and 2,4,6-trinitrophenol (picric acid) in methanol were prepared. A sample mixture containing each of the four phenols (12.5 μM for each phenol) was prepared by diluting the appropriate volume of each phenol stock solution in methanol. This mixture was split into three aliquots. The first aliquot was analyzed by negative ion ES-MS without addition of any base. Sodium hydroxide was added to the second (0.2 mM) and third (1.0 mM) aliquots prior to their analysis in negative ion mode.

C. Selective Detection of Amines in Triaminicol. Triaminicol (80 mL) (Sandoz Pharmaceuticals Corp., East Hanover, NJ) contains the amine salts phenylpropanolamine hydrochloride (**26**; 6.68 mM), dextromethorphan hydrobromide (**27**; 2.84 mM), and chlorpheniramine maleate (**28**; 0.515 mM) as the active ingredients. A solution of the untreated medication was analyzed by positive ion ES-MS following dilution in methanol. To extract the amines from the Triaminicol prior to derivatization, aqueous sodium hydroxide (6 mL, 7.0 M) was added to the medication, turning the solution cloudy and gradually changing the color from red-pink to orange. The neutralized amines were extracted from the aqueous phase with dichloromethane (3 × 20 mL), and the pink-tinged organic extracts were combined. After drying over calcium sulfate, the extract, which remained slightly cloudy, was evaporated. The residue was dissolved in dichloromethane (20 mL), and the solution was divided into three equal aliquots.

The first aliquot was analyzed by positive ion ES-MS before and after acidification with trifluoroacetic acid to protonate the amines. The second aliquot was used for the quaternization of the amines. The dichloromethane was evaporated, and then the residue was warmed (35 °C) with iodomethane and methanol (2 mL, 1:1 v/v) for 90 min and left to stand at room temperature for several days. The product, diluted in methanol, was analyzed by positive ion ES-MS without further purification. The third aliquot was used for the derivatization of phenylpropanolamine (**26**) with 3-nitrophthalic anhydride. The dichloromethane was evaporated, and then the residue, which contained ~0.1 mmol of phenylpropanolamine, was redissolved in dichloromethane. 3-Nitrophthalic anhydride (31 mg, 0.16 mmol) was added to this solution, followed by hexane (0.5 mL). The solution, which immediately turned cloudy, was warmed for 10 min. During this time, an off-white precipitate was formed as most of the solvent evaporated. The solid was dissolved in methanol containing sodium hydroxide and was analyzed in negative ion mode.

RESULTS AND DISCUSSION

Alkyl halides, alcohols, phenols, thiols, and amines can be considered to have the generic formula RX, where R is an alkyl or aryl group and X is a halogen, OH, SH, or NR_2 as appropriate. The reactivity of the polarized carbon–heteroa-

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Table 1. Summary of Routes to ES-Active Derivatives of Alkyl Halides

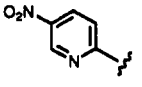
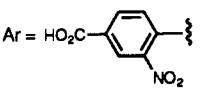
A. Derivatives via Nucleophilic Substitution[a]

overall reaction: $RX + Nu^- \rightarrow RNu + X^-$

eq	deriv reagent	product with RX	deriv ion detected	*m/z* deriv ion[b]	comment[c]
1	$(NH_2)_2C{=}S$	$RSC(NH_2){=}NH_2^+X^-$	$RSC(NH_2){=}NH_2^+$	(M + 76 − X)	works best for 1[y] alkyl bromides and iodides[44] fails for 3[y] halides
2	(1) $(NH_2)_2C{=}S$ (2) NaOH	RS^-	RS^-	(M + 32 − X)	works best for 1[y] alkyl bromides and iodides[44] fails for 3[y] halides
3[d]	$(C_2H_5)_3N$	$[RN(C_2H_5)_3]^+X^-$	$[RN(C_2H_5)_3]^+$	(M + 101 − X)	works best for 1[y] alkyl halides[39a] fails for 3[y] halides

B. Derivatives via Elimination and Modification of the Alkene Product[18]

overall reaction: $R_2CHCR_2X + (CH_3)_3CO^-K^+ \rightarrow R_2C{=}CR_2 \xrightarrow{'A\text{-}B'} R_2CACR_2B$

$^tBu = (CH_3)_3C$ Py =  Ar = 

deriv reagent	product with R_2CHCR_2X	deriv ion detected	*m/z* deriv ion[b]	comment[c]
(1) KO^tBu[e] (2) OsO_4 (3) $NaHSO_3$	R_2COHCR_2OH	$[(R_2COHCR_2OH) + Na^+]^+$	(M + 56 − X)	eliminations work best for 3[y] halides complex with Na^+[18]
(1) KO^tBu[f] (2) PySCl	R_2CClCR_2SPy	$[(R_2CClCR_2SPy - HCl) + H^+]^+$	(M + 154 − X)	protonate in acid[18]
(1) KO^tBu[g] (2) ArSCl	R_2CClCR_2SAr	$[(R_2CClCR_2SAr) - H^+]^-$ or $[(R_2CClCR_2SAr - HCl) - H^+]^-$	(M + 231 − X) or (M + 195 − X)	deprotonate in base[18]

C. Derivatives via Grignard Reaction

overall reaction: $RX + Mg \rightarrow RMgX \xrightarrow{CO_2} RCO_2H$

eq	deriv reagent	product with RX	deriv ion detected	*m/z* deriv ion[b]	comment[c]
4[h]	(1) Mg (2) CO_2	RCO_2H	$[(RCO_2H) - H^+]^-$	(M + 44 − X)	works for 1[y], 2[y], and 3[y] alkyl halides[45] deprotonate in base

[a] Reactions which occur by the S_N2 mechanism are preferred. [b] M, mass of original analyte. [c] Key: 1[y], primary, 2[y], secondary; 3[y], tertiary. [d] Other tertiary amines could be used. Tertiary phosphines (R_3P) could be used in place of tertiary amines. [e] The diol could also be produced with either $KMnO_4$ or a peroxy acid followed by opening of the epoxide with aqueous acid or base. [f] The product undergoes elimination of HCl before detection by ES–MS.[18] [g] The product might also undergo elimination of HCl.[18] [h] Reaction requires anhydrous conditions and will not work if the analyte bears any acidic hydrogens (e.g., O*H*).

tom bond ($C^{\delta+}$–$X^{\delta-}$) largely dictates the synthetic strategies that may be employed to convert these functionalities into ES-active moieties. Thus, for an analyte substituted with any one of these functional groups, there are three potentially valuable and general synthetic routes/strategies for the generation of ES-active derivatives.

The first of these routes is the generation of derivatives by nucleophilic substitution of X using a nucleophile that yields an ionic or readily ionized product. Typically, such nucleophilic substitutions occur via either the S_N2 or the S_N1 mechanism. It is preferable to carry out the reaction via the stereospecific S_N2 mechanism, which avoids the potential problems of skeletal rearrangement and elimination that accompany the S_N1 reactions. This route is especially important for alkyl halides, because halides are excellent leaving groups.

The second route is the generation of derivatives by modification of the X group, taking advantage of the characteristic chemical properties of the X group for each functionality. For instance, by simple modification of solution pH it is possible to generate ions of phenols and thiols, which are weak to moderate acids, and amines, which are weak to moderate bases. Alternatively, it is possible to take advantage of the nucleophilicity of the X group, as in the case of tertiary amines, which form quaternary ammonium salts with alkyl halides. The only compound class for which modification of the X group is of little value is the alkyl halides.

The third route involves the generation of alkenes by elimination of HX from the RX molecule. The only structural requirement for this route is the presence of a hydrogen on a carbon α to the C–X bond. The alkene may then be derivatized using methods discussed elsewhere (see Table 1).[18] Although this route is rather lengthy, it might prove useful for molecules where R is a tertiary alkyl moiety. Such compounds do not undergo S_N2 reactions, and often it is difficult to modify X because of steric hindrance.

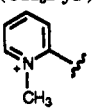
Although the general approach to forming ES-active derivatives of each of the functional groups discussed here follows one or more of the three routes presented above, the singular properties of each of the functional groups necessitate discussion of specific reactions and derivatives for each separately. The general synthetic strategies/routes used to form ES-active derivatives from the respective functionalities, the derivatives formed, as well as proposed derivatives, the derivative ions detected (or expected) in the ES mass spectrum, and the *m/z* value of the derivative ions, relative to the mass

Table 2. Summary of Routes to ES-Active Derivatives of Alcohols, Phenols, and Thiols

A. Derivatives via Nucleophilic Substitution of Alcohols and Thiols with Pyridinium Salts

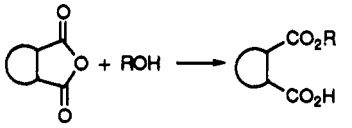
overall reactions: $ROH + (CH_3PyF)^+ \rightarrow (CH_3PyOR)^+$

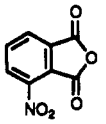
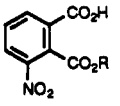
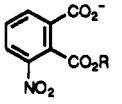
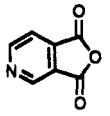
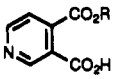
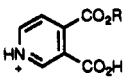
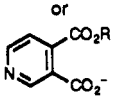
$RSH + (CH_3PyF)^+ \rightarrow (CH_3PySR)^+$

$CH_3Py =$ 

eq	substr	deriv reagent	product with ROH/RSH	deriv ion detected	*m/z* deriv ion[a]	comment[b]
5	ROH	(1) $(CH_3PyF)^+$ (2) $(C_2H_5)_3N$	$(CH_3PyOR)^+$	$(CH_3PyOR)^+$	(M + 92)	works for 1^y, 2^y alcohols[46,49] fails for 3^y alcohols
12	RSH	(1) $(CH_3PyF)^+$ (2) $(C_2H_5)_3N$	$(CH_3PySR)^+$	$(CH_3PySR)^+$	(M + 92)	works for 1^y, 2^y, 3^y thiols[51,52]

B. Derivatives via Reaction of Alcohols and Thiols with Cyclic Anhydrides

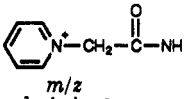
overall reaction:[c] 

eq	substr	deriv reagent	product with ROH	deriv ion detected	*m/z* deriv ion[a]	comment[b]
6	ROH				(M + 192)	1^y, 2^y alcohols work best[39b] 3^y alcohols need base catalyst[47] deprotonate in base
7	ROH				(M + 150)	1^y, 2^y alcohols work best[39b] 3^y alcohols need base catalyst[47] protonate in acid[d]
				or 	(M + 148)	deprotonate in base[d]

C. Derivatives of Primary and Secondary Alcohols via Oxidation

overall reactions: $RCH_2OH \xrightarrow{[O]} RCO_2H$

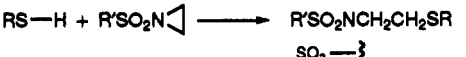
$R_2CHOH \xrightarrow{[O]} R_2C{=}O \xrightarrow{GNH_2} (R_2C{=}NG)^+$

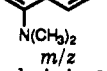
[O] = oxidant G = 

eq	substr	deriv reagent	product with ROH	deriv ion detected	*m/z* deriv ion[a]	comment[b]
8	RCH_2OH	$KMnO_4$	RCO_2H	$[(RCO_2H) - H^+]^-$	(M + 13)	works for 1^y alcohols only[39c] many other oxidants may be used
	R_2CHOH	(1) $KMnO_4$ (2) $(GNH_2)^+$	$(R_2C{=}NG)^+$	$(R_2C{=}NG)^+$	(M + 132)	works for 2^y alcohols only[39c] ketone product is derivatized with Girard's reagent P[34]

D. Derivatives via Reaction of Phenols and Thiols with Derivatives of Sulfonic acid

overall reactions: $ArOH + R'SO_2Cl \rightarrow R'SO_2OAr$



Ar = aromatic DANS = 

eq	substr	deriv reagent	product with ROH/RSH	deriv ion detected	*m/z* deriv ion[a]	comment
9	ArOH	DANS-Cl	DANS-OAr	$[(DANS\text{-}OAr) + H^+]^+$	(M + 234)	general derivatives for phenols[48] good derivative for optical detection[38]
14	RSH	DANS—N	$DANS\text{-}NCH_2CH_2SR$	$[(DANS\text{-}NCH_2CH_2SR) + H^+]^+$	(M + 276)	good derivative for optical detection[54]

Table 2 (Continued)

E. Derivatives via Deprotonation of Phenols and Thiols

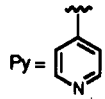
overall reactions: $ArOH + OH^- \rightarrow ArO^- + H_2O$

$RSH + OH^- \rightarrow RS^- + H_2O$

eq	substr	deriv reagent	product with ROH	deriv ion detected	m/z deriv ion[a]	comment
10	ArOH	NaOH	ArO^-	ArO^-	(M − 1)	can selectively prepare some phenoxides by modifying solution pH
11	RSH	NaOH	RS^-	RS^-	(M − 1)	can selectively prepare some sulfides by modifying solution pH

F. Derivatives of Thiols via Addition of 4-Vinylpyridine

overall reaction: $RSH + PyCH{=}CH_2 \rightarrow PyCH_2CH_2SR$

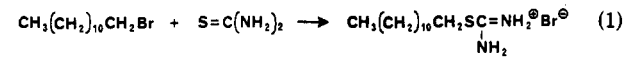
Py = 

eq	substr	deriv reagent	product with RSH	deriv ion detected	m/z deriv ion[a]	comment
13	RSH	(1) $PyCH{=}CH_2$ (2) $NaOC_2H_5$	$PyCH_2CH_2SR$	$[(PyCH_2CH_2SR) + H^+]^+$	(M + 106)	could quaternize with CH_3I, but product might be unstable[29,53]

[a] M, mass of original analyte. [b] Key: 1°, primary; 2°, secondary; 3°, tertiary. [c] The reaction was not carried out with thiols; however, the derivatization is well-known. [d] The 4-pyridinecarboxylic isomer may also be formed in significant amounts (see eq 7).

of the original analyte, are summarized in Tables 1–3.

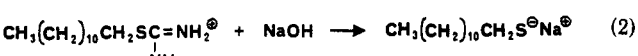
Alkyl Halides (Table 1). Primary and secondary alkyl halides can be derivatized readily to yield ionic products by nucleophilic substitution reactions. We found the formation of the *S*-alkylisothiouronium salt via reaction with thiourea to be an especially valuable reaction (eq 1).[44] Not only is it



$$CH_3(CH_2)_{10}CH_2Br + S{=}C(NH_2)_2 \rightarrow CH_3(CH_2)_{10}CH_2SC(=NH_2)^{\oplus}Br^{\ominus} \quad (1)$$

1

possible to obtain ES-MS spectra in the positive ion mode, but it is also possible to modify the salt to the produce the alkyl sulfide anion (eq 2), which provides the opportunity to

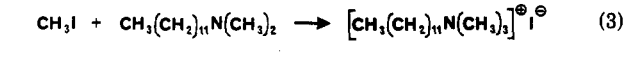


$$CH_3(CH_2)_{10}CH_2SC(=NH_2)^{\oplus} + NaOH \rightarrow CH_3(CH_2)_{10}CH_2S^{\ominus}Na^{\oplus} \quad (2)$$

2

analyze for the derivative in negative ion mode. The major ions observed in the ES mass spectra of each derivative (m/z 245 and 201, respectively) correspond in mass to the expected molecular ionic species, i.e., $(M + 76 - X)^+$ and $(M + 32 - X)^-$, respectively, where M is the mass of the original alkyl halide and X is the mass of the halide.

Preparation of the quaternary ammonium salt from an alkyl halide, using a tertiary amine as the derivatizing agent, is another valuable route to ionic derivatives. The reaction between iodomethane and *N,N*-dimethyldodecylamine shown in eq 3 provides an illustration of this derivatization. This

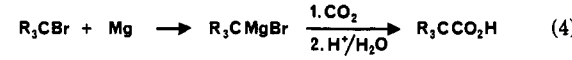


$$CH_3I + CH_3(CH_2)_{11}N(CH_3)_2 \rightarrow [CH_3(CH_2)_{11}N(CH_3)_3]^{\oplus}I^{\ominus} \quad (3)$$

reaction scheme is the reverse of the more common derivatization in which a tertiary amine is quaternized by reacting with an alkyl halide[19,21,22,27,42a] and can be carried out using a wide range of tertiary amines, including the readily available triethylamine. As expected, the positive ion ES mass spectrum of this derivative contains a single ion at m/z 228.

Alternatively, the derivatization in eq 3 could be carried out by quaternization of a tertiary phosphine (R_3P) in place of the tertiary amine. The product of this reaction would be the corresponding phosphonium salt. Such compounds have already been employed in ES-MS studies.[32]

Compared to primary and secondary alkyl halides, tertiary alkyl halides are more difficult to derivatize as they do not undergo any of the nucleophilic substitutions discussed above. One possible route to suitable derivatives of such compounds would start with the base-catalyzed dehydrohalogenation of the alkyl halide to yield the alkene. The resulting alkene may then be converted to an ionic species using one of the approaches discussed in detail elsewhere (see Table 1).[18] For instance, the 1,2-diol, which can be made ionic in solution through formation of a metal ion adduct, could be prepared by reaction with osmium tetraoxide or by hydrolysis of the oxirane generated by treatment of the alkene with a peroxy acid.[18] An alternative route is to convert the tertiary alkyl halide into a carboxylic acid via preparation of a Grignard reagent followed by reaction with carbon dioxide as shown in eq 4.[45] The effectiveness of this approach was demonstrated

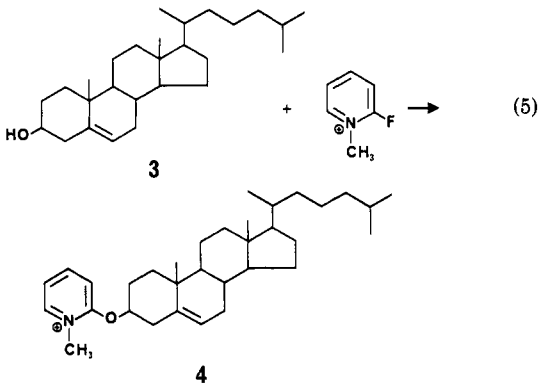


$$R_3CBr + Mg \rightarrow R_3CMgBr \xrightarrow[2.\,H^+/H_2O]{1.\,CO_2} R_3CCO_2H \quad (4)$$

by preparing the pentanoic acid derivative of the primary alkyl halide 1-bromobutane. The pentanoic acid derivative was detected in negative ion mode as the carboxylate anion at m/z 101, which corresponds in mass to $(M + 44 - X)^+$. Although this reaction sequence is rather lengthy, and requires anhydrous conditions, it provides a demonstrated route to derivatives of tertiary,[45] as well as primary and secondary, alkyl halides. In addition, it illustrates the potential value of organometallic chemistry for preparation of ES-active derivatives.

Alcohols (Table 2). Alcohols do not undergo S_N2 reactions very efficiently because OH is a poor leaving group. In addition, most alcohols are insufficiently acidic or basic to form stable ions by modification of solution pH. Therefore, the most useful and general routes to produce ES-active derivatives of alcohols make use of reactions in which the oxygen acts as a nucleophile.

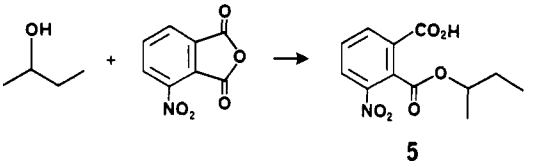
Primary and secondary alcohols can be converted into their *N*-methylpyridyl ether salts by nucleophilic displacement of the fluorine of 2-fluoro-1-methylpyridinium *p*-toluenesulfonate by the oxygen of the alcohol.[46,49] An example of this reaction, which occurs under mild conditions and is therefore suitable for the study of biological molecules, is shown in eq 5 for the



(5)

preparation from cholesterol (**3**) of its 1-methylpyridinium derivative **4**. The ES mass spectrum of **4** has as the major ion the expected molecular species at m/z 478, which corresponds to $(M + 92)^+$, where M is the mass of the original alcohol.

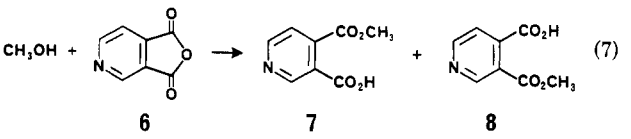
Reaction of primary, secondary, or tertiary alcohols with cyclic anhydrides provides a route to form a highly versatile class of ES-active derivatives. Selection of an appropriately substituted anhydride allows for formation of derivatives detectable in either positive ion mode or in negative ion mode or for formation of derivatives detectable in one mode or the other depending on solution pH (i.e., an amphoteric derivative). For example, anionic derivatives of primary and secondary alcohols may be generated by reaction between 3-nitrophthalic anhydride and 2-butanol as shown in eq 6. Although the



(6)

alcohol might be expected to attack either of the carbonyl moieties of the unsymmetrical anhydride, thereby yielding two isomeric products, one isomer, the 3-nitrobenzoic acid derivative **5** is formed almost exclusively.[39b] The carboxylic acid moiety of **5** is readily converted to the carboxylate anion in a basic solution and is observed in the negative ion ES mass spectrum at m/z 266, which corresponds to $(M + 192)^-$.

The use of 3,4-pyridinedicarboxylic anhydride (**6**) as the derivatizing reagent for alcohols results in formation of an amphoteric derivative (**7** and/or **8**; eq 7) that can be analyzed

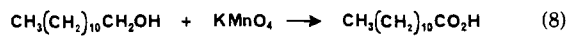


(7)

in either positive ion mode (by protonation of the pyridine ring in acidic solution) or negative ion mode (by deprotonating the carboxylic acid moiety in basic solution). In the positive ion mass spectrum of the product of the reaction of methanol with **6**, the major ion observed (m/z 182) is the protonated form of the derivative, corresponding to $(M + 150)^+$, relative to the mass of the original alcohol, M. In the negative ion mass spectrum, the major ion observed (m/z 180) is the deprotonated form of the derivative, corresponding to $(M + 148)^-$. Thus, with this amphoteric derivative, the ionization mode which provides the best detectability for the derivative (i.e., the best signal-to-noise ratio) can be selected merely by adjusting solution pH. Note that it is possible to generate two isomeric products (**7** and **8**) from the reaction in eq 7. However, the 3-pyridinecarboxylic acid isomer **7** may predominate as Ashcroft et al.[50] have reported this isomer to be the major product from the methanolysis of **6**.

Cyclic anhydrides are also important derivatizing reagents because they provide a means to form ES-active derivatives of tertiary alcohols. Tertiary alcohols are difficult to derivatize because steric hindrance makes it difficult to effect substitution reactions at the oxygen functionality. However, Davies et al.[47] demonstrated that derivatization of tertiary alcohols with cyclic anhydrides may be effected by prolonged heating in the presence of a slight excess of a tertiary amine. With this approach, we successfully derivatized *tert*-butyl alcohol using **6**. The major ion observed in the ES mass spectrum (m/z 222) is the expected ion for the deprotonated derivative, which corresponds in mass to $(M + 148)^-$, relative to the original alcohol, M. In positive ion mode, no peak was observed for the protonated form of the derivative. Apparently, the *tert*-butyl form of this derivative is not stable in an acidic solution.

Although derivatizations of alcohols by reactions in which the oxygen acts as a nucleophile are the most generally applicable, it is also possible to take advantage of the facile oxidation of primary and secondary alcohols to produce ionic derivatives. Primary alcohols are readily oxidized to carboxylic acids as shown in eq 8 for the oxidation of 1-dodecanol to



(8)

dodecanoic acid using potassium permanganate. The resulting carboxylic acid is readily detected as the carboxylate anion in negative ion ES-MS when sprayed from a basic solution.

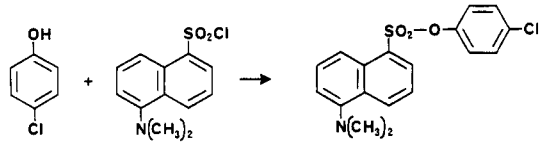
Secondary alcohols can be oxidized under similar conditions to form the corresponding ketones, but the ketone must be derivatized to yield an ionic product such as a Girard P

(49) Testino, S. A.; Busch, K. L.; Haseltine, J. N. In *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*, Washington, DC, May 31–June 5, 1992; pp 1539–1540.

(50) Ashcroft, W. R.; Beal, M. G.; Joule, J. A. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3012–3015.

derivative.[25,27,34] Although this route is rather time-consuming it provides the option of working with a water-soluble derivative.

Phenols (Table 2). A large number of the derivatization strategies applicable to the generation of ES-active forms of phenols closely resemble those strategies used with the alcohols, and therefore, they need not be discussed in detail. However, one derivatization specific for phenols (and also useful for amines) is the reaction with 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride, **9**), which forms a sulfonate that can be protonated in acidic solution. The reaction between the priority pollutant *p*-chlorophenol (**10**) and dansyl chloride in eq 9 illustrates the reaction. The positive ion ES mass



(9)

10 **9** **11**

spectrum of **11** shows a cluster of ions at m/z 362 as expected for the protonated form of this chlorinated derivative. These ions correspond in mass to $(M + 234)^+$ relative to the original phenol, M. Also observed in the spectrum is an ion at m/z 171 (55% relative intensity), which is probably formed by collision-induced dissociation of **11** in the atmospheric sampling interface.

It should be pointed out that dansyl derivatives, in addition to being ES-active, are fluorescent derivatives used to enhance optical detection of analytes in conjunction with condensed-phase separation methods such as HPLC.[38]

Another means to form ES-active derivatives of phenols takes advantage of the acidic nature of the hydroxy hydrogen. In general, phenols are more acidic than aliphatic alcohols and are of an acidity comparable to thiols. As a result, upon treatment of a phenol with the appropriate base, the ES-active phenoxide anion is generated in solution as shown for the fungicide *p*-phenylphenol (**12**) in eq 10. The negative ion

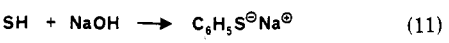
(10)

12

ES mass spectrum of *p*-phenylphenol, dissolved in and sprayed from a methanol solution containing ~1.0 mM potassium hydroxide, has as the major ion m/z 169, which corresponds to $(M - 1)^-$ relative to the mass of the original phenol, M.

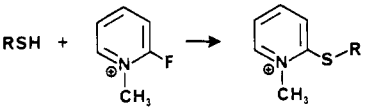
Thiols (Table 2). Thiols are chemically similar to both alcohols and phenols. Therefore, approaches to the generation of ES-active forms of thiols closely resemble those derivatization strategies discussed in detail above for alcohols and phenols. Since thiols are significant in biological chemistry, however, the thiol-specific reaction for production of an ES-active derivative discussed below may be of particular utility.

Thiols are similar in acidity to phenols. Thus, the alkyl sulfide anion or aryl sulfide anion is readily generated in base and detected in negative ion ES-MS. An example of this reaction is shown in eq 11 for formation of the phenyl sulfide anion from benzenethiol.



(11)

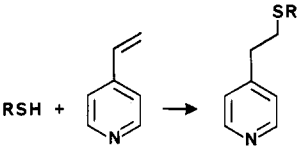
Other transformations of thiols to ES-active derivatives include formation of the *N*-methylpyridyl sulfide (**13**) by reaction with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate as shown in eq 12.[51,52] This reaction is amenable to the derivatization of primary, secondary, and tertiary thiols.



(12)

13

The derivatization of thiols using 4-vinylpyridine to produce the pyridyl sulfide derivative **14**, which can be protonated in acidic solution, is a promising route to ES-active thiol derivatives (eq 13). Indeed, Lam et al.[29] have used this reaction

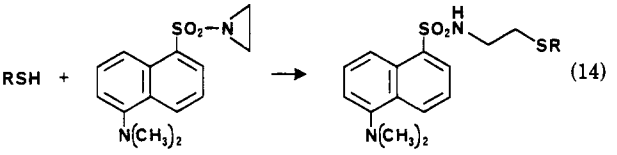


(13)

14

to modify the thiol functions on glycoproteins, thereby increasing the number of basic sites on the glycoproteins, which resulted in a decrease of the mass/charge ratio of the ions observed (i.e., greater charging). Katritzky et al.[53] have demonstrated the reaction to be applicable to a wide range of thiols.

A thiol-specific derivatization is shown in eq 14. The thiol reacts with dansylaziridine (**15**) to form a sulfonamide



(14)

15 **16**

derivative **16**, which is protonated at the dimethylamino group in acidic solution. This reaction is specific for thiols because only highly nucleophilic reagents can open the aziridine ring.[54] Amines and alcohols, which are weaker nucleophiles, do not react with the aziridine.

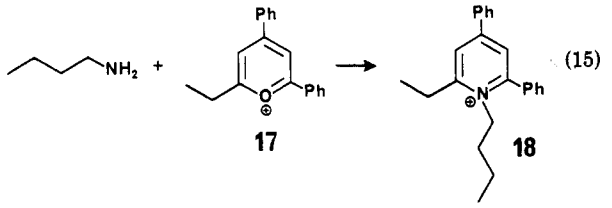
Amines (Table 3). Amines are typically amenable to ES-MS analysis because they are usually basic enough to be protonated in acidic solution. However, derivatizations that are selective for primary, secondary, or tertiary amines may

(51) Bald, E. *J. Chromatogr.* **1979**, *174*, 483–487.
(52) Bald, E.; Mazurkiewiczm, B. *Chromatographia* **1980**, *13*, 295–297.
(53) Katritzky, A. R.; Takahashi, I.; Marsou, C. M. *J. Org. Chem.* **1986**, *51*, 4914–4920.
(54) Scouten, W. H.; Lubcher, R.; Baughman, W. *Biochim. Biophys. Acta* **1974**, *336*, 421–426.

enable their differentiation. Derivatization can also afford detection of amines in negative ion mode, which may increase detectability or in some other way enhance an analysis. Furthermore, in addition to making an analyte ES-active, derivatization may be used to make the analyte more amenable to optical detection, which might be used with on-line condensed-phase separations in combination with ES-MS.

In attempting to derivatize amines to other ES-active forms, modification of the amine is generally the best synthetic strategy because the NR_2 functionality is a poor leaving group.

Primary amines can be readily, and specifically, reacted with a pyrylium salt to form a pyridinium salt as shown in eq 15 for the reaction of butylamine with 2,4-diphenyl-6-



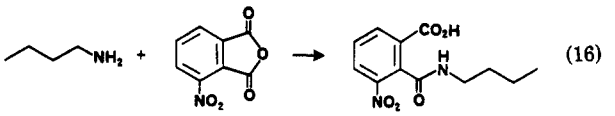
(15)

ethylpyrylium tetrafluoroborate (**17**).[39d] This derivatization has been used quite successfully to enhance the analysis of peptides by FAB mass spectrometry.[55,56]

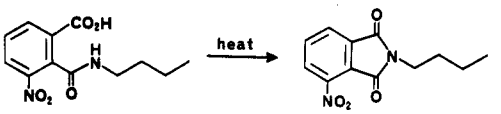
The positive ion ES mass spectrum of the pyridinum derivative of butylamine **18** has as the base peak the ion expected ion at m/z 316, corresponding in mass to $(M + 243)^+$, relative to the original amine, M. In addition, the unreacted derivatizing agent **17** is observed at m/z 261, which points out one of the potential drawbacks of using a derivatizing agent that is ionic in solution. Without purification of the reaction product or the use of an on-line separation, an ionic derivatizing reagent such as **17** may be observed in the ES mass spectrum of the reaction product and might also suppress the signal from the analyte derivative.

Primary and secondary amines react with cyclic anhydrides to form 3-nitro-2-[(alkylamino)carbonyl]benzoic acids, referred to here as "amic acids", as shown in eqs 16 and 18 for the reaction of 3-nitrophthalic anhydride with butylamine and dibutylamine, respectively. In addition to allowing for the negative ion analysis of amines, this derivatization provides, as discussed below, a means of distinguishing among primary and secondary amines.[39e] Ligon and Dorn[26] derivatized amines in this manner, using an anhydride that also imparted surface activity to the derivative, for their analysis by negative ion FAB mass spectrometry.

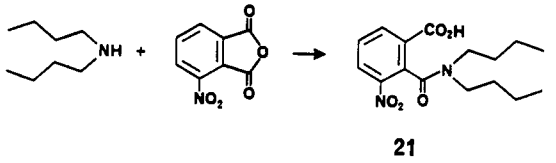
To demonstrate the utility of this derivatization for ES-MS, a 1:1 molar mixture of butylamine and dibutylamine was reacted with 3-nitrophthalic anhydride forming the amic acid derivatives **19** and **21**. Two ions were observed at m/z 265 and 321, corresponding in mass to the ions expected for each derivative (i.e., $(M + 192)^-$). In order to distinguish the primary from the secondary amine derivative, a second aliquot of the original reaction mixture was subjected to further heating. The negative ion ES mass spectrum of the more



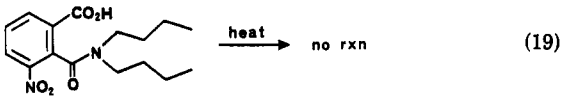
(16)



(17)



(18)

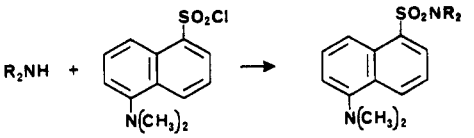


(19)

extensively heated sample clearly showed the complete disappearance of the ion at m/z 265, which was due to the amic acid derivative **19** formed from the primary amine. The absolute intensity of the signal owing to the secondary amine amic acid **21** at m/z 321 remained the same. The disappearance of the primary amine amic acids from the ES spectrum occurs because amic acids derived from primary amines undergo ring closure, with dehydration, upon prolonged heating to yield the phthalimide, which is not ES-active in the negative ion mode (eq 17). Amic acids derived from secondary amine cannot undergo this cyclization reaction and, therefore, remain ES-active (eq 19).

Of further utility is the fact that **19** and **21** are actually amphoteric derivatives and the imide formed upon heating the primary amine amic acids (e.g., **20**), although not detectable in negative ion mode, is ES-active in positive ion mode. Observed in the positive ion mass spectra acquired from acidified aliquots of the same samples used to acquire the negative ion mass spectra are the protonated forms of both **19** and **21** (m/z 267 and 323, respectively). A much less abundant ion is observed at m/z 249 due to the protonated phthalimide **20**, which apparently is present in this sample at a low level. The signal owing to **19** was less than that of **21**, possibly due to differences in the basicity and/or ES "ionization efficiencies" of these secondary and tertiary amides. In the heated sample, the protonated form of **19** is no longer detected, but the signal due to the protonated phthalimide **20** is increased because **19** is converted to **20** upon heating.

Another derivatization applicable to primary and secondary amines is the reaction with dansyl chloride (**9**) as shown in eq 20. The dansyl derivative, as described above for phenols



(20)

and thiols, not only provides a species ES-active in positive ion mode, through protonation of the dimethylamino moiety, but

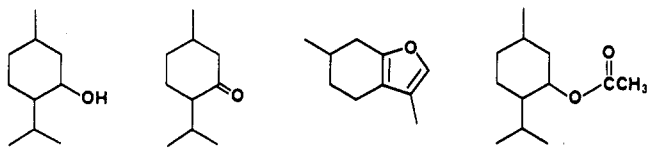
(55) O'Leary, M. H.; Samberg, G. A. *J. Am. Chem. Soc.* **1971**, *93*, 3530–3532.
(56) Busch, K. L.; Kroha, K. J.; Flurer, R. A.; DiDanato, G. C. In *Secondary Ion Mass Spectrometry, SIMS V*; Benninghoven, A., Colton, R. J., Simons, D. S., Werner, H. W., Eds.; Springer Series in Chemical Physics, 44; Springer-Verlag: New York, 1986; pp 512–514.

also provides for enhanced optical detection that might be used with an on-line separation in combination with ES-MS.[38,57]

Under mild conditions the reaction of an alkyl halide with an amine, as previously shown in eq 3, can be used as a relatively specific probe for tertiary amines. Methylation of the tertiary amine results in the formation of the quaternary ammonium ion which is detectable in positive ion ES-MS, even under basic solution conditions. Analysis of the products formed by reaction of an alkyl halide with a mixture of amines in positive ion mode, under basic solution conditions, inhibits the detection of primary and secondary amines or other species ionizable in solution by protonation. However, the quaternary ammonium ions formed from the tertiary amines will still be detected. Upon prolonged exposure to the alkyl halide, all amines in a mixture can be quaternized.

Selected Analytical Applications of Chemical Derivatization for ES-MS. The chemical derivatizations discussed above, when used in conjunction with ES-MS, can provide for analyte detectability or enhanced detectability (i.e., greater signal-to-noise ratio), analyte or functional group specific detection, and analysis flexibility (e.g., positive and/or negative ion detection). To demonstrate some of this utility, a series of derivatization ES-MS experiments were carried out using two "real-world" systems, viz., an essential oil and a commercially available medication, as well as a standard mixture of environmentally important compounds.

Detection of Menthol in Oil of Peppermint. The commercially important essential oils, such as oil of peppermint, find widespread use in flavoring and fragrances. Often, these oils are complex mixtures composed largely of relatively simple and low molecular weight terpenoid compounds isolated from plants. The four major constituents of oil of peppermint, viz., menthol (**22**; 33%), menthone (**23**; 24%), menthofuran (**24**; 11%), and menthyl acetate (**25**; 4%) comprise more than 70% (w/w) of the oil, but at least 300 other components have been detected in trace quantities.[58,59] While characterization of essential oils in recent years has been largely carried out using gas chromatography (GC) and GC/MS methods, derivatization used in combination with ES-MS might be of considerable utility in this area.



22 **23** **24** **25**

The potential utility of ES-MS in the analysis of essential oils is demonstrated here by a specific derivatization aimed at detecting the major primary and secondary alcohols that might be present in oil of peppermint. A positive ion ES mass spectrum of an acidified aliquot of the intact oil diluted in dichloromethane failed to detect any of the major constituents (**22–25**) of the oil. This was to be expected because these components are not ionic in solution, nor can they be readily ionized by acid/base chemistry. The derivatization strategy used to permit detection of the alcohols present was to react the intact oil with the 2-fluoro-1-methylpyridinium tosylate reagent, thereby selectively transforming primary and secondary alcohols in the mixture into ES-active 1-methylpyridinium derivatives (see eq 5 and Table 2). The positive ion ES mass spectrum of the derivatized oil of peppermint, shown in Figure 1, contains one intense ion at *m/z* 248. Since the 1-methylpyridinium derivative of an alcohol corresponds in mass to $(M + 92)^+$, the mass of the alcohol, M, must be 156 u, which is the mass of menthol. Thus, by this simple derivatization procedure and ES-MS analysis, the presence in the oil of one major primary or secondary alcohol of molecular weight 156 u was determined. Other alcohols are known to be present in oil of peppermint, but their concentrations are below 0.1% (w/w).[59] The inability to detect the known minor alcohol components in the current experiment is not unexpected given the large difference in concentration between the major and minor alcohols. Moreover, suppression of the ES signal from the derivatives of the minor components might occur owing to the high concentration of the menthol derivative in the sample.[60] Use of an on-line separation technique, such as capillary electrophoresis, with ES-MS might allow for detection of the derivatives of these minor components that might be present in the sample but are not observed currently.

Selective Detection of Phenols by Variation of Solution pH. Phenols play a significant role in many areas of industrial chemistry including the production of herbicides, pesticides, disinfectants, dyes, polymers, and pharmaceuticals. Unfortunately, phenols are rather toxic, with at least a dozen designated as priority pollutants. Many phenols may be analyzed by GC or GC/MS following derivatization,[61,62] but some highly polar phenols cannot.[63] Thermospray MS has been used successfully for the analysis of phenols, but the presence of solvent-derived ions tended to obscure the detection of lower molecular weight phenols.[64] In contrast, phenols are readily amenable to analysis by ES-MS because they are easily ionized in basic solution, as demonstrated in this paper and previously by Hughes et al.[63]

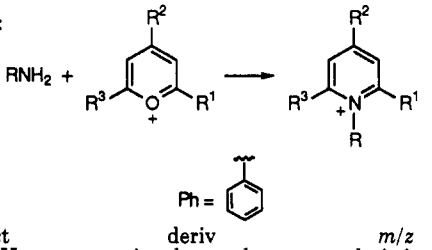
Not only are phenols amenable to analysis by ES-MS, but manipulation of solution pH allows for a degree of solution ionization selectivity and, therefore, detection selectivity that can enhance the analysis of phenol mixtures, with or without an on-line separation. This ionization/detection selectivity (i.e., selective derivatization) is afforded by the variation in phenol pK_a with the structure of the phenol. Thus, by controlling the pH of the solution, it is possible to control the degree of dissociation of the individual phenols to form their ES-active phenoxide anions. This selectivity was demonstrated using an equal molar mixture (~12.5 μM each) of 2,4,6-trinitrophenol (pK_a = 0.38), pentachlorophenol (pK_a = 5.26), 2,4-dichlorophenol (pK_a = 7.89), and phenol (pK_a = 10.02)

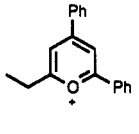
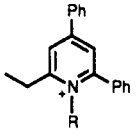
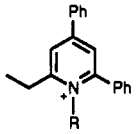
(57) Durden, D. A.; Davis, B. A.; Boulton, A. A. *Biomed. Mass Spectrom.* **1974**, *1*, 83–95.
(58) Sang, J. P. *J. Chromatogr.* **1982**, *253*, 109–112.
(59) Takahashi, K.; Someya, T.; Muraki, S.; Yoshida, T. *Agric. Biol. Chem.* **1980**, *44*, 1535–1543.
(60) Tang, L.; Kebarle, P. *Anal. Chem.* **1991**, *63*, 2709–2715.
(61) Korhonen, I. O. O.; Knuutinen, J. *J. Chromatogr.* **1983**, *256*, 135–142.
(62) Janda, V.; Van Langenhove, H. *J. Chromatogr.* **1989**, *472*, 327–330.
(63) Hughes, B. M.; McKenzie, D. E.; Duffin, K. L. *J. Am. Soc. Mass Spectrom.* **1993**, *28*, 597–603.
(64) Barcelo, D. *Chromatographia* **1988**, *25*, 295–299.

Table 3. Summary of Routes to ES-Active Derivatives of Amines

A. Derivatives of Primary Amines via Reaction with Pyrylium Ions

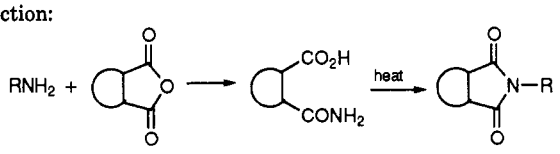
overall reaction:

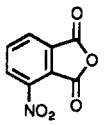
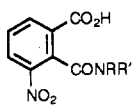
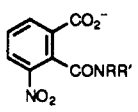
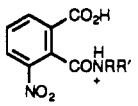
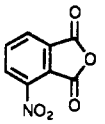
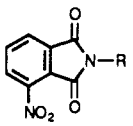
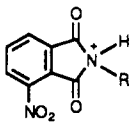


eq	deriv reagent	product with RNH_2	deriv ion detected	*m/z* deriv ion[a]	comment[b]
15				(M + 243)	works for 1^y amines only[39d,55,56]

B. Derivatives of Primary and Secondary Amines by Reaction with Cyclic Anhydrides[c]

overall reaction:

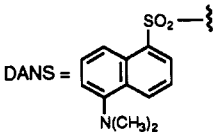


eq	substr	deriv reagent	product with RR'NH	deriv ion detected	*m/z* deriv ion[a]	comment[b]
16, 18	RR'NH[d]				(M + 192)	deprotonate in base works for 1^y or 2^y amines[39e]
				or		
					(M + 194)	protonate in acid
17	RNH_2[d]				(M + 176)	product formed on strong heating[39e] protonate in acid works for 1^y amines only

C. Derivatization via Reaction of Primary and Secondary Amines with Sulfonic Acid Derivatives

overall reactions: $RNH_2 + R'SO_2Cl \rightarrow R'SO_2NHR$

$R_2NH + R'SO_2Cl \rightarrow R'SO_2NR_2$



eq	substr	deriv reagent	product with RNH_2/R_2NH	deriv ion detected	*m/z* deriv ion[a]	comment
20	RNH_2	DANS-Cl	DANS-NHR	$[(DANS\text{-}NHR) + H^+]^+$	(M + 234)	good derivative for optical detection[38,57]
20	R_2NH	DANS-Cl	DANS-NR_2	$[(DANS\text{-}NR_2) + H^+]^+$	(M + 234)	good derivative for optical detection[38,57]

D. Derivatization via Protonation and Quaternization Reactions

overall reactions: $RNH_2 + H^+ \rightarrow (RNH_3)^+$

$R_3N + R'X \rightarrow (R_3NR')^+$

eq	substr	deriv reagent	product with amine	deriv ion detected	*m/z* deriv ion[a]	comment[b]
	RNH_2	TFA[e]	$(RNH_3)^+$	$(RNH_3)^+$	(M + 1)	1^y, 2^y, 3^y amines all protonate in acid
3	R_3N	CH_3I[f]	$(R_3NCH_3)^+$	$(R_3NCH_3)^+$	(M + 15)	works best for 3^y amines also used to derivatize alkyl halides (eq 3)

[a] M, mass of original analyte. [b] Key: 1^y, primary; 2^y, secondary; 3^y, tertiary. [c] This reaction fails with tertiary amines. [d] Other cyclic anhydrides including 3,4-pyridinedicarboxylic anhydride (**6**) could also be used. R' = H or alkyl. [e] TFA = trifluoroacetic acid. Other strong acids could be used. [f] Primary alkyl halides could be used.

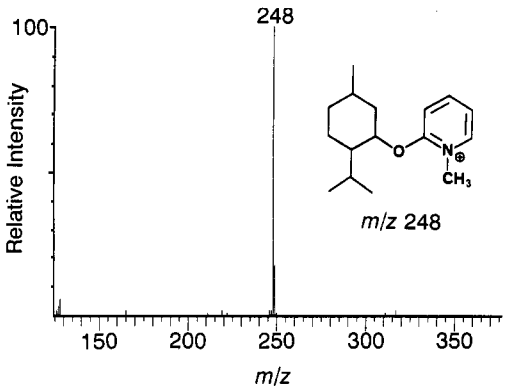
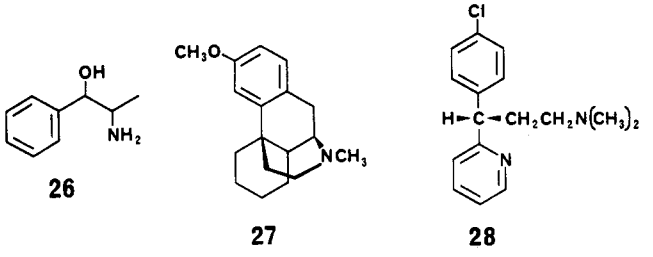


Figure 1. Positive ion ES mass spectrum obtained from a dichloromethane solution of oil of peppermint that was reacted with 2-fluoro-1-methylpyridinium tosylate to form 1-methylpyridinium derivatives of the primary and secondary alcohols in the oil (continuous infusion, 5 μL/min). The ion detected at *m/z* 248 corresponds in mass to the ion expected for the derivative of the secondary alcohol menthol (**22**), which is known to be the only alcohol that is a major constituent of the oil.

prepared in methanol.[65] Shown in panels a–c of Figure 2 are the negative ion ES mass spectra of this mixture acquired by spraying from solutions of increasing pH. As observed, at the lowest solution pH (no base added, Figure 2a) only the most acidic of the phenols in the mixture, viz., 2,4,6-trinitrophenol and pentachlorophenol, are detected. As the pH is increased by the addition of base (0.2 mM sodium hydroxide, Figure 2b) all four of the phenols are detected. By increasing the pH even further (1.0 mM sodium hydroxide, Figure 2c), the absolute intensity of the signal from each phenol is doubled, indicating more complete conversion to their respective phenoxide ions in solution.

Selective detection of phenols through manipulation of solution pH simplifies spectral interpretation and also yields structural information regarding the phenols. In general, the more acidic phenols (i.e., those detected at the lowest solution pH) are more likely to be substituted with electron-withdrawing groups, such as NO_2 or Cl, which tend to stabilize the phenoxide ion in solution.

Selective Detection of Amines in the Medication Triaminicol. The childrens' cold medication Triaminicol contains three amine salts as the active ingredients. Phenylpropanolamine hydrochloride (6.68 mM), a salt of a primary amine (**26**), acts as a nasal decongestant, dextromethorphan hydrobromide (2.84 mM), a salt of a tertiary amine (**27**), acts as a cough suppressant, and chlorpheniramine maleate (0.515 mM), also the salt of a tertiary amine (**28**), is an antihistamine. The positive ion ES-MS mass spectrum obtained from a



26 **27** **28**

(65) Prauss, P.; Dombeck, V. *Anal. Chim. Acta* **1993**, *277*, 97–101.

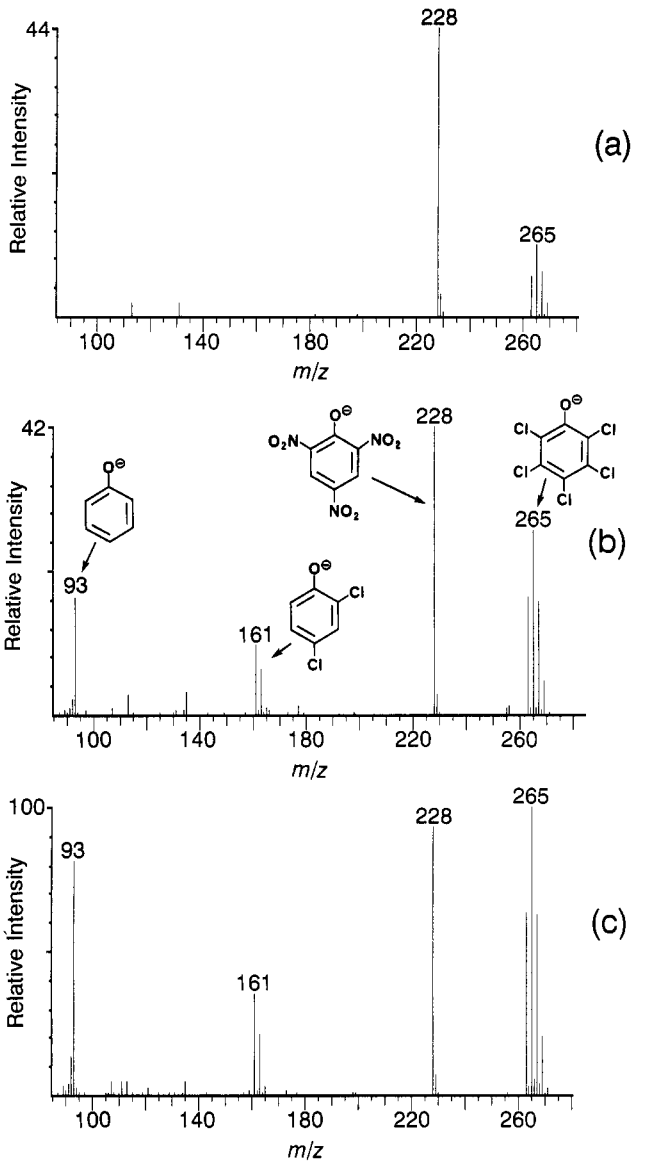


Figure 2. Negative ion ES mass spectra obtained from an equal molar mixture (~12.5 μM each, methanol) of 2,4,6-trinitrophenol (pK_a = 0.38), pentachlorophenol (pK_a = 5.26), 2,4-dichlorophenol (pK_a = 7.89), and phenol (pK_a = 10.02) using continuous infusion (5 μL/min). The individual spectra a–c were obtained by spraying mixture aliquots of increasing solution pH: (a) sprayed from methanol, no base added; (b) sprayed from methanol/0.2 mM sodium hydroxide; (c) sprayed from methanol/1.0 mM sodium hydroxide. The respective phenoxide ions detected correspond to $(M - 1)^-$ relative to the mass of the original phenol, M. Relative intensities in the three spectra are normalized to the intensity of the base peak in spectrum c.

methanol dilution of an aliquot of Triaminicol is shown in Figure 3a. The ions observed at *m/z* 152, 272, and 275 are those expected for the protonated molecules of **26**, **27**, and **28**, respectively. The relative intensities of these peaks (*m/z* 272 > *m/z* 152 > *m/z* 275) can be rationalized on the basis of the much lower concentration of **28** in the sample relative to the other two amines and on the lower solution-phase basicity of the primary amine **26** relative to the tertiary amine **27**. Thus, without any treatment of the sample, the known amines, which are ES-active in an acidic solution, can be observed. Despite this fact, without prior knowledge of the sample, the ions observed cannot be positively identified as amines. However, through a combination of solution pH manipulation and selective derivatization, it is possible to positively identify these

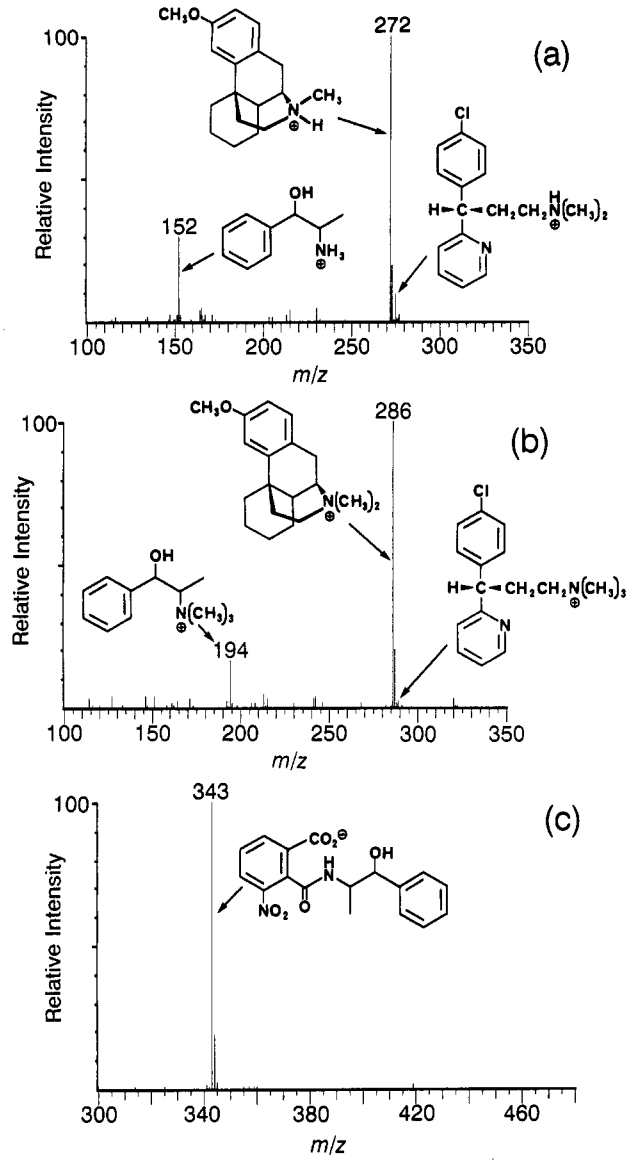


Figure 3. (a) Positive ion ES-MS mass spectrum obtained from a solution of untreated Triaminicol (~50 μM in phenylpropanolamine/methanol). (b) Positive ion ES-MS mass spectrum obtained from a solution of the neutralized extract of Triaminicol (dichloromethane/methanol, 90:10 v/v) following prolonged treatment of the extract with iodomethane. (c) Negative ion ES mass spectrum obtained from a solution of the neutralize extract of Triaminicol (methanol/1.0 mM sodium hydroxide) following treatment of the extract with 3-nitrophthalic anhydride. All spectra were obtained by continuous infusion at 5 μL/min.

sample components as amines and also possible to assign them as primary, secondary, or tertiary, using ES-MS.

In a first step toward this identification, the Triaminicol was treated with base and extracted with dichloromethane to isolate the organic compounds in the medicine that could be neutralized through acid/base chemistry. The positive ion ES mass spectrum of this neutralized extract revealed no ions, but upon acidification the same ions observed in Figure 3a were apparent. Thus, the ions at *m/z* 152, 272, and 275 are confirmed to be the protonated form of basic molecules in the sample, possibly amines, with molecular weights of 151, 271, and 274 u, respectively. In order to confirm the species as amines, and to identify them as primary, secondary or tertiary, the neutralized extract was treated for an extended period with iodomethane to quaternize all amines in the sample (eq 3, Table 3). Thus, *m/z* shifts of 14, 28, or 42 units in the peaks observed following quaternization as compared to the ions observed in Figure 3a identify the species as tertiary, secondary, or primary, respectively. Figure 3b is the positive ion ES mass spectrum of the neutralized extract following treatment with iodomethane. The peak observed at *m/z* 194 identifies the component with a molecular weight of 151 u as a primary amine (*m/z* shift 42). The components with molecular weights of 271 and 274 u are identified as tertiary amines (*m/z* shift 14), but the low abundance of the peak at *m/z* 289 makes that identification tenuous.

Supporting evidence for these identifications was obtained by treatment of the neutralized extract with the 3-nitrophthalic anhydride reagent to form the amic acid derivatives of any primary or secondary amines present in the sample (eqs 16–19, Table 3). These derivatives are amphoteric, but when analyzed in negative ion mode allow the detection of the derivative without the complication of spectral peaks arising from unreacted amines. Figure 5c is the negative ion ES mass spectrum of the extract from the neutralized Triaminicol following treatment with 2-nitrophthalic anhydride. The only ion observed, *m/z* 343, corresponds in mass to the derivative expected (i.e., $(M + 192)^-$) for reaction with the sample component of molecular weight 151 u. Therefore, this amine must be either primary or secondary. Disappearance of this peak from the spectrum upon treatment with heat, although not carried out, would confirm the derivative to be derived from a primary amine (eqs 16–19). Since derivative ions are not observed at *m/z* 463 or 466, the components of molecular weight 271 and 274 u, respectively, are confirmed to be tertiary amines.

SUMMARY

Simple alkyl halides and alcohols are not typically ES-active, i.e., not detectable by ES-MS, as they are not ionic in solution, nor can they be readily ionized in solution by acid/base chemistry. The derivatization reactions presented here transform these functional groups either into ionic or into solution ionizable moieties, affording their detection by ES-MS. While phenols, thiols, and amines may be ionized in solution by acid/base chemistry and are, therefore, normally detectable by ES-MS, the derivatizations presented are shown to enhance their analysis, as well as the analysis of the other RX type compounds. This enhancement results from functional group specific derivatization/detection and increased analysis flexibility. This flexibility is provided largely by derivatives that allow detection of a particular analyte in either positive ion or negative ion mode or, in the case of the amphoteric derivatives, in both ionization modes. Moreover, these derivatizations can also be chosen to enhance other methods of detection, such as on-line optical detection, by providing the analyte with a suitable chromophore as well as a charge. The majority of the derivatization reactions presented here are easy to perform, are well documented in the literature, utilize readily available reagents, and will be valuable for detection of trace levels of analytes. However, the precise methodologies for derivatization and detection at trace levels will depend heavily on the nature of the analyte. To optimize these methods, it will probably be best to incorporate a sample cleanup procedure and/or on-line

separation either before or after the derivatization step. Although not demonstrated in this work, these derivatizations should be applicable not only to the simple organic molecules discussed, but may also be used to alter ES-MS behavior of more complex molecules, thereby adding selectivity and flexibility to their analyses.

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