CHAPTER 11

AMINES

11.1. GC SEPARATIONS OF UNDERIVATIZED AMINES

Authors' Note: *Due to the current nonuse of packed columns in mass spectrometry laboratories, references to them have been removed from the first edition of this book; however, the original authors had a very strong opinion about their belief that packed columns were able to bring about superior chromatographs of amines. Based on this opinion of the original authors, the comments and information regarding packed columns have been retained for this chapter only.*

Although capillary columns are generally preferred, there are many examples where separation is better by using packed columns, especially for low-boiling amines.

A. Low-boiling aliphatic amines
   1. Amines from C_1 (methylamine) to C_6 (cyclohexylamine): 2-m 4% Carbowax 20M column + 0.8% KOH on 60–80 mesh Carbopack B, 75–150 °C at 6 °C min^{-1}.
   2. Isopropylamine, *n*-propylamine, diisopropylamine, di-*n*-propylamine: 2-m Chromosorb 103 column, 50–150 °C at 8 °C min^{-1}.
   3. Methylamine, ethyleneimine, dimethylamine, trimethylamine: 2-m Chromosorb 103 column, 60–180 °C at 6 °C min^{-1}.
   4. 1-Aminooctane, 2-aminooctane: 2-m Chromosorb 103 column at 135 °C.

B. Higher boiling aliphatic amines and diamines
   1. Diaminoethane, diaminopropane, diaminobutane, diaminopentane, diaminohexane, diaminooctane: 25-m CP-WAX column for amines and diamines (Chrompack cat. no. 7424), 75–200 °C at 6 °C min^{-1}.
   2. Diaminoethane, diaminopropane, 1-amino-2-propanol, diaminobutane, diaminopentane, *n*-decylamine: 25-m CP-WAX column for amines and diamines at 135 °C.

C. Aromatic amines and diamines
   1. Aniline, 2,3,4-picolines: 2-m Carbowax 20M column or Carbopack B column, 75–150 °C at 3 °C min^{-1}.

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2. Dimethylanilines and trimethylanilines: 25-m DB-1701 column, 60–270 °C at 5 °C min^{-1}.
3. Toluidine, nitrotoluene isomers, diaminotoluene, and dinitrotoluene isomers: 30-m DB-17 column, 100–250 °C at 8 °C min^{-1}.
4. Phenylenediamines
   a. Lower boiling impurities (sample dissolved in acetonitrile): Aniline (MW = 93 Da), quinoxaline (MW = 130 Da), dimethylquinoxaline (MW = 158 Da) from the phenylenediamines (MW = 108 Da). 25-m CP-WAX column for Amines (Chrompack) at 200 °C.
   b. Higher boiling impurities: Some impurities may be found under these GC conditions: quinoxaline, phenazine, tetrahydrophenazine, nitroanilines, hydroxyanilines, chloronitrobenzenes, hydroquinone, diaminophenazine, aminohydroxyphenazine. 30-m DB-17 column, 100–275 °C at 6 °C min^{-1}.
5. Diisopropylamine, diisobutylamine, dibutylamine, pyridine, dicyclohexylamine, aniline, 2,6-dimethylaniline. 25-m CP-WAX-51 column, 70–210 °C at 5 °C min^{-1}.

11.2. Derivatization of Amines and Diamines

MTBSTFA is the recommended reagent for silylation of the amine functionality because this reagent forms a more stable derivative than MSTFA, BSTFA, or BSA. The solvent used is important because amines can be difficult to silylate.

A. TBDMS and TMS derivatives
   Add 0.1 mg of the sample in 50 µL of acetonitrile (or THF) to 50 µL reagent. Let the solution stand at room temperature for 10–20 minutes.
   Reagents: MTBSTFA (recommended)
   MSTFA (recommended for amine hydrochlorides)
   BSTFA
   BSA
B. Preparing Methyl-8® derivatives: Add less than 0.1 mg of sample to 50 µL of acetonitrile and then add 50 µL of Methyl-8® reagent [$(CH_3)_2NCH(OCH_3)_2$]. Heat at 60 °C for 30 minutes or at 100 °C for 20 minutes.

11.3. GC Separation of Derivatized Amines

A. Diamines
   1. TBDMS or TMS derivatives of diamines: 30-m DB-225 column, 75–225 °C at 8 °C min^{-1}.

2. Methyl-8® derivatives of diaminohexanes and diaminooctanes: 30-m DB-5 column, 80–270 °C at 8 °C min^{-1}.

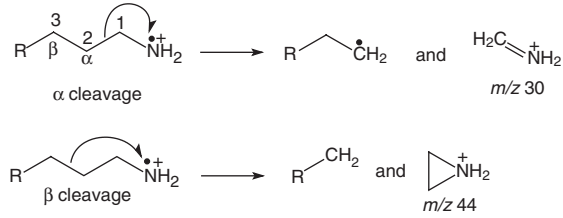
11.4. Mass Spectral Interpretation of Amines

A. Underivatized

Organic compounds with an odd number of nitrogen atoms will have an odd nominal mass; therefore, the $M^{+\bullet}$ peak in the mass spectrum of compounds having an odd number of nitrogen atoms will be at an odd *m/z* value. There will be fragment ion peaks at even *m/z* values as long as the ion is an EE^+ ion peak and retains the odd number of nitrogen atoms. When a fragment ion is formed from a molecular ion containing an odd number of nitrogen atoms and a nitrogen atom is lost, this ion will have an even *m/z* value now that it contains an odd number of nitrogen atoms. For aliphatic amines, the most important fragmentation is cleavage of the bond that is alpha to the carboamino carbon atom (the carbon atom attached to the nitrogen) with the charge remaining on the nitrogen-containing fragment. In electron ionization, when the molecular ion of an aliphatic amine is formed, the most likely site of the charge and radical will be on the nitrogen atom due to the loss of one of the two nonbonding electrons associated with that atom. The first reaction involving fragmentation of the molecular ion is a cleavage of the bond between C_1 and C_2. This is called α cleavage. Aliphatic amines will also undergo β cleavage, which is the breaking of the bond between C_2 and C_3 (Scheme 11.1).

Examination of the EI mass spectrum of *n*-octyl amine in Figure 11.1 shows that there are peaks representing these two ions (*m/z* 30 and 44).

The mass spectrum for this same compound in the first edition of this book showed a relative intensity of about 25% for the peak at *m/z* 44. In this mass spectrum, the intensity of the peak at *m/z* 44 is ~5% of base peak. The same is true for all the spectra of this compound in the NIST08 Database. Beta cleavage does play a significant role in the mass spectra of 2° and 3° aliphatic amines; however, for straight-chain



Scheme 11.1

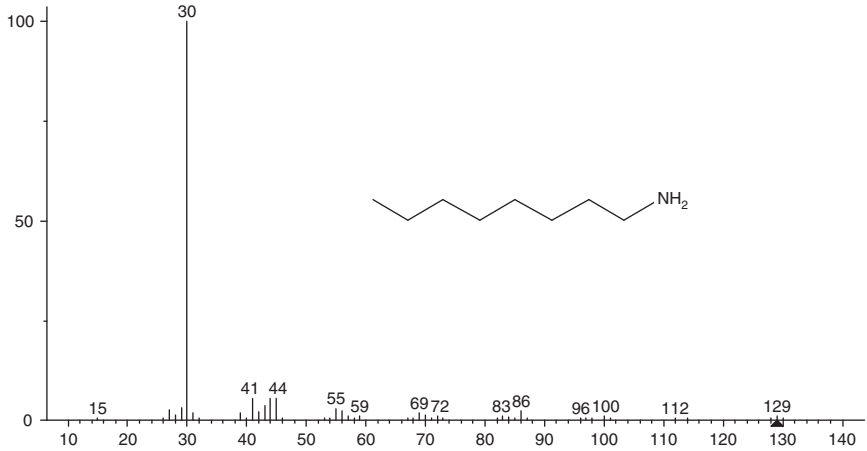
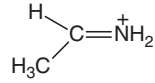


Figure 11.1 EI mass spectrum of *n*-octylamine.

compounds with six or more carbon atoms, the ion with *m/z* 44 is primarily the result of a complex series of rearrangements. The ion has the structure rather than the structure shown in the above example of β cleavage. The tendency for this rearrangement fragment ion to form is highly dependent on analyte ion-source pressure. The spectrum in the first edition of this book was from the authors' laboratory and was probably acquired using a direct insertion probe. This could well have meant that the partial pressure of the analyte was very high, which would favor the formation of the rearrangement product [1].

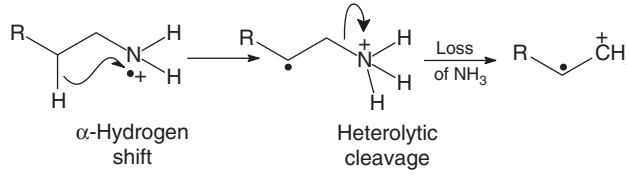


Although EI mass spectrometry is considered to be the most reproducible of all the mass spectrometry techniques, this example is a good illustration of variabilities that can exist and have to be watched for in all GC/MS analyses.

Underivatized aliphatic diamines are difficult to identify by their mass spectra alone because of the low abundance of the molecular ion (<3%). However, in some cases, an $[M-17]^+$ (loss of NH_3) peak representing a common fragment ion formed by a hydrogen shift from the α-carbon followed by a heterolytic cleavage as shown in Scheme 11.2.

1. Primary amines

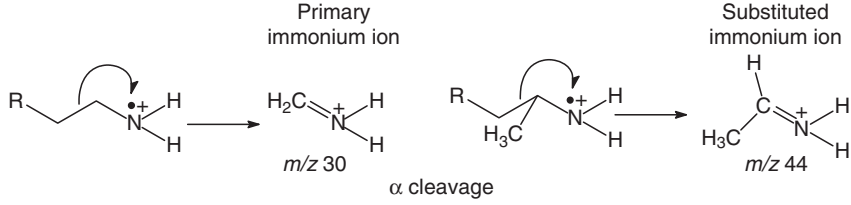
   The mass spectra of primary aliphatic amines show characteristic peaks at *m/z* 18 ($[NH_4]^+$ difficult to distinguish from the $[H_2O]^+$) and *m/z* 30 $[H_2C{=}NH_2]^+$. If the carboamine carbon is alkyl substituted, then intense peaks are observed at *m/z* 44, 58, or 72,



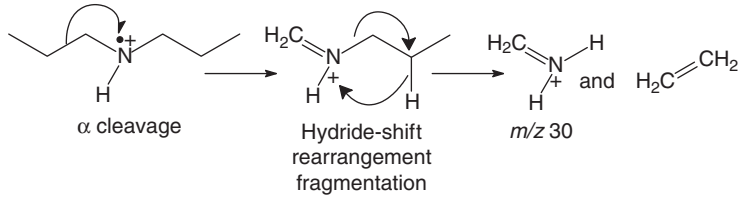
Scheme 11.2

and so on. If the unknown amine reacts with acetone or Methyl-8®, then it is a primary amine (Scheme 11.3).

2. Secondary amines
   The molecular ion of a secondary amine will undergo α cleavage to form a secondary immonium ion, which in turn will undergo a hydride-shift rearrangement fragmentation to lose an olefin and result in a primary immonium ion, provided both chains have at least two carbon atoms (Scheme 11.4 and Figure 11.2).
3. Tertiary amines
   Tertiary amines will undergo α cleavage, preferentially in the loss of the largest hydrocarbon chain. If there are two or more carbon atoms present in both of the other two chains, then two hydride-shift rearrangements occur to result in the primary immonium ion (Scheme 11.5). The peak representing the primary immonium ion is not as intense as in the spectra of primary and secondary amines because all of the intermediate ions are not converted as can be seen by the intensity of the peaks that represent each of them (Figure 11.3).



Scheme 11.3



Scheme 11.4

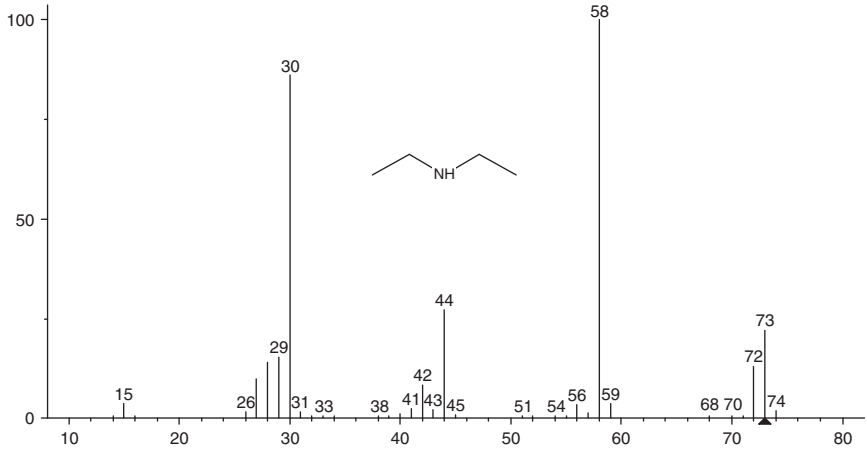
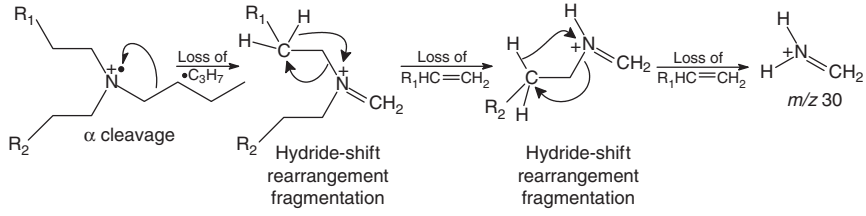


Figure 11.2 EI mass spectrum of *N,N*-diethylamine exhibiting peaks at *m/z* 58 (α cleavage) and *m/z* 30 (a primary immonium ion); the result of a hydride-shift rearrangement fragmentation of the ion with *m/z* 58.



Scheme 11.5

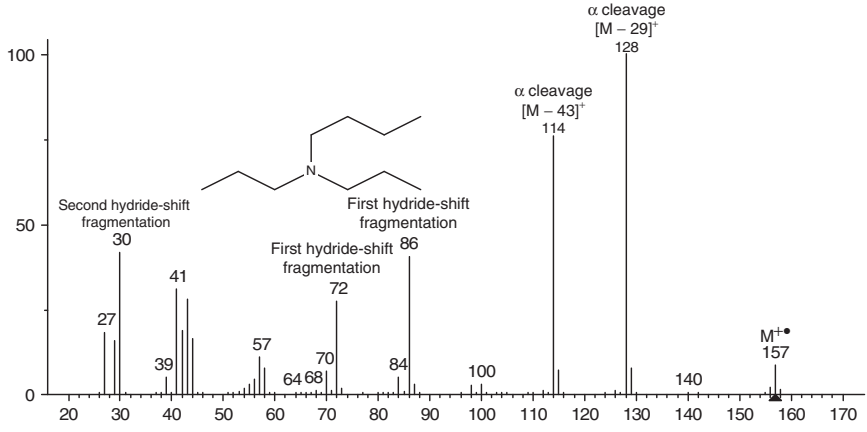
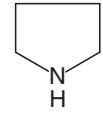


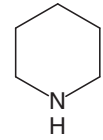
Figure 11.3 EI mass spectrum *N,N*-dipropyl-1-butanamine.

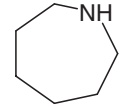
Summary: If the molecular weight is odd, then the compound contains an odd number of nitrogen atoms. Fragment ions observed at even-mass values suggest the presence of nitrogen. The loss of ammonia is fairly common in nitrogen compounds and may not indicate exclusively that an amine is present. Chemical derivatization will easily determine if the unknown is a tertiary amine.

4. Mass spectra of cyclic amines
   $M^{+\bullet}$ peaks as well as peaks representing $[M - H]^+$ ions are observed in the mass spectra of cyclic amines.

Ethylenimines  $[M - H]^+$ $[M - CH_3]^+$

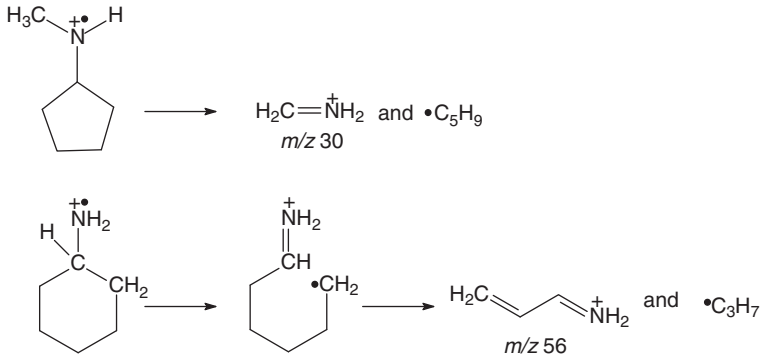
Pyrrolidines  $[M - H]^+$ $[M - 28]^+$

Piperidines  $[M - H]^+$ $[M - 29]^+$ (a peak at *m/z* 84 is present in the mass spectrum of alkylpiperidines)

Hexamethyleneimines  $[M - H]^+$ $[M - 29]^+$ (a peak at *m/z* 112 is present in the mass spectrum of alkylhexamethyleneimine)

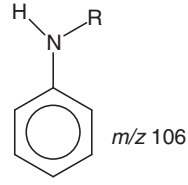
The peak at *m/z* 28 in the mass spectra of cyclic amines represents the $H_2C{=}N^+$ ion. The peak at *m/z* 30 is fairly intense in the mass spectra of nonsubstituted cyclic amines.

5. Mass spectra of cycloakylamines
   $M^{+\bullet}$ peaks are easily detected in the mass spectra of most cycloalkylamines; the spectra of these compounds also have a characteristic peak at *m/z* 30 representing a primary immonium ion. In the mass spectrum of methylcyclopentylamine, *m/z* 30 is the base peak; whereas in the mass spectrum of cyclohexylamine, a peak representing an ion formed by a rearrangement fragmentation is the base peak (Scheme 11.6).



Scheme 11.6

6. Mass spectra of aromatic amines
   Aromatic amines show intense $M^{+\bullet}$ peaks (characteristic of all aromatic compounds). When alkyl side chains are present, the $M^{+\bullet}$ peak intensities decrease with increasing alkyl chain length, but the $M^{+\bullet}$ peaks are still fairly intense. The mass spectra of aromatic amines (including the naphthylamines) exhibit peaks representing ions formed by the loss of 1, 27, and 28 from the molecular ion, but the peaks represented by these losses also decrease in intensity as the alkyl side chain increases in size. From the mass spectrum alone, it is difficult to determine whether the alkyl group is on the ring or on the nitrogen. A peak at *m/z* 106 represents an abundant ion when one alkyl group is on the nitrogen:



   An important member of this class of compounds is drugs related to amphetamine. These compounds have both a benzyl ($C_6H_5CH_2$–) and an aliphatic amine moiety (R–CH_2–N(R_1)R_2) where R_1 and R_2 can be a H or another alkyl group. If the benzylic carbon and the carboamino carbon are the same, the fragmentation is dominated by benzylic cleavage (cleavage following the benzylic carbon resulting in the formation of a tropylium ion; see Chapter 21). If the benzylic carbon and the carbinamino carbon are separated by one or more other alkyl carbon atoms, the fragmentation is dominated by α cleavage initiated by the charge and radical sites being on the

nitrogen atom of the molecular ion. This is clearly shown in Figures 11.4 and 11.5.

A third class of compounds that must be considered in this category is the halo anilines.

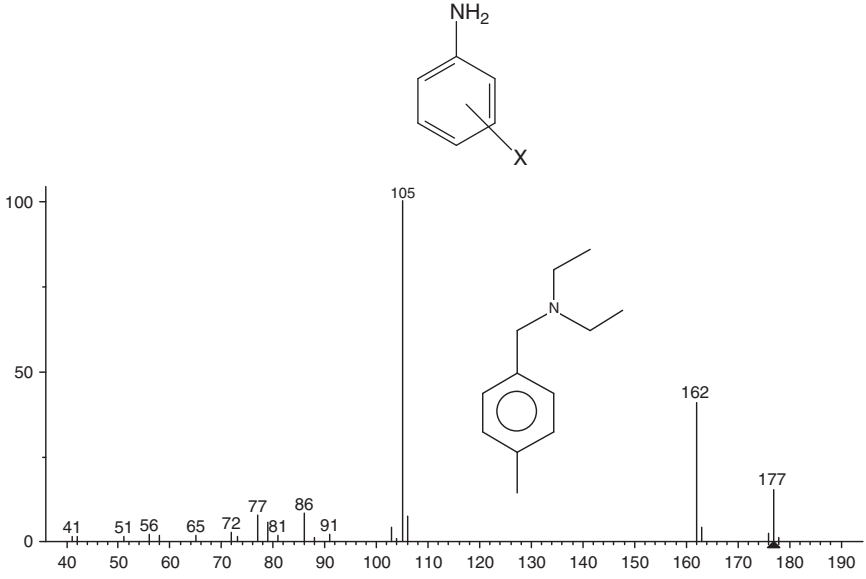


Figure 11.4 EI mass spectrum of diethyl(4-methylbenzyl)amine. The benzyl and carbinamino carbon is the same atom. The spectrum has all the characteristics of one produced by an aromatic compound (intense $M^{+\bullet}$ peak, very little fragmentation, the base peak represents a methyl-substituted tropylium ion).

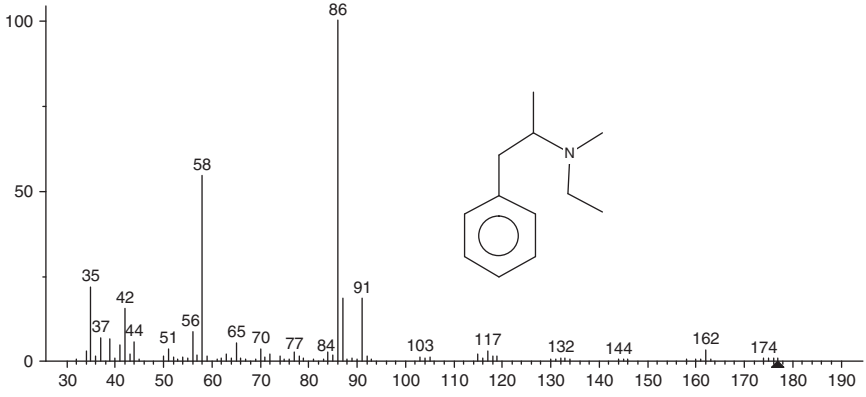
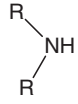


Figure 11.5 EI mass spectrum of *N*-ethyl-*N*,α-dimethyl-benzeneethanamine. The benzyl and carbinamino carbon atoms are not the same in this molecule. The spectrum is dominated by fragmentation characteristic of an aliphatic amine with the charge and radical sites on the nitrogen atom.

The mass spectra of bromo- and chloroaniline are distinguishable from one another by the unique X+2 patterns exhibited by their $M^{+\bullet}$ peaks. The iodo- and fluoroanilines have unique molecular weights, and the $M^{+\bullet}$ peak for all four compounds is the base peak. For any one given compound, there is no real difference in the mass spectra of the three regioisomers (*o*-, *m*-, and *p*-); however, these isomers can be distinguished from one another by the differences in their retention indices.

These compounds can be reacted with organic acids to form halophenyl N-substituted amides [2]. The mass spectra of the *ortho*-isomers of the chloro-, bromo-, and iodo-isomers of the acetyl, formyl, and benzoyl derivatives all exhibit a peak with a significant intensity representing the loss of the halo radical. The mass spectra of various compounds with *ortho*-substitution will exhibit an ortho effect which manifests itself in the loss of a molecule and the formation of odd-electron fragments, whereas the mass spectra of the *meta*- and *para*-isomers do not exhibit such behavior. This conventional ortho effect is used to distinguish the *ortho*-isomer from the other two regioisomers of many multiple-substituted aromatic compounds. Apparently this variation on the ortho effect that occurs under electron ionization of these derivatized haloanilines can also be used in a similar manner (Figure 11.6 and Figure 11.7). The loss of a halo radical is not observed when the halogen is a fluorine.

7. Mass spectral fragmentation

Amine	Characteristic Fragments	Rearrangement Ions	Characteristic losses From the Molecular Ion
RNH_2	*m/z* 30	*m/z* 18 (NH_4)	$[M - NH_3]^+$ (especially diamines)
	α cleavage longest chain	*m/z* 30 ($H_2C{=}NH_2^+$)	
	α cleavage longest chain	$(NHRCH_2)^+$	

B. Derivatized

Preparing derivatives of amines can make the identifications much easier.

Functional Group	Derivative	Increase in MW
—NH_2	—NH–$Si(CH_3)_3$ (TMS)	72
—$(CH_2)_3NH_2$	–$N[Si(CH_3)_3]_2$ (TMS)	144
—NH_2	NH–$Si(CH_3)_2C(CH_2)_3$ (TBDMS)	114
—NH_2	$NHCOCF_3$ (trifluoroacetyl)	96
—NH_2	$N{=}CHN(CH_3)_2$ (Methyl-8®)	55

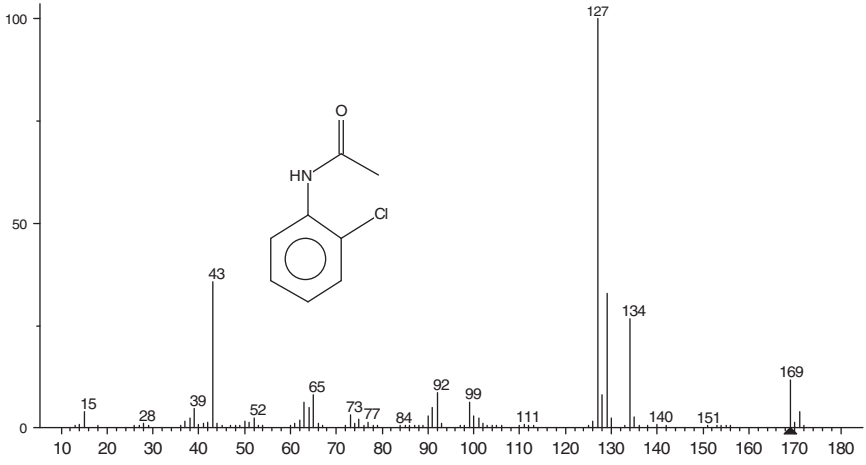


Figure 11.6 EI mass spectrum of the acetyl derivative of *o*-chloroaniline. The peak at *m/z* 134 represents the loss of a chlorine radical from the molecular ion, an ortho effect.

If at least three CH_2 groups are present between the amino group and another functional group, it is possible to add two TMS groups to the amine functional group. The presence of an intense peak representing an ion with *m/z* 174 confirms the addition of two TMS groups on the same nitrogen. By adding 15 to the *m/z* value of the peak with a significant intensity that represents the highest mass ion, the molecular weight of the TMS derivative is determined. The molecular weight of the amine is then determined by subtracting 114 from this value. The Methyl-8® derivative is excellent for use in analyzing diaminohexanes, diaminooctanes, and so forth. Only one Methyl-8® derivative adds per nitrogen so that multiple derivatives are not obtained as is the case with TMS. The $M^{+\bullet}$ peaks of these derivatives are relatively intense.

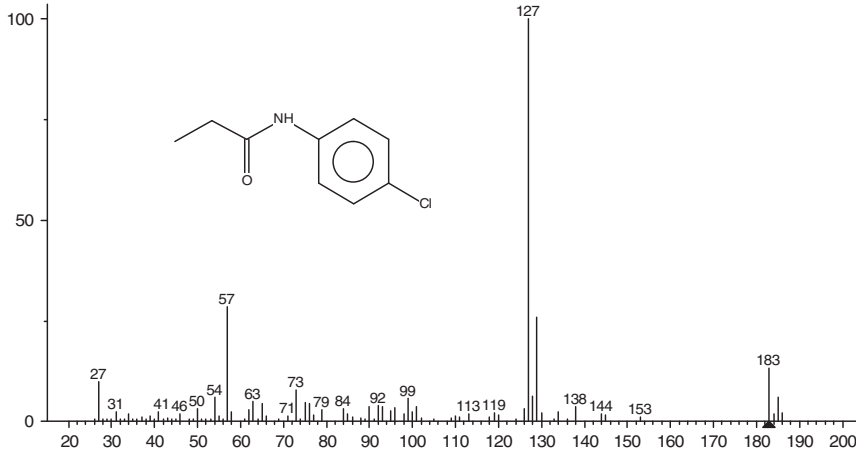
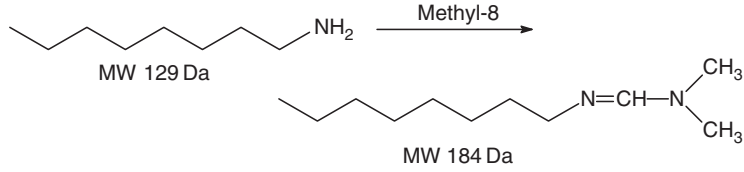


Figure 11.7 EI mass spectrum of the acetyl derivative of *p*-chloroaniline. The peak at *m/z* 134, seen in Figure 11.6, is not present in this spectrum or the spectrum of the *meta*-isomer. This supports this unusual variation on the ortho effect.



Scheme 11.7

C. Sample mass spectrum
The most prominent peak in the mass spectrum of 1-octylamine is at *m/z* 30 (Figure 11.1). From Appendix Q, a very intense peak at *m/z* 30 suggests a primary or secondary amine, or a nonsubstituted cyclic amine. A very small $M^{+\bullet}$ peak is barely visible at *m/z* 129. If the unknown reacts with Methyl-8® reagent, it is a primary amine (not a secondary amine) or a cyclic amine (Scheme 11.7).

11.5. Amino Alcohols (Aliphatic)

A. GC separation of underivatized amino alcohols
1. Monoethanolamine, diethanolamine, triethanolamine, and impurities: 30-m, 1.0-μm Rtx-35 (Restek) column, 40 (2 minutes) 0–250 °C at 6 °C min^{-1} (hold 15 minutes).

2. 1-Amino-2-propanol, 3-amino-1-propanol, 2-amino-2-methyl-1-propanol, and similar compounds: 25-m CP-WAX-51 column or CP-WAX column for amines, 50–210 °C at 5 °C min^{-1}.

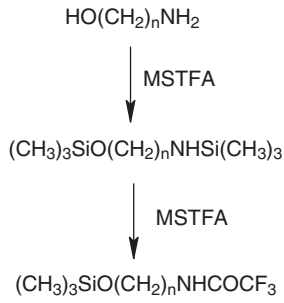
B. GC separation of derivatized amino alcohols
   1. 30-m DB-1701 column, 45 (10 minutes) 250 °C at 10 °C min^{-1}.

C. Mass spectra of amino alcohols
   In the mass spectra of aliphatic amino alcohols, the peak at *m/z* 30 is intense, whereas the peak at *m/z* 31 has a low intensity. The amino group dominates the fragmentation, making it difficult to recognize the alcohol group. If only an *m/z* 30 peak is found in the mass spectrum of an unknown, it does not mean that no alcohol group is present. Sometimes the peak at *m/z* 31 due to the presence of an alcohol moiety can be mistaken for the ^{13}C isotope peak relative to the peak at *m/z* 30. The presence of a distinguishable peak at *m/z* 31 suggests the presence of a terminal alcohol group. The mass spectra of these compounds usually do not exhibit an $M^{+\bullet}$ peak of a reasonable or even discernible intensity.

To identify the presence of an amino alcohol, use the following procedure. Prepare a TMS derivative of the unknown using MSTFA reagent and obtain a mass spectrum of the resulting TMS derivative. Prepare a second TMS derivative of the unknown using TRI-Sil Z reagent. When the molecular weight of the unknown increases by 144 mass units using MSTFA reagent and the TMS derivative using TRI-Sil Z reagent only increases the molecular weight by 72 mass units, this suggests the presence of both an amino group and a hydroxyl group in the unknown—TRI-Sil Z reagent silylates alcohols and carboxy hydroxyl groups but not amino groups.

A method to determine the number of amino groups present in the molecule requires the formation of a TMS derivative with MSTFA which silylates both hydroxyl and amino groups [3]. First, obtain a mass spectrum of the unknown using GC/MS. Next, add MBTFA reagent to the previously prepared TMS reaction mixture and let stand approximately 30 minutes. Obtain a mass spectrum of the resulting reaction product. A trifluoroacetyl group will replace each TMS group on primary and secondary amino groups because the amino-TMS group is less stable. From the mass differences obtained before and after the reaction with MBTFA (24 for each amino group), the number of amino groups present can be determined.

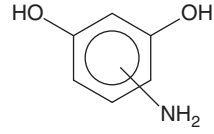
MBTFA $CF_3CON(CH_3)COCF_3$
N-methyl-bis (trifluoroacetamide)
MSTFA $CF_3CON(CH_3)$-TMS
N-methyl-*n*-trimethylsilyl trifluoroacetamide



11.6. AMINOPHENOLS

A. GC conditions
   1. Aminophenols (underivatized): *o*-aminophenol, *p*-aminophenol, and acetanilide: 30-m DB-1701 column, 45–250 °C at 10 °C min^{-1}.
   2. Aminophenols (as acetates; see Chapter 35.1.C for acetate derivatization procedure) acetanilide (C_8H_9NO), *o*- and *p*-aminophenol ($C_{10}H_{11}O_3N$) 30-m DB-1 column, 100–250 °C at 10 °C min^{-1}.

B. Mass spectra of underivatized aminophenols
   $M^{+\bullet}$ peaks of unsubstituted aminophenols are intense. The peaks representing the $[M - 28]^+$ and $[M - 29]^+$ ions are observed. Peaks of prominent intensities are seen at *m/z* 80 and 109 ($M^{+\bullet}$ in the mass spectrum of all three regioisomers of aminophenol). There is no apparent ortho effect exhibited by the mass spectra of any of the three isomers of aminophenol; however, the mass spectrum of 2-aminoresorcinol exhibits an $[M - H_2O]^+$ at *m/z* 107, which has an intensity that is ~40% of that of the base peak. This peak is not present in the mass spectrum of 4-aminoresorcinol, the only other amino resorcinol spectrum in NIST08. This is a clear example of the ortho effect.



C. Mass spectra of aminophenols as acetates
   The $M^{+\bullet}$ peaks of the unsubstituted phenols are observed in the mass spectra of these compounds (acetate adds to amino group), but they have

a smaller intensity than the $M^{+\bullet}$ peaks in the spectra of the underivatized aminophenols. The $[M - 42]^+$ peak is characteristic of acetates. Peaks with prominent intensities are observed at *m/z* values of 109, 151, and 193. The mass spectrum of the *ortho*-isomer of the derivative does exhibit an $[M - H_2O]^+$ peak, which is missing from the mass spectra of the acetate derivatives of the *para*- and *meta*-isomers of aminophenol. Again, this is a good example of the ortho effect.

11.7. Solvent Consideration

Amines tend to be very reactive compounds. Long-chain aliphatic primary amines like stearylamine ($C_{18}H_{37}NH_2$) will form $C_{17}H_{35}CH_2–N=CH_2$, which has a molecular weight that is 12 Da higher than the molecular weight of stearylamine. The compound is difficult to identify because there is no $M^{+\bullet}$ peak, just a very discernable $[M - 1]^+$ peak. When 3,4-(methylenedioxy)-phenethylamine hydrochloride was dissolved in methanol at a level of about 10–100 ng μL^{-1} and injected at an injection-temperature of 290 °C, there was almost a 100% conversion to the $R–H_2C–N=CH_2$ analog. The first time this was encountered, it was unexpected and resulted in quite a surprise. When ethanol was substituted, the corresponding $R–H_2C–N=CH_2CH_3$ analog was formed. Similar results have been reported [4]. According to this citation, secondary amines appear to undergo methylation under these conditions.

In the GC/MS analysis of amines, care must be taken to avoid these potential reactions with solvent.

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