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Review Article

Spectroscopy of Amines of Pharmaceutical Interest

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ALTHOUGH THE USE of alkaloidal amines of plant origin as therapeutic agents extends well back into the history of mankind, it was not until the advent of synthetic organic chemistry in the 19th century that the first successful attempts were made to simulate and improve upon nature. Early in this century it was discovered that many body substances either are amines, are metabolized to amines, or can be simulated by amines. Certainly by its brilliant work elucidating the structure and function of epinephrine (1), norepinephrine (2), ephedrine (3), and the other sympathomimetic amines now so widely used in medicine, pharmaceutical chemistry has to a great extent been amine chemistry.

Amines are, of course, basic nitrogen-containing groups derived from ammonia. Amino groups can be found or placed on aromatic as well as aliphatic skeletons; they can be fused into alicyclic rings, be attached to atoms other than carbon, be quaternized to increase their basicity, or be located adjacent to other functional groups that make them neutral or even acidic. It is not the purpose of this review to cover all possible amino group combinations. For the purpose of this review we will restrict our discussion to basic amino-containing compounds in which the amino group is attached directly to an aliphatic carbon with no other substitution on the nitrogen other than alkyl groups or hydrogens. There may of course, be aromatic, or heteroatom-containing groups elsewhere in the molecule.

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The various forms of spectroscopy, UV, IR, NMR, Raman, mass, *etc.*, are all well-established techniques for elucidating gross and fine structure of organic compounds, for evaluating compound interactions, and under appropriate circumstances for quantitatively determining compounds in mixtures. It is the purpose of this paper to review specific applications of the various forms of spectroscopy for amine-containing compounds that have been or are likely to be of interest to people working in the field of pharmaceutical chemistry. Our primary purpose is to describe how spectroscopy can help elucidate the amine function but secondarily also to attempt to relate spectroscopy to the whole molecule.

Of the various spectroscopic techniques covered here, ultraviolet and infrared absorption are best known and most widely applied in structure identification and quantitative analysis. Nuclear magnetic resonance is now perhaps the most useful and most rapidly growing branch of spectroscopy for gross and fine structure determination. Mass spectroscopy shows great promise for rapidly unraveling difficult structures particularly when only small sample sizes are available, as for example, in the effluent from a gas chromatograph. Raman spectroscopy has much promise for structure assignment work, but it still awaits development of simple inexpensive equipment before its potential can be adequately realized. The most promising of all developments in organic structure identification is the combined use of all spectroscopic techniques to attack a single problem (4, 5). This very powerful pro-

cedure can become even more so when applied in conjunction with an interpretive computer. This certainly is the direction of future development in this field.

There are a number of good review articles and books that have been published on the use of the various spectroscopic techniques in analyzing amine-containing organic structures (6–8). Previous reviews in this journal have covered certain aspects of the subject quite adequately and should be referred to for further information on the subject.

NUCLEAR MAGNETIC RESONANCE

To attempt to review all of the literature for every report of nuclear magnetic resonance data on amines would be an impossible task. The number of scientific papers including NMR data has increased at an almost unbelieveable rate. Five years ago 12% of the reports in the *Journal of Organic Chemistry* contained NMR data either as the major topic of discussion or as supporting data for structure assignment. Today, it would be safe to say that no more than 12% do not contain NMR data. It has been reported (9) that in 1964 NMR was mentioned in every sixth paper appearing in the chemical literature of the United States. In other words, every journal reporting synthesis or structure elucidation of organic compounds serves as a repository for NMR data. Realizing the difficulties inherent in undertaking a search of such dimensions and the practical limits of time and space allotted here, this reviewer has chosen to present a broad review of the techniques developed specifically for amines. These data will be supplemented with references to pharmaceutical and biologically important amines.

The use of nuclear magnetic resonance in the elucidation of the structure of amines has found wide application. The identification of *N*-alkyl groups by NMR has been described by several workers (10–13), and the technique has largely replaced other methods for identifying *N*-alkyl groups. Ma and Warnhoff (14) and others (15–18) have discussed the application of NMR for detection of *N*-methyl groups in particular. The NMR method for *N*-methyl analysis is described as being dependent on a pronounced downfield shift of the resonance peak of basic *N*-methyl groups in solvents of increasing acidity. The effect is due to the decreased shielding of the methyl protons of the positively charged ion relative to those of the free amine. This has also been mentioned by earlier investigators (17–19). Ma and Warnhoff have presented the NMR spectral data on a series of 116 compounds containing *N*-methyl and/or other methyl groups and have recorded the spectra in deuterochloroform, perdeuteroacetic acid, and trifluoroacetic acid. *N*-Methyl protons in secondary amines (aliphatic) have been found to be decidedly less deshielded in strong acid than *N*-methyl groups in tertiary amines. The explanation offered for this behavior is that the charge on the protonated secondary amine is more dispersed (20), and therefore, the smaller charge has a smaller deshielding effect on the *N*-methyl protons. A large number of chemical shift values and spin-spin splitting constants was given.

An extension of the method to the identification of primary, secondary, and tertiary aliphatic amines has also been reported (16, 21). Once again it is found that the effects of strongly acidic solvents on the chemical shift and spin-spin splitting constants of the protons α to the amine ion are characteristic for the particular functional group. The protons adjacent to the primary amine ion, NH_3^+, are split into a quartet; those adjacent to secondary ion, NH_2^+, into a triplet, and those adjacent to tertiary ion, NH^+, into a doublet. This phenomenon is also reported by Loewenstein and Meiboom (22) and Jackman (23). The nuclear magnetic resonance spectra of amino acids have been reported. Among the more interesting studies that have been reported are those on the correlation of NMR spectra with the L- and *meso* structures of cystine (24) and of the effect of optical activity of amino acids on their NMR spectra (25). There have been comprehensive analyses of many specific amino acid spectra using NMR (26–29); much detail on the spectra of the individual acids is given. In addition, Thompson *et al.* (21) have shown that spectra of *N*-substituted amino acids examined in alkaline deuterium oxide and in trifluoroacetic acid show chemical shifts and first-order spin-spin splitting patterns that are useful for identification of *N*-methyl, *N*-methylene, *N*-phenyl, and other groups attached to the nitrogen of an amino acid.

Data on aromatic amines are not as plentiful as those on aliphatic amines unless one wishes to consider the spectra of individual amines reported in the literature. The spectrum and chemical shift of the aromatic protons in aniline have been reported (30), and Ma *et al.* (14) have included data on 13 aromatic and *N*-heterocyclic amines in their work. Identification of *N*-phenyl amines by acidic solvent effects on the chemical shift and spin-spin splitting constants of the phenyl protons were reported (31) to provide the basis for the identification of the *N*-phenyl groups

in primary, secondary, and tertiary amines.

In addition to these rather general techniques for identification of amines, there have been collections and compilations of NMR data on nitrogen-containing compounds. One of the most recent and extensive papers is presented by Slomp and Lindberg (32), in which the structural and chemical shift data for 2306 hydrogens influenced by nitrogen in a variety of ways have been correlated and charted. There are also publications on specific classes of nitrogen-containing compounds such as the alkaloids (33). Other heterocyclic systems which have been recorded and analyzed are the pteridines (34), pyridines (35), piperidines (36), purines (37), pyrrolines (38). A general treatment of heterocyclics by NMR is also available (39). Reports on pharmaceutical applications of NMR have been presented (40), and Jardetzky and Jardetzky (41) give many examples from the biological field.

For those who have found that trying to keep abreast of all the current NMR literature is impossible, but who would like to stay up to date, there remains the abstract services (42, 43).

Techniques

Spin-Decoupling—Equipment is commercially available for heteronuclear spin-decoupling and has been used with success. Anderson and Silverstein (16) have described the decoupling of the NH^+ protons from the nitrogen in amine salts. The actual performance of a spin-decoupling experiment is straightforward, and an experienced operator can accomplish it in a relatively short time. For a complete and, excellent review of spin-decoupling see Baldeschwieler and Randall (44). Heteronuclear spin-decoupling equipment can be purchased either from the various instrument manufacturers or from Nuclear Magnetic Resonance Specialties, Inc., New Kensington, Pa.

Micro Sampling—Most instrumental techniques are taxed to the limit of sensitivity in the pharmaceutical and biological areas where a constant problem is paucity of sample. In the study and identification of metabolites, for instance, frequently there is an insufficient amount of sample to obtain spectral data by routine methods. This is especially true in NMR work, but the use of a CAT (computer of average transients) has eliminated much of the problem of small sample sizes. A spectrum, is scanned repeatedly a preselected number of times. The signal output from the spectrometer is stored and accumulated by the computer which presents a final spectrum with a total signal proportional to the number of passes through the spectrum and with the noise accumulated as the square root of the number of passes. This gain in signal to noise ratio and the buildup of sample signal allows one to obtain an NMR spectrum on microgram quantities of sample. Applications of the technique to biological systems have been reported (45).

INFRARED SPECTROSCOPY

An earlier review in this Journal by Carol (46) presented the fundamental theoretical concepts and techniques of infrared spectrophotometry as applied to pharmaceutical analyses. In the present report we shall confine our interest to the relatively narrow range of amines. The areas of interest in the infrared spectra of amines concern themselves primarily with the stretching and bending or deformation vibrations of the amine function. These regions have been assigned (47–49) as follows: N—H stretching region for primary and secondary amines from 3500–3300 cm.$^{-1}$; NH_2 bending or deformation approximately 1650 cm.$^{-1}$. Primary amines are identified by two absorption bands in the 3500–3300 cm.$^{-1}$ region (47–52). A third band is often found at slightly lower frequency due to hydrogen bonding. This third band is generally broader than the two bands at higher frequency (53). The 1650 cm.$^{-1}$ region is also useful in identifying primary amines in that it contains the NH_2 deformation band. This absorption band is usually broad and of medium intensity (47, 54) and shifts to higher frequency on hydrogen bonding in a similar manner as the NH_2 stretching vibration (53). Because of its location, the absorption band can often be assigned only with difficulty when the complexity of the molecule increases, and aromatic and other functional groups such as secondary amides are present. In such cases a variety of techniques may be brought to bear on the problem. The two techniques most commonly used are deuteration of the NH_2 groups (55, 56) and/or formation of an amine salt (57). Deuteration causes a shift of the NH_2 absorption to lower frequency, and salt formation involves a shift to lower frequencies as well as other changes in the absorption bands. Although for secondary amines one might expect only one absorption band in the 3500–3300 cm.$^{-1}$ range, there are often two bands observed due to association (53). The band from the associated NH is broader than that of the free NH, just as in the case of the associated NH_2 band. However, the integrated absorption intensity of the absorption bands of primary amines is approximately double that for secondary amines.

Tertiary amines, lacking the NH function, offer no absorption bands in the areas 3500–3300 or 1650 cm.$^{-1}$. They are difficult if not impossible to identify as free bases by infrared spectroscopy. There exists the possibility of identifying the group attached to the nitrogen atom, *i.e.*, CH_3N, C_2H_5N, *etc.*, but in the final analysis it would appear that the best method of identification by infrared would be to form a derivative of the amine.

From what has been said thus far, one might get the impression that differentiation of primary, secondary, and tertiary amines could be made quite simply. However, such is not always the case. Duval (58) describes how various factors such as molecular association, interactions with solvents, and poor dispersion in some instruments make identification and differentiation of amine classes an extremely difficult proposition.

A great amount of effort has been exerted in the study of inter- and intramolecular interactions of amines (53, 59). The conclusion reached almost unanimously is that spectra, to be compared qualitatively or quantitatively, should be measured in the same solvent under the same conditions.

For positive identification of amines it has been found useful in many cases to form an amine salt. This is particularly true of pharmaceutical amines which are generally administered in the form of a salt. The formation of the NH_3^+, NH_2^+, and NH^+ ions results in a shift of the amine absorption band to lower frequency. The wavenumbers at which the resulting absorption bands occur are specific for each class of amine. Primary amine salts have been reported to show a series of weak peaks with a broad absorption band of medium to weak intensity located between 1960 and 2080 cm.$^{-1}$. Secondary amine salts have been found to have a series of three bands between 2860 and 2040 cm.$^{-1}$; splitting of one or more of these bands has been observed in some cases. Tertiary amine salts, which are by far the most common class of amines used pharmacologically, have the simplest absorption spectra since they contain only one hydrogen on the nitrogen. The distinguishing feature of NH^+ spectra is a broad and intense peak located in the range of 2770–2380 cm.$^{-1}$. The exact location of the absorption band depends on whether the molecule contains water of hydration (64). There is of course strong hydrogen bonding in all of the crystalline amine salts and very little absorption found at the normal N—H frequency range 3500–3300 cm.$^{-1}$.

One thing to be remembered in dealing with amine salts is to avoid preparation of alkali halide disks whenever possible. Changes in spectra arising from hydrogen bonding and interaction with halide disks have been reported (65).

Near IR—Near infrared spectrophotometry in this discussion refers to the area of the spectrum between 1.0 and 2.5 μ. We are eliminating the fundamental N—H region because it has been adequately covered in the preceding section. The area deals largely with stretching vibrations involving hydrogen bound to a variety of other atoms. The absorption bands in this region are overtones or combination bands of vibrations originating in the fundamental region. The principal use of the area is in quantitative analysis; however, it is possible to differentiate primary, secondary, and tertiary amines on the basis of their absorption here. In addition to other absorptions, primary amines have a peak at about 2.0 μ, which is due to a combination band resulting from N—H bending and stretching vibrations in the fundamental region (66). Secondary and tertiary amines lack the 2.0 μ band, but secondary amines have an absorption in the first overtone region which is located at about 1.5 μ. Tertiary amines have no absorption due to NH and contribute nothing to this area of the spectrum as free bases.

The area of the combination band (1.95–2.0 μ) has great quantitative value in determining primary amines alone or in combination with secondary and tertiary amines (67, 68). The chief disadvantages to more extensive use of this region of the spectrum is the increased sample size required for significant absorption in the overtone region and the lack of suitable solvents. Carbon tetrachloride is the only solvent transparent in the region from 1.0 to 3.0 μ. Carbon disulfide has only one peak in the area of 2.2 μ, which makes this a useful solvent also. However, because of solubility problems on one hand and possible solvent interaction on the other, it is sometimes difficult to operate under the most desirable conditions. Whetsel *et al.* (53), in describing solvent and concentration effects on the near infrared NH bands of primary amines state that the absorption bands tend to broaden and shift to longer wavelength with increasing concentrations. Intensities and band widths vary markedly from solvent to solvent and great care should be exercised in comparing qualitative as well as quantitative data on the same material unless the data have been collected under similar conditions.

The spectroscopy of amine salts in the near infrared region has recently been described (69). A technique using samples in the solid state is described by which qualitative and quantitative

data on the salts were obtained. Primary amine salts were distinguished from secondary and tertiary amine salts on the basis of the presence of a band at 2.18 μ and the absence of a band at 2.05 μ.

For a review of near infrared spectrophotometry in general, the reader is referred to the chapter by Goddu in "Advances in Analytical Chemistry" (75).

Far IR—During the last few years instrumentation extending beyond the 650 cm.$^{-1}$ region has become commercially available, and many laboratories have begun investigating the range of 700–200 cm.$^{-1}$ for potential application. The techniques and methods of analysis used in the 4000–7500 cm.$^{-1}$ region are generally applicable to the far infrared. Window materials and solvents are not used interchangeably from one region to another, but there is an adequate supply of these for both regions. For example, the range to 400 cm.$^{-1}$ can be covered with potassium bromide cell windows and disks, while cesium bromide and cesium iodide transmit reasonably well to about 300 cm.$^{-1}$. Polyethylene has been shown (76) to be a useful window material for far infrared work. Good solvents for the far infrared are available although not as common as those available for the infrared region. Wyss *et al.* (77) have published a collection of 12 organic solvents for possible use in the far infrared.

For solid state samples the common alkali halide disks have been found to be most useful. Cesium iodide has also been used to press disks. Polyethylene and hydrocarbon wax have also been suggested as possible disk matrices (78). The information obtained in this range of the spectrum is related to the skeletal vibrations in the amines. Although the amount of information available on amines in this range is small relative to the 4000–7500 cm.$^{-1}$ range, some work has been reported on NH_2 vibrations (70). Tsuboi *et al.* (71) and Fukushima (72) have investigated amino acids in some detail in this region and have reported on NH_2, ND_2, and NH_3^+ vibrations. While the potential of the far infrared for providing a wealth of information for routine analysis is low, the area will undoubtedly provide more supplementary information that will aid in identifying molecular vibrations.

Attenuated Total Reflectance—The technique of attenuated total reflectance should be mentioned in this review of pharmaceutical amines because of its ability to obtain spectra with relative ease on samples which would ordinarily be difficult or impossible to handle. While the technique of ATR is known principally for its application to surfaces, it is also extremely useful in obtaining spectra on small amounts of sample. Microgram quantities of sample spread out as a thin film on an ATR sample plate or crystal yield spectra of excellent quality. The practical applications of the technique include analysis of metabolites isolated from biological systems and fractions from thin-layer and gas–liquid chromatograms, in amounts that heretofore would have precluded any attempt at identification by infrared spectra.

The principles of attenuated total reflectance have been set forth by Fahrenfort (73) and Harrick (74). The equipment in the form of attachments for present infrared spectrometers is available commercially from several manufacturers, including Wilks Scientific, the Perkin-Elmer Corp., and Barnes Engineering Co.

ULTRAVIOLET SPECTROPHOTOMETRY

Ultraviolet spectrophotometry has become an established, fundamental technique in pharmaceutical research and analysis as well as in the broad area of organic and inorganic analysis. Its widespread use and acceptance is such that for a general review of theory and techniques the reader is referred to several recent texts and not necessarily to papers appearing in current journals. The possible exception to this statement is the review of ultraviolet spectrometry appearing biannually in *Analytical Chemistry*. The theory and applications of ultraviolet spectrometry have been treated in detail in such recent books as "Absorption Spectroscopy," by Bauman (79), "Theory and Applications of Ultraviolet Spectroscopy," by Jaffe and Orchin (80), "Molecular Spectroscopy: Methods and Applications in Chemistry" (81), "Ultraviolet and Visible Spectroscopy: Chemical Applications" (82), "Advances in Analytical Chemistry and Instrumentation" (83). Special topics have been covered in other volumes such as "Interpretation of the Ultraviolet Spectra of Natural Products" (84), "Spectrophotometric Analysis of Drugs" (85), and a review and tabulation of the ultraviolet spectra of alkaloids (86). The latter review is especially useful since it contains all the available ultraviolet data on alkaloids up to and including 1964. The data are arranged according to structural type for more convenient study.

There are several collections of spectra and spectral data available which provide quantitative as well as qualitative data on amines as well as other organic classes. Perhaps the most useful of these is the four-volume work "Organic Electronic Spectral Data" (87). This collection

provides qualitative and quantitative data, often in several solvents, for each compound. The data are derived from a literature search involving some 69 journals. It is probably the most comprehensive work of its kind.

Ultraviolet spectrometry has found application in the study of aromatic amines in many areas of pharmaceutical analysis. The technique has been used in organic analysis and structure elucidation as well as to study stability, kinetics, ionization constants, mixtures, drug levels, and metabolites.

Organic Analysis—It has been mentioned previously that ultraviolet spectrophotometry is an established, fundamental technique in pharmaceutical analysis. Because of its general use in all phases of analysis it is often shunted aside and neglected in one of its most basic applications—structure elucidation. Many people bypass ultraviolet spectra completely in favor of the latest or what they consider to be more sophisticated techniques in the solution of organic structure problems. While other, newer techniques undoubtedly make important contributions in structural analyses, ultraviolet spectra can give much in the way of complementary or supplementary evidence for a specific structure proof. As a matter of fact, the determination of small amounts of organic material is very often done more conveniently by ultraviolet spectrometry than by any other method. Recent examples of the use of ultraviolet spectra in structural analysis are described in the work of Kamlet, Adolph, and Hofsommer (88) who showed that comparison spectra of 2,4-dinitroaniline and its *N*-alkyl and *N,N*-dialkyl derivatives permit discernment of the spectral effects of steric enhancement and inhibition of resonance. Baker *et al.* (89) have shown that it is possible to identify positional isomers in *N*-substituted uracils containing additional substituents in the 5- and 6-positions. Ultraviolet spectra taken in neutral and 0.1 *N* alkaline solvents show that substitution in the N-1 position results in a shift of less than 2 mμ. Substitution in the N-3 position results in a shift of 24–36 mμ.

In the quantitative analysis of aromatic amines, ultraviolet spectrometry plays a major role. Standard methods of analyses of aromatic amines invariably include UV as one of the methods of choice. Recent reports of the use of the technique include a spectrophotometric method for the determination of poldine methylsulfate (90), phenethanolamine after oxidation with periodate to benzaldehyde (91), and ephedrine, phenylpropanolamine, phenyramidol, and phenylephrine (92). In addition, Brandstaetter-Kuhnert *et al.* (93) have described a combination thermomicroscopic and ultraviolet spectrophotometric method for the qualitative determination of a series of antihistamines. Determination of thiobarbiturates by simultaneous recording of two spectra of the same material, differentiated by a spectrum shift due to regulated acidification of an alkaline solution of the thiobarbiturate, has been reported by Williams *et al.* (94). This use of differential UV spectrophotometry allows determination without interference from regular barbiturates. Needless to say it would be impossible to include here all of the articles and papers relating to the UV spectra of amines. For methods and applications for specific amines, the reader may consult the applications review articles in *Analytical Chemistry* or *Chemical Abstracts.*

Kinetics—Reaction rates and mechanisms of reactions in solution are conveniently followed by ultraviolet spectroscopy. An added advantage of following reactions this way is the detection and identification of intermediates and/or other unexpected products. Repetitive scans of the spectra of reaction mixtures at fixed time intervals permit observation and calculation of the amount of product as it is formed. The method, of course, is dependent on either formation of a new absorption band, which increases with time, or the decrease in intensity of the band of the starting material as it reacts to form a product. This method has been used by Eisenthal and Katritsky (95) to measure the rate of hydrolysis of 1-methoxypyridinium salts. A study involving oxidation and bromination of phenothiazine showed that the phenothiazine sulfoxide was the first stable product formed (96).

Szulczewski, Shearer, and Aguiar (97) studied the kinetics and mechanism of isomerization and hydrolysis of 4,6-diamino-1-(3,5)-dichlorophenyl-1,2-dihydro-2,2-dimethyl-1,3,5-triazine in dilute aqueous solution. In this study, which may be considered a typical kinetic study involving UV spectroscopy, a standard solution was prepared and diluted into various buffers held at the temperature of the run. Samples of the thermostated solutions were withdrawn periodically, and the ultraviolet spectrum of the intact starting material was obtained. The absorbance of the solution was read and the amount of unchanged material was calculated from a standard plot of the starting material.

Stability—The method for stability studies is the same as that used for kinetic work. The method provides stability data in a relatively short space of time, and the stability of the

material in question can be checked under a variety of conditions, *e.g.*, heat, light, time, *etc.* Recent applications of this method are reported by Garrett and Schraeder (98) and Simpson and Zappala (99). Riegelman and Fisher (100) have used the method to measure the stability of epinephrine. UV may also be used in stability studies in conjunction with thin-layer chromatography where decomposition products are isolated and determined by UV.

Amine Complexes—The formation of amine complexes showing absorption in the UV range has been used in the determination of amines. A number of articles describing formation of these UV-absorbing complexes have appeared recently. Ferric chloride has been found to complex with pyridine (101). The complexes of mercury and aniline have been studied (102), and the interaction of mercuric acetate with indole derivatives has been reported (103). Iodine has been found to complex with aniline (104), tertiary amine *N*-oxides (105), and 2,2-bipyridines (106). In a report by Lim *et al.* (107), a study of the solubilization and stabilization of phenobarbital by aminoalcohols is described. The increased solubility of phenobarbital by aminoalcohols is attributed to the formation of a "complex" or salt. An ultraviolet method for quantitative analysis of phenobarbital was used throughout the study.

Ionization Constants—The changes in the spectra of aromatic amines brought about by change of the pH of the solution reflect the changes in electronic state in going from free base to ion. These spectral changes are useful in studying tautomeric equilibria and for determining ionization constants of amines. The procedure generally followed in determining pKa values is to obtain the absorption spectra of the amine in basic solution, the amine ion in acidic solutions, and mixtures of the two forms in suitable buffer solution. The pH of the buffer solution is measured, and this in addition to the absorbance values at a given wavelength permits calculation of the pKa value from the following relationship:

$$\mathrm{pKa} = \mathrm{pH} + \log \frac{A_B - A_{OH^-}}{A_{H^+} - A_B} + \log \gamma^+$$

where A_B, A_{OH^-}, and A_{H^+} are the absorbance in the buffer, basic, and acidic solutions, respectively, and γ^+ is the activity coefficient of the ion in the buffer solution. This procedure or variations and modifications of it have been used to calculate the pKa values of many aromatic amines. For ultraviolet or pKa data on individual amines, the reader is referred to *Chemical Abstracts.* Recently pKa values of 2-(2′-thienyl)-pyridine and of methyl-2-picolyl sulfide were determined by Kahmann, Sigel, and Erlenmeyer (108) and of the dichloroanilines by Robinson (109). Apparent dissociation constants of some phenolic amines reported also (110), and there are reports of special techniques for individual amines and/or circumstances. For instance, determination of ionization constants on submilligram amounts of sample has been reported by Rinehart and Stafford (111).

Mixtures—Ultraviolet spectrophotometric analysis of mixtures is carried out routinely on two- and three-component systems. This is made possible, of course, by the fact that absorbance is linear with concentration and that the absorbances of various components in a mixture are additive. This concept is treated extensively in the text by Bauman (79) and others. Examples of its practical applications are found in the recent literature. Endriz (112) has described an ultraviolet spectrophotometric determination of heroin hydrochloride, methapyrilene hydrochloride, and quinine hydrochloride mixtures. The spectra for heroin, methapyrilene, and quinine show points of maximum absorption at 280 mμ, 313 mμ, and 348 mμ, respectively. Methapyrilene and quinine do not seriously interfere with the heroin maximum, and methapyrilene in dilute acid solution absorbs only slightly at the quinine maximum of 348 mμ. The procedure is fast and accurate enough for forensic purposes.

Analyses of chloramphenicol and tetracycline hydrochloride mixtures (113) and phenobarbital and pentobarbital in pharmaceutical mixtures (114) are further examples of the utility of UV spectrophotometry in this area.

Metabolite and Drug Levels—Because of the sensitivity of the ultraviolet method, the amount of sample required for spectral identification is very small; drug levels and metabolites have been determined in biological tissues and fluids after separation and extraction. The method is used extensively and routinely. Since most materials are not photosensitive, the technique is nondestructive, and the metabolite or drug can be recovered for further testing. Reports have been published for most pharmaceutical amines whose metabolites have been investigated discussing the UV spectra of starting material and metabolites. For UV data on specific metabolites, once again the reader is referred to *Chemical Abstracts* data on the specific parent amine.

RAMAN SPECTROSCOPY

Raman spectroscopy is presently undergoing a revitalization after years of relative inactivity. After the discovery of Raman spectroscopy in 1928, a wealth of information regarding molecular structure of organic and inorganic compounds was obtained. A large number of scientific papers was presented and published in the 1930's dealing with the analysis of molecular vibration by Raman techniques. Subsequently, however, infrared spectroscopy eclipsed Raman and took the lead in widespread use and application in industrial laboratories. The principal difficulty with Raman technique was the great care and time-consuming aspects associated with obtaining good data. In spite of the fact that good, reliable, and revealing information could be obtained with Raman, the experimental difficulties were too great for most people. Industrial chemists, especially, were reluctant to contend with the weak lines produced by Raman scatter, which took hours to produce a photographic spectrum of sufficient intensity, when they could prepare and obtain an infrared spectrum in a few minutes. Cost of equipment was also a factor which limited its use in most laboratories. The result of these experimental and cost disadvantages was the decline and overshadowing of Raman by infrared.

The development of the continuous laser as a source for Raman spectroscopy has revived and stimulated new interest in this technique. The laser, by increasing the intensity of the Raman scatter lines, has once more made the technique attractive. The additional development of electronic devices has made possible photoelectric readout and in a few minutes a complete vibrational spectrum from 4000–50 cm.$^{-1}$ can be recorded. The spectrum is recorded as intensity *versus* wavelength, and since the intensity is directly proportional to the concentration, the technique lends itself very well to quantitative analysis. Other advantages of laser-Raman are that the scatter lines produced by a laser source are sharper and easier to read than those from other sources. Focusing and collimating are simplified. Ultraviolet radiations from conventional sources which cause photodecomposition of some molecules is eliminated. Use of the ionized noble gas laser allows different laser lines of excitation to be used, and in some cases this eliminates the problem of fluorescence which often prevents obtaining a spectrum when a conventional Hg lamp is used. A sample containing water, which would obscure a good part of the infrared spectrum, can be run successfully by the Raman technique because the spectrum of water is weak in the Raman. The whole range from 4000–50 cm.$^{-1}$ can be seen without instrumental changeover (prisms, gratings, *etc.*). Selection rules for the Raman effect permit observations of some vibrations which do not make themselves known in infrared spectroscopy. For example, rotational transitions of molecules are observed for molecules which do not possess a permanent dipole moment. Vibrations involving groups such as $R_1R_2C{=}CR_1R_2$ which have very weak infrared absorptions at best can be seen clearly with Raman. The advent of the laser source has not only stimulated interest in academic and industrial scientists but has stirred the commercial world as well. With low-cost equipment available and more research on low-cost instrumentation promised, the outlook for increased use of Raman spectroscopy is very favorable.

For the best comprehensive summary of Raman spectroscopy up to 1960, see Brandmuller and Moser (115). Other review articles on the subject are those by R. N. Jones and M. K. Jones (116), A. C. Jones (117), and Green (118). A number of books devoted in whole or in part to Raman have appeared recently (119–122). The recent impetus given to Raman is indicated not only in the increased number of publications covering the subject but also in the beginnings of a shift of Raman from the academic laboratories exclusively to more industrial laboratories. The use of Raman to provide information on the C—C, C=C, and C—H skeletal bands of complex molecules will provide more information leading to the elucidation of structures. If the recent interest in Raman can be sustained and nurtured by more organic and analytical chemists, the amount of information involving group frequency-structure correlations in Raman will assume an importance on a par with infrared correlations used routinely in nearly every organic and analytical laboratory.

The principal applications of Raman in the area of organic chemistry have been in the field of hydrocarbons and amino acids. Studies have been made on glycine (123, 124), diglycine barium chloride monohydrate (125), and triglycine selenate (123). Stretching frequencies of N—H hydrogen bonding of these compounds have been investigated and reported by the same authors. Raman spectra of glycine have also been reported by Ghazanfar (126). Methyl, ethyl, and propylamine have been studied by Wolff and Staschewski (127). A number of other molecules have been investigated singly or in conjunction with infrared studies on the same molecule. It is often difficult to locate the

Raman spectra of the material in such cases because the spectra are often listed under infrared headings with little or no attention given to the Raman data in the journal index. In addition to this more recent data, there are, of course, data on individual amines listed in collections such as the Landolt–Bornstein (128) tables which include assignments for spectra. There are many amines and nitrogen-containing heterocyclics which are included in this collection. While the collection and accumulation of this data were slow and painstaking over the years, the data and assignments are of good quality and can be of great service not only in Raman work but in infrared as well.

MASS SPECTROMETRY

Introduction—The history of structural organic chemistry is filled with methods used to resolve complex molecules by breaking them into small pieces, which can be easily and unambiguously identified, and then assembling these pieces in a logical sequence to determine the original compound. Obviously as molecules become larger and more complex the task becomes more formidable. This differs in principle from infrared spectroscopy where functional groups present in the molecule can be identified and the positions of the groupings in respect to one another obtained. Isolated nuclear magnetic resonance data also provide fragmentary data and, if unsupported, does not permit unambiguous identification of many chemical species.

Just as NMR has become a valuable tool to provide unique structural information about unknown molecules and to complement infrared spectroscopy, mass spectrometry has also become a valuable complementary technique to provide additional unique information about molecules, and to facilitate the assignment and verification of chemical structures. Unlike ultraviolet, nuclear magnetic resonance, and infrared spectroscopy where compounds are recovered after analysis, mass spectrometry is a destructive technique; however, one might be consoled in knowing that fractional parts of a microgram of sample, which otherwise might be insufficient for other instrumental methods, are sufficiently large to obtain a perfectly good mass spectrum. Another important feature of mass spectrometry, in addition to its sensitivity is the fact that, even though a sample is impure, considerable structural information can be obtained, and although the sample is destroyed, a permanent record of the data can be obtained in an extremely short period of time. Scans of masses up to approximately 500 atomic mass units can be made in a matter of seconds.

There has been an aura of mystery associated with mass spectrometry because of the complex nature of a mass spectrum which can comprise a hundred or more peaks, and the lack of a simplified theoretical approach for interpreting these data. In spite of the immensity of data provided by a mass spectrometer, this technique has come of age and has become an equally important partner of the other spectroscopic techniques. This rise in importance and popularity is due, for the most part, to the efforts of such men as Professor F. McLafferty, Purdue, Dr. Klaus Biemann, M.I.T., Dr. R. I. Reed, Glasgow University in Scotland, Professor Carl Djerassi and his co-workers, Stanford, Professor Einar Stenhagen, Dr. Raynor Ryhage, Dr. J. H. Beynon, Dr. J. S. Shannon, and several others who have contributed significant data to the literature of this subject.

Mass spectra of compounds containing the amine function have been studied extensively (7, 129–136, 138). In the work of Gohlke and McLafferty (132), 67 aliphatic amines were studied, fragmentation patterns were presented, and methods for identifying amines were given. Collin (129) reported on the mass spectra of aliphatic amines including appearance potential data, likely structure, and additional mass spectral correlation data. Judson and Struck (153) have also correlated mass spectral data on aliphatic amines. The theory of unimolecular decomposition as applied to mass spectra electron bombardment of aliphatic amines has been discussed by Hurzeler, Ingraham, and Morrison (154). Budzikiewicz and co-workers (7) have discussed the mass spectra of aliphatic, cycloalkyl, aromatic, and steroidal amines as well as other nitrogen functions in their book and publications. A correlation of the mass spectral data of alicyclic amines has been reported by Saunders and Williams (155). This class of amines has been studied by several other investigators (155–159). The fragmentation process of some cyclic amines (158) and the location of the rearranging atom in some alicyclic amines (156) was established by deuterium labeling. The usefulness of dimethylamine derivatives in the study of steroidal fragmentation was presented by Pelah and co-workers (160). Geminal amine mass spectra (134), derivatives of amines (161, 162), and the mass spectra of amino acids (163–167) and derivatives (164) of amino acids have been studied extensively. Aromatic amines (140–143) and heteroaromatic *N*-containing compounds (168) have been studied extensively because of their significance in the pharmaceutical industry.

A discussion of all the above aspects of amine mass spectrometry is a formidable and quite impractical plan in this type of report; however, this is an attempt to present a practical coverage of pharmaceutically important amine-type functions, the factors that have provided the basis of mass spectral study of these compounds, and an understanding of the factors involved in using mass spectrometry for further study and future research in these areas.

In its most simplified version, mass spectrometry of organic molecules involves the bombardment of a hypothetical molecule, ABCD, in the vapor state with a high energy electron beam. If the ionization potential of the molecule is less than the energy of the electron bombardment, a single electron will be expelled from the molecule and a molecular ion ($ABCD^{+\cdot}$) is produced (*A*):

$$ABCD + e^- \longrightarrow ABCD^{+\cdot} + 2\,e^- \quad (A)$$

If this ion ($ABCD^{+\cdot}$) is accelerated without further breakdown and deflected in the magnetic field of the mass spectrometer, it will be collected at a radius that corresponds to the molecular weight. This, in fact, is the basis of molecular weight determinations by mass spectrometry. Further and more intense electron bombardment will break bonds and produce additional ions and smaller fragments of the original molecule. As more bonds are broken a multiple stepwise process will occur, producing one positively charged ion and additional neutral species:

Fragmentation:
$$ABCD^{+\cdot} \longrightarrow ABC^+ + D\cdot \quad (B)$$
$$ABC^+ \longrightarrow BC^+ + A\cdot \quad (C)$$
$$BC^+ \longrightarrow B^+ + C\cdot \quad (D)$$
$$BC^+ \longrightarrow C^+ + B\cdot \quad (E)$$

Rearrangement:
$$ABCD^{+\cdot} \longrightarrow AD^+ + BC\cdot$$
$$ABCD^{+\cdot} \longrightarrow AC^+ + BD\cdot$$

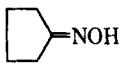
Ionization:
$$ABCD \longrightarrow ABCD^+ + 2\,e^-$$
$$ABCD \longrightarrow ABCD^{2+} + 4\,e^- \quad \text{(multiple ionization)}$$

The ions and fragments which form will depend upon the bond broken, its dissociation energy, and the stability of the charged and neutral species formed. This formation of a charged particle is analogous to carbonium ion and multiple bond formation, with the consequent stabilization of the ion by formation of positively charged particles having considerable stability, such as tropylium, azatropylium, acylium, oxonium, and ammonium ions.

$R'-CH=\overset{+}{O}H$ (oxonium)

$R-C\equiv O^+$ (acylium)

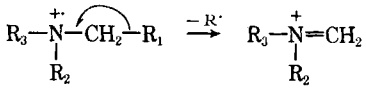

(tropylium)


(cyclic oxonium)

$$R'-CR''-NH_2^{+\cdot} \longrightarrow R'-CR=NH_2^+$$

(ammonium)

In general, the stability of a molecular ion will be enhanced by any structural feature which permits spreading of the odd electron charge over the molecular ion rather than concentrating it at any one atom. Thus charge delocalization leads to greater stability. In aromatic compounds, conjugated unsaturated systems, and functional groups having unsaturated groups, stable bonds are produced. In alicyclic systems where two bonds have to be broken to produce a fragment, additional stability is encountered. In amine compounds with which we are concerned here, the most easily removed electron in the ionization process is from the lone pair (nonbonding) electrons of the nitrogen atom. The process may be represented as in Scheme I.



Scheme I

[Homolytic processes will be indicated by a fishook (↷) which implies a one-electron shift, whereas an arrow (↷) represents a heterolytic (two-electron shift) (7).]

In dealing with the mass spectra of amine compounds we are dealing with a molecule having a nitrogen atom which provides more information than a nonnitrogen-containing molecule. In compounds having an odd number of nitrogen atoms, the molecular weight is odd, and in those having an even number of nitrogens, the molecular weight is even. In organic molecules containing carbon, hydrogen, and oxygen, fragments arising from the cleavage of *one bond* without hydrogen, transfer will always appear at odd mass numbers. The reverse of this occurs in compounds with an odd number of nitrogens, *e.g.*, one-bond fission of an odd nitrogen-containing compound will produce an even fragment. Elimination of neutral stable molecules is a frequent fragmentation mechanism. Nitrogen forms such stable species as HCN and NH_3 which are removed from amine-containing compounds. Amines frequently constitute a more volatile group than other nitrogen species, *e.g.*, NO_2^-, amido, *etc.*, and conversion to an amine permits easy handling and specific fragmentation,

which permits facile identification of unknown species. Deuteration of amino nitrogens also permits convenient study of reaction pathways and rearrangement processes. These points will be elaborated in the context of this paper.

The principles of mass spectrometry are dealt with in great detail in several textbooks (7, 169–174) and will be summarized here only briefly. To record the mass spectrum of a compound the sample has to be admitted into the ionization chamber of a mass spectrometer at pressures of 10^{-6} to 10^{-8} torr. For easily volatile materials this is done by placing the sample in a reservoir of the inlet system either as a gas or vapor at pressures of about 10^{-2} torr. and allowing it to "leak" through a very small orifice into the ionization chamber where it is bombarded with a high energy electron stream having a potential of about 10 to 100 e.v. (usually 70). The pressure in the chamber is kept low by a series of mercury diffusion pumps. Both positively and negatively charged particles and neutral fragments are produced. Only the positively charged particles are accelerated in the chamber, where the ion beam is focused magnetically and electrostatically (only in double focusing instruments) into a circular path. The radius of this path depends upon the ratio of the mass to charge of the cations (m/e). This radius is related to the accelerating voltage, V, and the magnetic field by the expression: $R = (2mV/eH^2)^{1/2}$. The mass spectrometer is designed so that only particles which describe a radius R will pass through the exit slit and be recorded by the ion collector. The current of the particles is of the order of 10^{-15} to 10^{-8} amps. This current is amplified and recorded *via* an electrometer and recording galvanometer which produces signals according to the relative abundance of the ions. The m/e value of the collected ions will be $R^2H^2/2V$ and will depend upon instrument design and applied electrical and magnetic field. The complete m/e spectrum is obtained by changing either the magnetic field, H, or the accelerating voltage, V, during the recording of a spectrum. It should be noted that mass spectra are recordings of discontinuous signals, unlike the continuous recordings in the infrared or ultraviolet region, since atomic masses vary in integral units. The units of m/e of atomic mass to charge in units of electric charge are termed "mass number" or atomic mass units (a.m.u.).

Instrumentation—The recent availability of moderately priced mass spectrometers (about $25,000) has permitted many smaller pharmaceutical laboratories and universities with limited budgets to enter the mass spectrometric field. As a result of this interest and activity, current literature on this subject has increased logarithmically in the number of published articles over the last few years and has significantly advanced the science. The lower priced instruments have resolutions of 500–850 with a 10% valley over the 1–300 mass range (Perkin-Elmer model 270) and up to 2500–3000 (AEI Model 15 and the Hitachi Model RMU6E).

High resolution instruments with a capability of 1 part in 30,000 or better are much more expensive ($80,000+). These instruments permit the analysis of fragments of about 5 p.p.m. or better. To be considered as high resolution, an instrument must have the capability of making mass measurements with a resolving power ($m/\Delta m$) of 10,000 or better with a 10% valley definition and at the same time have a precision of better than ± 10 p.p.m. measuring capability.

Much of our present mass spectrometric instrumentation is designed for use in qualitative organic structural analysis, and differs from earlier instrumentation which was used for the determination of isotope masses and, later, for relative abundances of these masses. These very early instruments were developed in the 1930's into precision equipment for mass/charge determinations which led to exhaustive studies using stable isotope tracers. About 1940 commercial instrumentation was first placed on the market.

There are two major types of commercially available high resolution mass spectrometers, the Mattauch-Herzog and the Nier-Johnson type. In both of these instruments there is an electrostatic field used as an energy selector, which controls the energy spread in the ion beam produced by the initial kinetic energy of the ions. The differences are in the methods of focusing the ions. In the Mattauch-Herzog system all the ions are focused simultaneously in one plane, and a collector slit can, therefore, be placed along any point in the plane. By scanning the magnetic field a complete mass spectrum of the ions can be obtained. This can be displayed either on a photographic plate or a high speed recorder. These, of course are permanent high resolution mass spectrum recordings. For very high precision mass determination of peaks, a peak matching procedure is used incorporating an oscilloscopic screen. In the Nier-Johnson type system the ion beam is refocused at a single point, and it is at this point where the collector slit is placed. In either system, of course, a complete mass spectrum can be recorded within a fraction of a second to a few minutes, depending upon the resolution desired and also on the particular spectrum being

obtained. A third type of instrument, the "time of flight" mass spectrometer, works on a different ion separation principle. Here a pulse of ions which has been produced by the ion bombardment is accelerated down a straight evacuated tube. In the course of their flight, the ions are separated according to the momentum they have received during their acceleration. These ions arrive at the ion collecter plate in order of increasing mass, the lightest arriving first and the higher ones follow in increasing order of mass. An instrument with capabilities of unit mass resolution of 700 ($m/\Delta m$) can be used for general organic structural analysis and identification. It has the advantage of attaining high scan speeds of a few milliseconds and is useful for thermodynamic, combustion, and fast reaction studies. It does not, however, meet the requirements for high resolution work.

The newest entry into the mass spectrometer field is the double focusing cycloidal medium resolution mass spectrometer (Varian Associates) which produces a cycloidal ion trajectory in which focusing depends only on the m/e of the ion and not on the direction or velocity of the charged particle.

Gas chromatograph attachment to the mass spectrometer permits mixtures to be separated and analyzed while the chromatograph is being run without the need for collecting individual peaks. This aspect will be discussed more fully later. Many instruments are equipped with detection systems which allow detection of negative ions produced in the fragmentation process. All the instruments mentioned here are suitable for use with pharmaceutical amines.

Sample Requirements and Manipulation—Boiling points and volatilities of organic amines range from the low boiling liquid to solid. Many of the amines are conveniently isolated and recrystallized as salts. Introduction of these materials into a mass spectrometer involves injection *via* a gas chromatograph, a gas inlet system, or a solid direct insertion probe. Modification by derivatization as silyl derivatives, trifluoroacetyl, or acetyl derivatives also provides convenient procedures for volatilizing the more intractable or heat-sensitive compounds. Probably the most elegant feature of the mass spectrometric method is the sensitivity of the instrument; submicrogram quantities of sample are sufficient for a mass spectrum (176). Biemann (175) has used 0.25 mcg. (1.5×10^{-9} moles) of phenylalanine and was able to get unambiguous identification of the compound. Elliot and co-workers (176) have obtained excellent accuracy of mass measurements with as little as 0.005 mcg. (5×10^{-9} Gm.) of ibogaine.

It is obvious, of course, but important that pure uncontaminated samples be used for the most valuable retrievable information. This presumes the use of high purity and sufficiently volatile solvents, which can be pumped out of the instrument before volatilization of the sample, when a direct sample insertion technique is used or when samples are being isolated from mixtures for insertion as liquids. This precaution is usually not necessary when good separation is possible *via* a gas chromatographic sample separation and insertion technique. Occasionally when an amine sample does not give a good molecular ion, introduction of a salt will help. If this is a hydrochloride it presents other problems by corroding equipment and being difficult to pump out completely. Nonvolatile compounds may sometimes be safely placed directly in the ion beam where, under the effect of high vacuum and temperature, small amounts are flaked off into the ion beam to produce a good spectrum. When a smoke or aerosol can be created in the ion source, highly intractable compounds inert to chemical modification are frequently analyzed successfully.

Molecular Weight—The molecular ion of amine compounds can be obtained if the compounds do not decompose under the influence of heat and electron bombardment at the low pressures (about 10^{-6}–10^{-8} torr.) that they will experience in the sample chamber of the mass spectrometer. In cases where decomposition does take place, more stable derivatives may be prepared by silylation, acetylation, trifluoroacetylation, *etc.* (discussed later). In most cases with amines, as with other compounds the "parent" or "molecular" ion may be found in the normal fragmentation pattern. It usually appears as the largest peak of the highest molecular weight multiplet of peaks in the mass spectrum, and represents a single electron removed by electron bombardment of the original compound: $M \rightarrow M^+ + e$.

McLafferty (132) examined the spectra of 67 aliphatic amines and found that the molecular ion was present, although in small relative abundance, in amines containing up to 16 carbon atoms. With primary amines the molecular ion intensity reaches a minimum (about 0.2–0.4%) in the region of nine to 12 carbon atoms. When the α-carbon atom is substituted, the molecular ion intensity is greatly diminished. McLafferty points out that in α-substituted primary amines containing four or more carbon atoms, the molecular ion is useless for analytical purposes; whereas, with α-substituted secondary amines, the molec-

ular ion is indistinguishable when there is a total of approximately eight carbon atoms. In cases where the longest chain of an α-substituted amine has more than four carbon atoms, the molecular ion cannot be used for molecular weight determinations.

In compounds where the amine function is attached or is part of an aromatic or alicyclic system, there is greater ion stability (177) and the molecular ion abundance increases significantly.

As the intensity of the parent peak of higher molecular weight amine compounds becomes indistinguishable precluding its use for determining molecular weight, there is increased possibility of observing the ion occurring at a molecular weight + 1, $(M + 1)^+$. This, of course, is now an even mass ion for compounds containing an odd number of nitrogen atoms, and as such is a diagnostic device, which helps to pick out this ion. This anamolous type of ion can also be found in alcohols, ethers, sulfides, glycols, and nitriles and is due to a bimolecular reaction which occurs in the ion stream. A striking feature of this ion which makes it useful for analytical purposes is the fact that it is pressure sensitive. The relative abundance of this $(M+1)^+$ peak increases with increased sample pressure and with decreased ion repeller potential. This peak is especially valuable with high molecular weight amines, and its presence can be demonstrated by rescanning the spectrum of these compounds at the higher mass range after reducing the repeller potential, thereby allowing longer ion residence time in the ion stream for reaction to occur. Increasing the sample pressure by adding another organic compound will also increase the relative amount of this peak. Munson and Field (178) have used chemical ionization mass spectrometry for certain amines and indicate that the ions in the ionization chamber of the mass spectrometer will produce a spectrum, which is more useful for determining the structure and the molecular ion for certain amines, and they indicate that the ions in the mass spectrum are predominantly high molecular weight fragments; whereas, the converse is true for electron impact spectra. Since the ionization potentials of primary aliphatic amines are greater than 8.5 v. and between 7 and 8 v. for tertiary aliphatic amines, the authors point out the differences indicated for the parent ion of these types of compounds are sufficiently different to allow isomers to be distinguished by this technique.

The $M+1$ ion indicated above was first reported by Wertzeler and Kinder (135) and later work (129) by Collin indicates that the $M+1$ ions arise from a reaction such as the following:

$$RNH_2 + RNH_2 \longrightarrow RNH_3^+ + RNH$$

To recognize the molecular ion in amines, certain tests may be applied to the highest mass peak that can help to distinguish whether this ion is not the molecular ion. These tests cannot determine if the converse is true.

Where the possibility exists that impurities are present, contributions from background or other artifacts, suitable steps such as partial ionization, examination of the compounds by thin-layer chromatography or gas chromatography should be used to complement these data. A combination of gas chromatography and mass spectrometry (discussed later) may provide a suitable system for obtaining a meaningful mass spectrum of the compound.

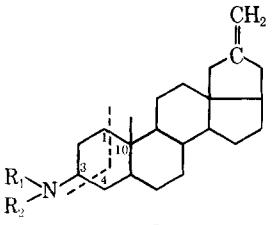
Since the molecular ion is produced by electron impact and the loss of one electron from the molecule, the ion produced must be an odd-electron ion, M^+, and appear at an odd mass. An even electron ion, M^+, appearing at high mass at an even mass number is usually the molecular ion. For an even mass ion to be a molecular ion it must contain an odd number of nitrogen atoms. In cases where major ions appear at positions separated from the highest mass ion by anomolous mass values or elemental composition by high resolution, mass measurement is illogical; the molecular ion is probably not the molecular ion or else there probably is an impurity in the sample. Mass losses of 3 to 14, 21 to 25, 33, 37, and 38 are highly improbable, and an examination of the masses compiled by McLafferty (179) in his mass spectral correlations will help to establish whether such an ion is probable. To prevent possible mistakes in considering the molecular weight of compounds, which have atoms where isotopes are naturally present, such as with bromine or chlorine, the mass should be calculated on the basis of the lower mass of the two isotopes. Low energy bombardment may also help to establish the identity of the molecular ion. Since the molecular ion is the precursor ion, it requires less energy for its formation, and as the electron energy is lowered, it should be the last one to disappear. In the case of some amines this would be difficult because of the low abundance of the molecular ion, but for derivatives, this should be suitable. An ion with the lowest appearance potential is not necessarily the molecular ion; only the converse would be true. Empirical formulas can frequently be determined from the two small peaks at $(M+1)^+$ and $(M+2)^+$, which are due to the isotopes of C, H, O, N, Cl, Br, S, *etc.*, present in the compound. If an illogical mass is obtained,

further investigation of other peaks, sample purification, *etc.*, are in order. Molecular weight determinations may also be field ionization (180) and rate of effusion techniques. Once the molecular ion has been identified, interpretation of the remainder of the spectrum can start.

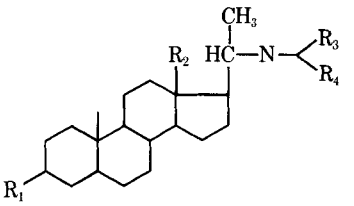
Use of Isotopes—Radioactive and stable isotope use in chemical, biochemical, and physiological fields has increased over the years very markedly. The availability of ^{18}O, deuterium, ^{13}C, and ^{15}N has provided the stimulus for research in biological and chemical fields where only small amounts of isolated compounds are available for study, and where incorporation of these materials into a molecule provides an elegant way to determine the mode of action, as well as to provide a means of following the compound in a living system. It is, therefore, not unusual to incorporate a stable isotope in a drug and use the mass spectrometer to assist in identifying and following the metabolic fate of the drug in an animal or microbial system. Because many pharmaceutical compounds have an amine function associated with them, it is not uncommon to exchange the active hydrogen atoms attached to a nitrogen with deuterium or to synthesize an amine or other nitrogen-containing pharmaceutical with ^{15}N.

Mechanisms (7, 158) of mass spectral fragmentation have been studied *via* deuterium exchange in many phases. The replacement of —N—H with N—D or NH_2 with —ND_2 leads to fragment ions of one and two atomic mass units greater than the parent; however, in the case of tertiary amines there is no exchange, and hence no effect.

Deuteration is achieved readily by equilibration of the sample with deuterium oxide. Deuteration has been useful to study fragmentation patterns, to determine structure, to examine ionization potentials. After deuteration of an amine, comparison of the fragments and the molecular ion permits identification of the fragments which have the nitrogen containing the deuterium. In cases where there are two nitrogens present in the molecule, the procedure becomes particularly useful. Systematic studies have been made by Pelah *et al.* (147) upon the migration mechanisms of hydrogen migration in amides and amines, and for the selective replacement of certain hydrogen atoms in *N*-acetylcyclohexylamine. Fragmentation of steroidal amines was also studied after deuteration. Incorporation of deuterium in the amine nitrogen of *Holarrhea* alkaloids of types I and II by Dolejs *et al.* (181) permitted these workers to ascertain the number of methyl groups bound to the individual nitrogen atoms.

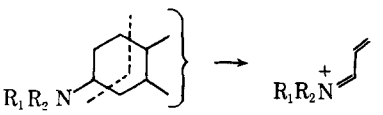


I



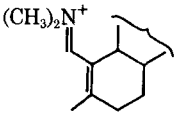
II

By the fragmentation process in Scheme II, cleavage in these molecules was shown to occur by rupturing of the C_3—C_4 bond (β-bond to the nitrogen atom) and the C_{1-10} to give fragments as those in the scheme. The nitrogen ion which was formed was stabilized by splitting off or transfer of hydrogen atoms. Adequate metastable peaks in sufficient abundance were also found to verify the fragmentation. Where R_1 and R_2 are methyl the inductive effect of the methyl group stabilizes the positive charge on the nitrogen atom (*Reference 175*, p. 88) and produces high abundance fragments. Additional studies on the structure and stereochemical relationships of steroidal dimethylamines (152) done by Pelah and co-workers supported these studies by deuterium labeling experiments. This work showed the most abundant ion in the fragmentation of steroidal dimethylamines corresponds to the immonium ion (III) and they predicted this



m/e = 56
70
84

Scheme II



III

species in terms of charge localization on the nitrogen of the molecular ion and subsequent fragmentation by rational bond homolysis and hydrogen transfer. They also showed in their studies that dimethylamino compounds were desirable derivatives for mass spectral purposes.

The exchange of active hydrogen for deuterium in amines can very conveniently be carried out by injection of D_2O into a gas chromatograph, and then immediate injection of the amine with the replaceable hydrogen. Alternatively, this can be done with the sample in contact with D_2O in the heated inlet system of the mass spectrometer.

Ambiguities arising from the splitting out, for example, of an M-28 peak, which could be due to the loss of H_2CN, CO, C_2H_4, or the appearance of a M-27 peak due to the loss of C_2H_3 or HCN, can be resolved in many cases with deuterium labeling.

Aside from the labeling aspect of isotope mass spectral data, the isotope peaks $M+1$ and $M+2$ can be used to calcuate the molecular formula of a compound. Details may be found in the textbooks mentioned above (170–175).

Elemental Analysis and High Resolution Mass Spectrometry—In addition to the information obtained from the molecular ion determination, information on molecular structure is obtained from an elemental analysis of secondary ions which provides information on the arrangements of the atoms in the molecule. Compounds which contain several isotopic elements (polyisotopic) provide unique peak abundances in proportion to the abundance of the isotopes normally associated with an element. These data are useful both qualitatively as well as quantitatively. They not only indicate the presence of these elements but often tell how many of the atoms are present in the molecule. The monoisotopic elements found with organic compounds include fluorine, phosphorus, and iodine. These are also useful in studying fragmentation mechanisms and compound structures. Perfluorokerosene, for example, is one of the most widely used internal standards for peak identification and peak matching to obtain very accurate mass values.

Microcombustion analysis for the elements, because of its inherent errors, falls short of the capabilities of the mass spectrometer; however, the data provided by such determinations very often complements mass spectrometric data and also provides information on the compound such as inorganic ash content, compound stability, nature and ease of compound decomposition, volatility, ease of sublimation, *etc.* Many researchers use combustion data to verify or support their mass spectral findings. One must, however, recognize that unit mass measurements with low as well as high resolution instruments provide accurate means for establishing exact molecular formulas. Because of this high degree of accuracy, high resolution mass spectral determination has removed the ambiguities that arise from combustion analysis data and has simplified this aspect of structural elucidation problems. This has been extremely useful in pharmaceutical applications for high molecular weight amine compounds, alkaloids, polypeptides, *etc.* (151), which differ from other possible structures by mass units less than one. Microelemental analysis would have been completely inadequate to assign exact empirical formulas for such compounds. Another advantage with mass data of this type is for determining molecular formulas of mixtures of compounds, if there is supporting additional information available on a sample. When molecular species present or suspected as being pressed in a sample have sufficiently different masses, they can often be recognized and determined because of unusual fragment ions which are not present in normal fragmentations. By changing parameters on the instrument such as ionization voltages to below ionization potentials of some of the compounds present in a mixture, distinction can be made between several compounds; by changing temperature, a fractionation is often possible; or by coupling a gas chromatograph, separate compounds in a mixture can be determined as they elute from a column. These are discussed later.

High resolution mass spectrometers which use both electrostatic and magnetic focusing systems are capable of determining and distinguishing between molecular formulas of monoisotopic molecular weights to values of less than a few parts per million of the actual mass on as little as 0.2 mcg. of sample. The modern approach to determining elemental formulas for all the ions in a mass spectrum with high resolution mass spectrometers was introduced by Biemann and co-workers (182). High resolution mass spectra may be recorded on magnetic tape, photographic plates, recorder charts, or obtained by peak matching techniques using an oscilloscope (171), or by combinations of all these techniques. These data may be fed into a computer (184), and the information may be displayed as an "elemental map" containing all the ion species arranged in columns according to mass, elemental composition, intensity of peaks, and elemental combinations (183). Element mapping makes use of all the peaks in a mass spectrum, unlike

that in normal spectra where many of the low abundance peaks are disregarded. These lesser abundance peaks very often provide much of the information, which allows a complete structure determination, that otherwise would either be impossible or extremely time consuming to obtain. Biemann (185, 186) has provided several examples of this "elemental map" technique for the analysis of high resolution mass spectra of alkaloids, steroids, amines, and many other compounds. Powers and Wolstenholme (183) used element mapping to determine the structure of linear peptides, cyclic peptides, and cyclodepsipeptides. They report that computer interpretation of the high resolution mass spectra of peptides completely removes the necessity for quantitative amino acid analysis. For linear peptides, acylation provides sufficient information for the computer to identify all the peaks by their atomic composition. Spectra at a resolution of 10,000 can be run in as little as 10-sec. intervals thereby permitting this technique to be used simultaneously with gas chromatography. The result is that unambiguous assignments of atomic composition of molecular and fragment ions are possible even in complex mixtures.

The accuracy of measurement is limited to the capability of the instrument to resolve very close peaks, and this determines, to a large extent, how unique a differentiation can be made to select two or more possible elemental combinations to fit an empirical formula. In cases where it is necessary to determine the differences in mass of a compound containing a CH_4 (m/e = 16.031300) or an oxygen (15.994915) where the difference in mass is (0.036385) or 36.4 micro mass units (m.m.u.) it would require an instrument with a resolving power ($m/\Delta m$) = 16/0.036385 = 440. If this difference existed in a molecule with a mass of 160, an instrument with a resolution of 4400 would be required (*e.g.*, 160/0.036385 = 4400), and, as the case may very well be with pharmaceutical compounds, a mass twice this amount, *e.g.*, 320, would not be unusual. This, of course, would require a resolution of 8,800 or very nearly 10,000 which approaches the resolution limits for many "high resolution" instruments. For a compound containing C, H, N where a distinction between CH_2 *versus* N is required (Δm = 12.6 m.m.u.) an instrument with a resolving capability of ±5 m.m.u. would be necessary. Other more demanding resolution capability (±1 m.m.u.) would not be unusual.

Biemann (186) has reported many of the techniques where high resolution mass spectrometry can be used. The voluminous data accumulated from mass measurements have made element mapping a very desirable technique and have made interpretation of spectral data an attractive technique. Suitable computer programs (183, 184) have been developed to handle such mass spectrometric data, and frequently when such data are presented, interpretation may be made by use of a few significant peaks which show the parent ion, the important fragments, and the fragmentation process with reasonable certainty. Many aliphatic amines (187) are determined this way. Several approaches to the interpretation of high resolution spectra have been reported and should be consulted for more details and the rationale used. The usefulness of high resolution mass spectrometery used in conjunction with element mapping or exact fragment determination has been reported in several reviews (188–194).

Sample Modification—*Derivatives*—Incorporation of stable and radioactive isotopes in compounds for mass spectral analysis was described earlier. Amine compounds very often can be successfully analyzed by means of their more stable derivatives. These compounds may be acetylated with acetic anhydride or other suitable anhydride, trifluoroacetylated, or converted into silyl derivatives (195).

Many easily obtainable and efficient silylating reagents such as bis-trimethylsilyl, acetamide (BSA), hexamethyldisilizane, trimethylchorosilane, bromomethyldimethylchlorosilane (197), and chloromethyldimethylchlorosilane may be used. The halogenated compounds are useful for work at low concentrations of sample, using gas chromatographs incorporating electron capture detectors at low or only moderate temperatures. Amino acids (198,199) are readily converted into volatile, stable derivatives with BSA, *N*-trimethylsilydiethylamine, *N*-trimethylsilydiethylamine.

Hill (170) and Reed (169) give detailed instructions on the utilization of chemical reactions to convert many compounds to amines to obtain characteristic fragmentation patterns for structural elucidation. They give examples for converting amides and nitro compounds to amines, nitriles to primary amines, isonitriles to secondary amines, *etc.*, to assist in characterizing them. Thus treatment of primary aromatic amines with nitrous acid and heat converts them to phenols, secondary amines to N-nitroso and tertiary amines to C-nitroso derivatives. The mass spectra of these compounds are usually quite easily distinguished because of characteristic peaks. In the case of C-nitroso *versus* *N*-nitroso compounds,

the *N*-nitroso compound should lose NO (M-30) easily. Reed (169) has treated the subject of distinguishing compounds containing one, two, or more nitrogens adequately. Budzikiewicz, Djerassi, and Williams (7) have illustrated the usefulness of the information in compound identification and reaction mechanisms of fragmentation patterns. They have also shown the utility of using stable isotopes in such studies. Several reviews on this subject are also available (131, 188).

Gas Chromatography–Mass Spectrometry—Resolution of many of the difficult problems associated with isolation of components in a mixture, especially when they are present in trace amounts, has been greatly facilitated when the advantages of a gas chromatograph have been combined with those of a mass spectrometer. The capability of rapid scanning of peaks emerging from a gas chromatograph, even those small trace peaks or peaks which have shoulders or tails, has provided information which previously has been extremely difficult and time consuming to obtain. Because of the speed at which a spectrum can be obtained on a mass spectrometer, a peak appearing in the gas chromatographic recording can frequently be scanned several times to determine if it is, indeed, a single component, or whether it is an unresolved mixture. It is easy, of course, to see the potentialities of this technique for studying the reaction products of an organic synthesis, microbiological transformation, metabolic transformation, or identification of the components in a simple mixture. Reaction mechanisms and much of the physical chemistry of compounds may be deduced by the procedure. Because mass spectrometers handle small samples (as little as 0.2 mcg.) with relative ease, the high plate efficiency of capillary gas chromatographic columns can be exploited. Thus, materials obtained from human, animal, or agricultural isolations can be examined easily. Research in the study of drug metabolites of chlorpromazine found in human blood (200) have been monitored by Holmstedt, Hammar, and Ryhage, Karolinska Institute. Their studies have indicated that chlorpromazine disappears from the blood in about 4 hr., but the drug metabolites continue to circulate for an indefinite time, and rather significant to medical and pharmaceutical scientists is the fact that breakdown products differ from person to person.

In addition to the advantages described above, the gas chromatograph provides a convenient way to introduce volatile gases, liquids, and volatile solids into the mass spectrometer. Rapid volatilization and hence low residence times at high temperatures often permit heat-sensitive compounds, such as steroids and their derivatives, to be examined without thermal decomposition.

In the past few years mass spectrometer–gas chromatographic combination instruments have become available commercially. These instruments couple gas chromatographs to a mass spectrometer by three popular separators (201), the Biemann-Watson, the Becher-Ryhage (202), and the Llewelyn types (203). The first two are based on the concept of sample enrichment by gas diffusion, whereas the Llewelyn, a two-stage separator, is based on the use of a permeable barrier separation where an elastomer barrier sets up a condition somewhat similar to the liquid phase of a gas chromatographic column. The gas not being very soluble does not permeate the barrier greatly, whereas the organic liquid, on the other hand, does. The result is an enriched gas on the opposite side of the barrier to the one in which the gas passes. For additional enrichment a two-stage separator is used. These elastomeric membranes are made of silicone rubber and permit very rapid organic liquid permeability while enriching the gas at the same time. Since permeation of different organic materials varies considerably, the gas chromatograph–separator cannot be used strictly as a quantitative tool; however, the gas chromatograph itself should be considered the quantitative tool (see above), and as such, does not affect the utility of the system. The operating parameters in using fast scanning, high resolution mass spectrometry in tandem with gas chromatography have been reported by McMurray, Greene, and Lipsky (204), by Watson and Biemann (205), and many others (206-208). A direct application for the separation and identification of optically resolved amino acids is reported in the work by Halpern and coworkers (209).

Quantitative Analysis—Quantitative mass spectral analysis was pioneered and certainly exploited by the petroleum industry. While many of the applications of mass spectrometery are for qualitative analysis or structural elucidation, quantitative mass spectrometric analysis of a 30-component mixture can be done rapidly and accurately ($\pm 1\%$) (210). The method used in quantitative mass spectrometric analysis is similar to that in infrared or ultraviolet spectrometry. Peaks are chosen from the spectrum of pure compounds and used for calibration. Ideal peaks are those which have high intensity and freedom from interference from other components in the mixture being analyzed. After choosing these peaks, measure-

ments are made for all the compounds present in the mixture and a calibration curve is prepared. Simultaneous linear equations are set up, one for each compound. Thus, a 30-component mixture would require 30 × 30 sets of equations (211). These are best handled using an inverse matrix and a computer program (213). A desk calculator is quite satisfactory for small systems once a matrix has been set up (175). Where occasional analyses, say a few a week, are required, mass spectrometry would probably be impractical; however, in cases where hundreds of analyses on multicomponent systems are needed, the mass spectrometer is a very efficient technique. Calculations for quantitative analysis are detailed in texts on mass spectrometry (169, 212, 213).

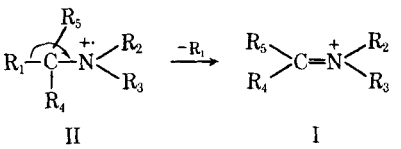
When mass spectrometry is combined with gas chromatography, the mass spectrometer sees only fractional parts of the sample because of the variances due to separator efficiencies and varying efficiencies of ion collection. This is due to the fact that ions pass through a variety of electrical and magnetic fields before reaching the collector. Exact conditions, careful control of temperature, and careful calibration are necessary for good mass spectral quantitative data. It is much more reasonable to use the gas chromatographic data for quantitative analysis and the mass spectrometer for the qualitative identification of the components present in the mixture.

Interpretation of Mass Spectra of Amines—After the molecular ion of a compound has been located and an empirical formula established from the mass spectral evidence, identification of structure begins. This involves the assemblage of the spectral fragments, a rational picture of the elements, functional groups, and other structural features into a chemical structure which will stand the tests of all or any other evidence that is presented, *e.g.*, spectral or chemical. The whole sequence of events involved in breaking down a molecule into smaller, more simple fragments must, in effect, be reversed. An understanding of these fragmentation processes has been the subject of extensive investigation by many serious individuals (145, 148, 154, 196, 214–218), but their work, for the most part, has been limited to practical, mechanistic, and semiempirical approaches. Their efforts, on the other hand, have permitted a logical interpretation of compounds based on spectral evidence. There has, however, been only limited effort made on the development of the theoretical aspects of mass spectrometry.

Many of the relationships in the fragmentation of simple amine structures may be extended to more complex pharmaceutical amine compounds, and an understanding of these will help to identify the structure of the more complex pharmaceutical amine structures. These structural features which appear in the mass spectra of amines are considered below on an individual basis. It should be remembered that identification and interpretation of mass spectral data is much easier and more meaningful if the data are plotted under high resolution conditions, and the formula of each peak determined from accurate mass measurement or an element map is prepared. Lacking such capabilities, much structural evidence may be gleaned from low (unit) or medium resolution instruments (231).

Aliphatic Amines—In considering the spectra of aliphatic amines, considerable information about the fragmentation processes may be deduced by a comparison with the spectra of known ethers and alcohol compounds (133). It might well be imagined that the spectra of amines would be more complex because of the nature of a nitrogen-containing molecule where there exists the possibility of three rather than two side chains. Primary aliphatic amines (129–133, 214) fragment by a C—C cleavage at the bond β to the nitrogen, by alpha cleavage, and by rearrangement and cleavage. Many examples of this type of fragmentation are found in the references given earlier (129–134). Rules regarding the fragmentation of aliphatic amines are included which were derived from a large number of amine compounds, many of which had been tagged with deuterium.

Important in the primary fragmentation process of an aliphatic amine (132) is the removal of the lone pair of electrons of the heteroatom, followed by cleavage of the carbon-carbon bond adjacent to the nitrogen atom (α-cleavage) to give a positive ion (I) and an alkyl fragment (R_1) (Scheme III).



II I

Scheme III

Ion I is the most intense peak in the spectra of primary, secondary, and tertiary alkyl amines (II, $R_4 = R_3 = H$) and α-mono- and α-disubstituted primary amines (II, $R_2 = R_3 = H$, R_4 = alkyl or H). When R_3 and/or $R_4 = H$, an M-1 peak is usually visible. The largest alkyl group is lost preferentially and hydrogen is eliminated only when no other alternative exists. Since the

amino group usually directs the fragmentation process (215) in preference to other functional groups because of the ease with which it loses an electron and because of the stronger resonance stabilization of the ion fragment, the only structural information available is that from the low mass region.

Examples of the fragmentation which occur in several amines of biological importance were reported by Beckett and co-workers (136) and by others (137) and are shown in Scheme IV. This information has been proposed to aid in the identification of metabolites as well as parent compounds in the urine of dosed individuals.

One mass unit differences in possible fragments differentiate between these two isomers (137).

Amino Acid Esters—Fragmentation of α-amino acid esters (217–223) occurs according to the general Scheme V, where β-cleavage is the preferred mode of fragmentation. The charge is carried on the amine fragment as expected. If the ester is an ethyl, the ester fragment will have a mass of 102 and can be used to identify the NH_2 position in other amino acid esters. From the molecular weight, the amine fragment, as well as the ester fragment, any substitution in the α-carbon position readily can be deduced.

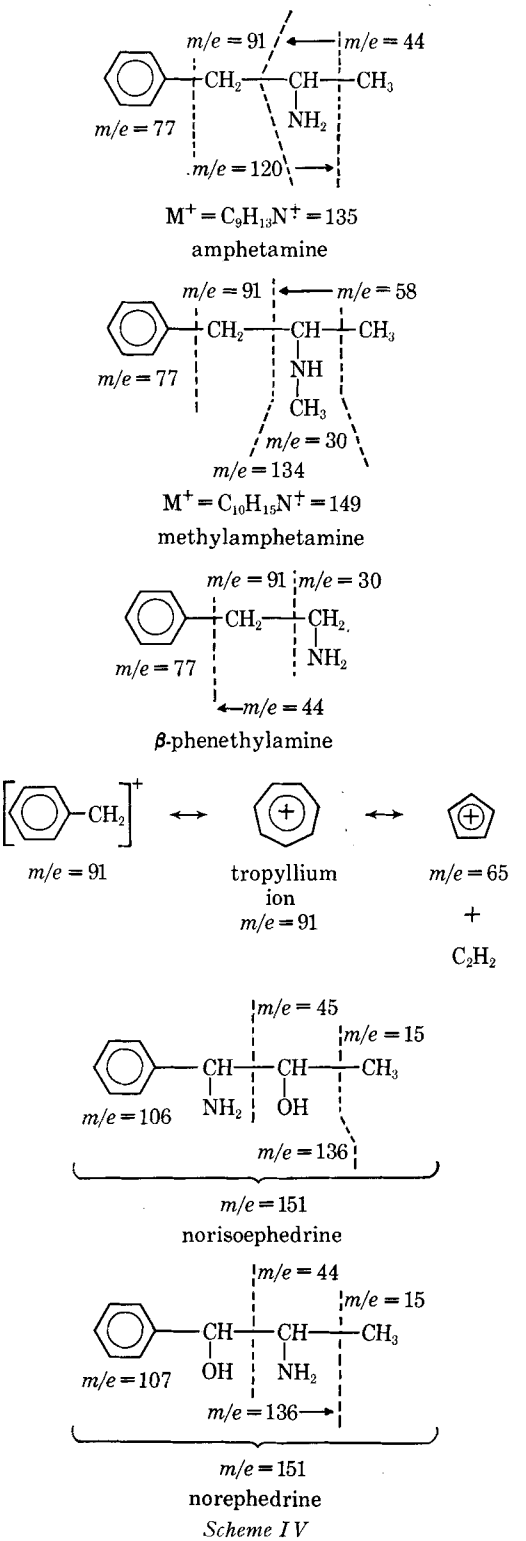
Just as in the aliphatic amine compounds, many amino acid esters have weak molecular ions but do have a characteristic M+1 peak detected by changes in pressure (*vide supra*).

Using the information obtained by studying known amino acid structures, it is possible to assign structures to new amino acids. Thus, Biemann *et al.* (224, 225) established the structure of lysopine, the lysine analog of octapine, which contains an arginine moiety. The processes leading up to the identification of new amino acids by mass spectrometry are elegantly described by Biemann (139).

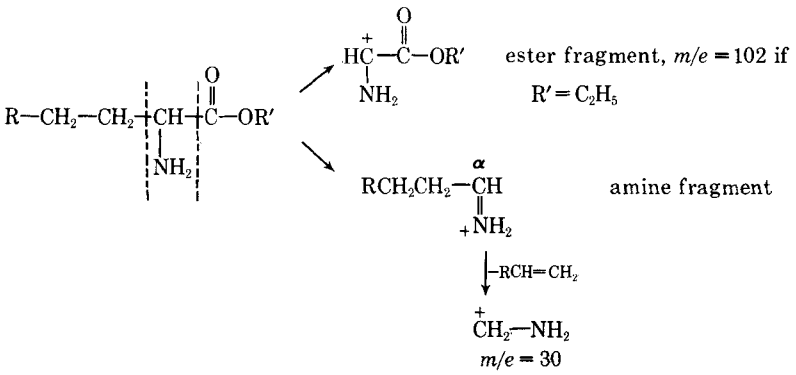
Aromatic Amines—The mass spectra of primary aromatic amines show that there are no bonds which are easily broken, therefore aniline loses one of the amine hydrogen atoms to give a moderately strong peak at M-1, also a peak in which HCN is lost. *N*-Alkyl anilines decompose by cleavage of a C—C bond next to a nitrogen to give an ion fragment of *m/e* = 106 (Scheme VI).

The mass spectra of aromatic amines have been reported by several authors (226–229).

Other Amine Structures—Reports on the mass spectra of cyclic amines include the work of Duffield and co-workers (230) and Gallegos and Kiser (231). Geminal amines (134) are described by Kostyanovskii and Pan'shin. Mass spectrometry has been successfully used to determine the amino acid sequence in oligopeptide methyl esters (146) and has proved ex-



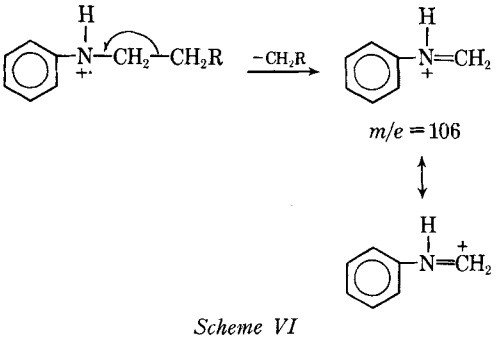
Scheme IV



Scheme V

tremely useful in structural and stereochemical problems of steroidal dimethylamines (147). Nitrogen in a steroid very strongly and consistently directs the mass spectral fragmentation, so it is very desirable to use mass spectrometry for the study of many steroidal amines to complement other spectral techniques. The fragments have diagnostic value for determining both the size and number of alkyl groups attached to nitrogen and also the nature of carbons in other functional groups in related molecules. Smith and Grostic (230) have described a procedure for mass spectrometric identification of the metabolites of an orally active diuretic agent, methyl *N*-(*o*-aminophenyl)-*N*-(3-dimethylaminopropyl) anthranilate. The mass spectra of enamines (149), and acetylene-amine adducts (150) have been studied. The recent review by Spiteller (216) on the mass spectrometry of heterocyclic compounds includes information on many amine-containing alkaloids, heterocyclic, and other compounds and should be consulted for an excellent description of the uses to which mass spectrometry may be put in the study of many biologically related compounds. Rearrangements (133) and hydrogen transfer reactions (132) in amides and amines are also reported.

In the field of pharmaceutical chemistry mass spectrometry has made a significant and important impact in the last 10 years and it promises



Scheme VI

to pioneer even greater pathways in the future. It should contribute much understanding of the processes involved in microbial as well as animal metabolism, and potentially supply valuable information for the development and advancement of new drug designs by providing the means for identification and elucidation of new structures of matter.

SUMMARY

The various branches of spectroscopy hold much promise for organic amine structure elucidation and analysis. Procedures and techniques for deducing information about these compounds are now quite well established, although new approaches are being developed daily. Future developments in this field are likely to include development of new sophisticated techniques and equipment, simplification of known techniques and equipment, and, most of all, wider use of computers to allow the research worker to learn more with less effort.

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Keyphrases

Amines–spectroscopy review
NMR spectrometry–structure
IR spectrophotometry–structure
UV spectrophotometry–analysis
Raman spectroscopy
Mass spectrometry

Research Articles

Role of Wetting on the Rate of Drug Release from Inert Matrices

By PARVINDER SINGH*, SAURABH J. DESAI*, ANTHONY P. SIMONELLI, and WILLIAM I. HIGUCHI

This investigation has shown that matrix permeability and rates of permeation of the matrix by the solvent can individually limit drug release rates. This was found to be a function of the pore size distribution of the matrix and the permeation pressure of the release media defined by its surface tension and contact angle. Methods useful in separating the various roles of the above were developed and are presented. They include the correlation and use of external pressure, vacuum techniques, surface tension and contact angle measurements, and porosimeter data. The results have been used to develop models to illustrate the possible systems that can be encountered. Concepts such as rates of pore permeation, varying solubility dependence, and tortuosity are developed and applied to these models.

PREVIOUS release studies have indicated that the polyethylene matrix gave excellent results in the presence of surfactant (1). The experiments in water, however, gave comparatively poor results as release rates were very slow and apparent tortuosities very high. It was speculated at that time that polyethylene was hydrophobic in nature and that wetting was not fully achieved.

Studies involving the effect of increasing concentrations of dibasic potassium phosphate on salicylic acid release from polyethylene matrices surprisingly showed no rate increase due to potassium phosphate even though the solubility increased by a factor of 45. There was, however, a dramatic rate increase in the presence of surfactant, even though the solubility remained constant.

These studies prompted this investigation in order to further clarify the possible mechanisms which can be operative under various conditions.

EXPERIMENTAL

Release Rate Determinations—The general procedure used for investigating the release rates has been previously described (2). It was necessary, however, to redesign the apparatus to allow the pressure to be kept constant at different pressures, as illustrated in Fig. 1.

A water-jacketed (C) glass conical flask with 2

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