Atmospheric Pressure Ionization (API) Mass Spectrometry. Solvent-Mediated Ionization of Samples Introduced in Solution and in a Liquid Chromatograph Effluent Stream

by **E. C. Horning, D. I. Carroll, I. Dzidic, K. D. Haegele, M. G. Horning** and **R. N. Stillwell,**
Institute for Lipid Research, Baylor College of Medicine, Houston, TX 77025

Abstract

Atmospheric pressure ionization (API) mass spectrometry is a novel form of mass spectrometry in which ions are generated in a reaction chamber external to the low pressure region of a quadrupole mass analyzer. Samples may be introduced in solvent-free fashion (by vaporization in a stream of preheated carrier gas, or in the effluent stream from a gas chromatograph) or with solvents (by injection of solutions, or in the effluent stream from a liquid chromatograph). Solvent-mediated ionization reactions leading to positive ions were studied. A liquid chromatograph-mass spectrometer-computer (LC-MS-COM) analytical system was constructed; the entire effluent stream from the chromatograph was vaporized through the API source.

Introduction

Atmospheric pressure ionization (API) mass spectrometry (1,2) is a novel type of mass spectrometry in which ionization is carried out in a reaction chamber external to the low pressure region of a quadrupole mass analyzer. Ions (and molecules) present in the source enter the mass analyzer region through a small aperture. The usual forms of ion analysis involve mass scans to obtain ion profiles (used chiefly in qualitative studies) and selective ion monitoring (used in quantitative work). Two different primary sources of electrons have been employed: a ^{63}Ni foil and a corona discharge.

Solvent-free samples may be introduced through direct vaporization in a stream of preheated carrier gas, or as eluates from a gas chromatographic column. Samples may also be injected in solution; in this case, the solvent(s) and compound(s) under study are vaporized simultaneously and are present at the same time in the ionization chamber. It has been found advantageous to employ solvents that can be ionized, and when this is done the terminal ionization step usually involves reaction of solvent-derived ions and the compound(s) under study.

These circumstances suggest that each mode of sample introduction can be employed in the design and operation of gas chromatograph-mass spectrometer-computer (GC-MS-COM) or liquid chromatograph-mass spectrometer-computer (LC-MS-COM) analytical systems in which either gas chromatography or liquid chromatography is used for separation purposes. The work described here was carried out to obtain additional information about solvent-mediated ionization processes, and to establish the principles of operation of a liquid chromatograph-mass spectrometer-computer (LC-MS-COM) analytical system in which the effluent stream is analyzed continuously by API mass spectrometry.

Experimental

Instrumentation and Operation

Details of the design and operation of an API mass spectrometer have been published (1,2). Two types of ionization chambers were employed. A low-volume reaction chamber containing a ^{63}Ni foil as a primary source of electrons has been described (2). A corona discharge source was also constructed; details of the design will be published separately. This source accepts samples in the same fashion as the ^{63}Ni source.

The size of the reaction chamber and the manner of sample introduction affect the limiting sensitivity in detection displayed by the instrument. With the low-volume reaction chamber described previously (2), samples in the femtogram range were detected with syringe introduction of solutions. The upper limit of linear response for this source was determined by the total ion current available for ionization purposes, and was about 10-20 nanograms. For experimental studies of LC-MS-COM analytical system design, it was considered desirable to start work with a high pressure liquid chromatograph of commercial origin which included a conventional ultraviolet photometer as a detector. By employing a larger reaction chamber, and a corona discharge as the primary source of electrons, the upper limit of response was increased. Samples of 5-100 nanograms were used in most experiments with serial detection by the ultraviolet detector and the API mass spectrometer.

1. Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I. and Stillwell, R. N., Anal. Chem. 45, 936 (1974).
2. Carroll, D. I., Dzidic, I., Stillwell, R. N., Horning, M. G. and Horning, E. C., Anal Chem., in press.

Two modes of ion analysis were used. Scanned mass spectra provided ion profiles showing all ions present in the reaction chamber during the scan period. Selective ion monitoring, based on narrow range scanning, was used to detect specific ions.

The liquid chromatograph was a Waters Model ALC-202 high pressure liquid chromatograph with ultraviolet detection capability at 254 nanometers. A short length of narrow bore stainless steel tubing was used to connect the effluent line of the LC unit to the vaporizer-source assembly of the API mass spectrometer. The columns were standard Waters one-eighth inch tubing; the length was 30 cm and the packing was Corasil II. Samples were introduced by syringe injection. Flow rates of solvents were 0.5-2.0 ml/min at pump pressures of 400-1600 psi.

The vaporizer-reaction chamber assembly was maintained at 200°C for studies of ion-molecule reactions. When continuous solvent flow (effluent from the LC unit) was employed, the temperature of the heated block was 280-300°C. The temperature gradient along the vaporizer tube was not defined, but the effect of continuous solvent flow was to increase the requirement for heat supply. The carrier gas (nitrogen) flow rate was 8-10 ml/min when continuous solvent flow was employed (operation of the LC-MS-COM system) with the corona source, and 30-40 ml/min when samples were injected by syringe in the fashion of gas chromatography (MS-COM operation).

Solvents and Reference Compounds

Solutions were prepared as described earlier (1,2). Benzene and benzene solutions generally showed few impurities. Chloroform solutions usually showed many impurities present in low concentration.

Biological Samples

Urinary extracts were obtained by extracting 5 ml of urine with 5 ml of solvent (benzene or chloroform), as described by M. G. Horning, Gregory, Nowlin, Stafford, Lertratanangkoon, Butler, W. G. Stillwell and Hill (3) for drugs and drug metabolites. The resulting solutions were transferred to clean, dry sample tubes, but were not treated with a drying agent. Derivatives were not prepared.

Results and Discussion

Primary Ionization Reactions Leading to Positive Ions

The positive ions present in the reaction chamber with both ^{63}Ni and corona discharge sources, when nitrogen is used as the carrier gas, are shown in Figure 1. Studies carried out at pressures up to 4 Torr by Good, Durden and Kebarle (4) indicate that the following sequence of reactions occurs:

$$N_2 + e \rightarrow N_2^+ + 2e$$
$$N_2^+ + 2N_2 \rightarrow N_4^+ + N_2$$
$$N_4^+ + H_2O \rightarrow H_2O^+ + 2N_2$$
$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH^\bullet$$
$$H_3O^+ + H_2O + N_2 \rightarrow H^+(H_2O)_2 + N_2$$

In the absence of water, the ion which would be present is N_4^+; however, traces of water cannot be easily removed from a reaction chamber maintained at atmospheric pressure, and the principal positive ions observed in studies with carrier gas alone are those derived from water. The distribution of cluster ions of the general structure $H^+(H_2O)_n$ is dependent upon the temperature and concentration of water in the reaction chamber. Ions of this kind have been observed previously in α-particle irradiated gases (5) (200 Torr pressure) and in a corona discharge (6) in nitrogen at atmospheric pressure.

At 200°C, with nitrogen as the carrier gas and without added water, the principal cluster ion is $H^+(H_2O)_2$. The ions H^+H_2O and $H^+(H_2O)_3$ are also present in lower concentration. The ion NO^+ (and its mono- and dihydrate) is also present.

Ionization of Solvents

The introduction of common organic solvents into the reaction chamber normally results in the formation of multiple ions. Alcohols (methanol, ethanol) react in the same way as water. Solvated protons with the structure $H^+(CH_3OH)_n$ or $H^+(C_2H_5OH)_n$ are produced. Figure 2 shows the positive ion profile observed at 200°C for chloroform stabilized with 0.75% ethanol. The chief ion is $H^+(C_2H_5OH)_2$, corresponding to the dihydrate observed when water is ionized. Protons with up to six solvating molecules have been observed when ethanol alone is vaporized through the source assembly.

Benzene, when injected into the reaction chamber at 200°C, gives the ion profile shown in Figure 3. The principal ions are $C_6H_6^+$ and $C_{12}C_{12}^+$. The dimer ion was described by Wexler (7); it is produced by col-

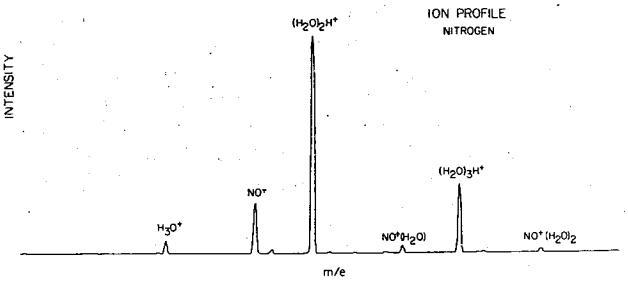

Figure 1. Ion profile showing positive ions present in the reaction chamber with a ^{63}Ni foil as the primary source of electrons and nitrogen as the carrier gas. Source temperature, 200°C.

3. Horning, M. G., Gregory, P., Nowlin, J., Stafford, M., Lertratanangkoon, K., Butler, C., Stillwell, W. G. and Hill, R. M., Clin. Chem. **20**, 282 (1974).
4. Good, A., Durden, D. A. and Kebarle, P., J. Chem. Phys. **52**, 212 (1970).
5. Kebarle, P. and Hogg, A. M., J. Chem. Phys. **42**, 668 (1965).
6. Shahin, J. M., J. Chem. Phys. **45**, 2600 (1966).
7. Wexler, S. and Clow, R. P., J. Am. Chem. Soc. **90**, 3940 (1968).

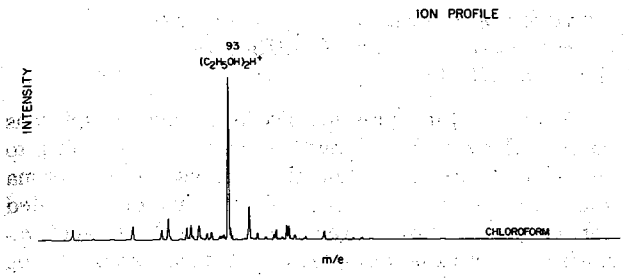

Figure 2. Ion profile showing positive ions present in reaction chamber (^{63}Ni source) after injection of chloroform (nitrogen carrier gas). Source temperature, 200°C.

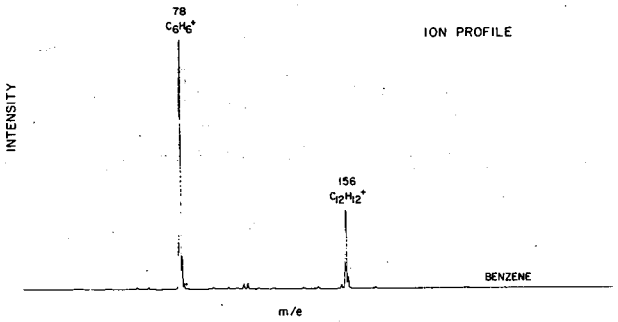

Figure 3. Ion profile showing positive ions present in reaction chamber (^{63}Ni source) after injection of benzene (nitrogen carrier gas). Source temperature, 200°C.

lisions of neutral benzene molecules with $C_6H_6^+$. Ions derived from water are not observed when benzene is present in high concentration in the reaction chamber. The formation of H_2O^+ may occur as a transient process, but this ion would be expected to react with benzene to produce $C_6H_6^+$ by charge transfer. In effect, an energy cascade occurs; the ionization potential of benzene is between 9.5-10.5 eV (8), and the only compounds which will ionize by charge transfer when introduced with benzene are those with a lower ionization potential.

A low concentration of the ion $C_6H_7^+$ is present along with $C_6H_6^+$. The route of formation of this ion is not known with certainty. Benzene is a stronger gas phase base than water, methanol or ethanol, and it is possible that the proton needed to form this ion is derived from traces of water in the reaction chamber.

Ions due to compounds of low molecular weight are frequently found in solvent ion profiles. Some of these may be due to substances absorbed from room air when vessels are opened; others are impurities present in low concentration in the solvents. The chief mode of ionization is believed to be protonation. These effects are illustrated in Figures 2 and 3. Benzene normally shows very few ions due to impurities. Chloroform ion profiles usually show multiple small peaks which are believed to be derived primarily from room air components which dissolve in the solvent and which are protonated in the reaction chamber.

Sample Ionization by Solvent-Mediated Reactions. Proton Transfer With or Without Molecular Association

The protonation of a gas phase base in the reaction chamber is analogous to the protonation of a base in solution in a liquid phase. Solvation, however, occurs to a limited extent in gas phase chemistry, and the observed order of base strengths for organic compounds does not always correspond to that observed in liquid phase studies. Amines (primary, secondary, tertiary) are bases in the gas phase, but a variety of non-nitrogenous compounds also show gas phase basicity. The relative intensity of each peak in the ion profile depends upon the structures of the base and reacting ions, the temperature of the reaction chamber, and the concentrations of the proton acceptor and the compound(s) involved in associative structures which may be present.

Many types of organic compounds are stronger bases in the gas phase than methanol or ethanol. The formation of ions corresponding to MH^+ is therefore frequently encountered when these alcohols are used as solvents, or are added to the commonly used chlorinated solvents (methylene chloride, chloroform, carbon tetrachloride). Ions corresponding to associative structures are usually present as well; $(MHM)^+$ ions usually do not occur under conditions of high dilution (very small samples), but ions of the type $(MHROH)^+$ are very likely to be present when protonation is carried out in the presence of alcohols. Fragmentation has not been observed as a consequence of solvent-mediated protonation of gas phase bases. Solvent-mediated reaction conditions have also been established for the ionization of gas phase acids; the work described here, however, is limited to ionization reactions which yield positive ions as products.

Charge Transfer

Carbocyclic and heterocyclic compounds with aromatic character generally have ionization potentials below that of benzene, while aliphatic compounds have higher ionization potentials. When benzene is used as a solvent, the effect is that of establishing an upper limit of energy available for ionization by charge transfer. The result which is observed is that of selective ionization of compounds with aromatic character. Nicotine and caffeine, for example, form M^+ ions when injected in benzene solution.

Solvent-Mediated Ionization of Human Urinary Bases

The upper chart in Figure 4 is an ion profile observed for a chloroform extract of human urine. All peaks are due to compounds with gas phase basicity greater than that of ethanol. Charge transfer does not occur under these conditions (see Figure 2; the reacting ions are solvated protons) and the only ions expected are those corresponding to MH^+. Only a few of

8. J. L. Franklin, *et al.*, "Ionization Potentials, Appearance Potentials and Heats of Formation of Gaseous Positive Ions," NSRDS-NB 526 National Bureau of Standards, Washington, DC, 1969.

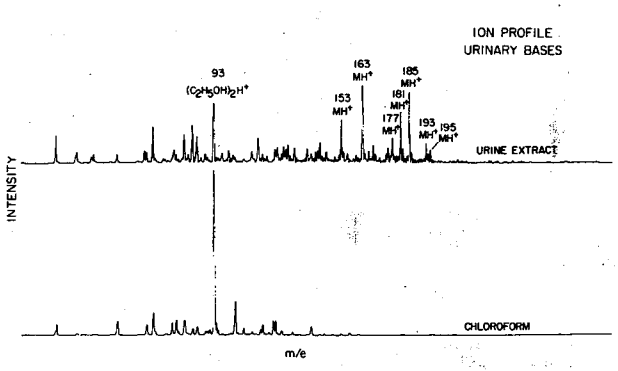

Figure 4. Ion profile of human urinary bases. A chloroform extract of urine was injected into the vaporizer-source assembly. The proton-donating ion at mass 93 is derived from ethanol present in the solvent. Source: ^{63}Ni; carrier gas: nitrogen; source temperature: 200°C. The bases which have been recognized are nicotine (MH^+ at 163 amu); cotinine (MH^+ at 177 amu), and caffeine (MH^+ at 195 amu).

these urinary bases have been identified at this time. The ion at 163 amu is due to nicotine (the subject was a smoker); cotinine forms MH^+ at 177 amu; caffeine form MH^+ at 195 amu.

Figure 5 shows a similar positive ion profile observed for a benzene extract of human urine. A number of bases, including nicotine (162 and 163 amu) and caffeine (194 and 195 amu) yield both M^+ and MH^+ ions. The compound responsible for the ion pair at 180-181 amu has not been identified, but it is most likely a base with aromatic character. Many of the same compounds are present in both extracts, but the degree of extraction of all bases is not the same for benzene and chloroform.

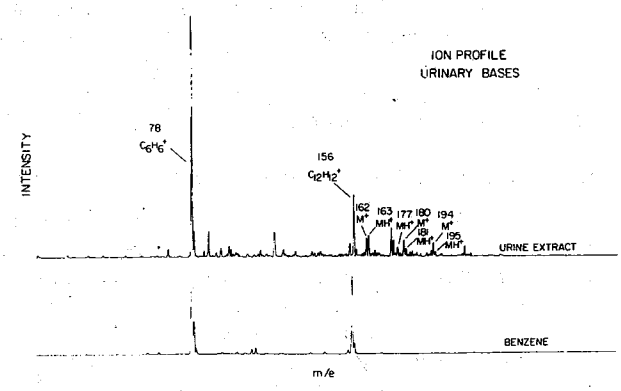

Figure 5. Ion profile of human urinary bases. A benzene extract of urine was injected into the vaporizer-source assembly under the same conditions that were used for Figure 4. The bases which have been recognized include nicotine (MH^+ at 163 amu, M^+ at 162 amu) and caffeine (MH^+ at 195 amu, M^+ at 194 amu).

Operation of a Liquid Chromatograph-Mass Spectrometer-Computer (LC-MS-COM) Analytical System

A Waters high pressure liquid chromatograph was connected by a short length of narrow bore tubing to the API vaporizer-reaction chamber assembly (corona source). A low flow (about 10 ml/min) of preheated carrier gas (nitrogen) was used to aid the solvent vaporization process. Samples of dibenzalacetone (5 ng and 25 ng) were injected, and the elution of the compound with chloroform was monitored both with the ultraviolet adsorption detector and with the API mass spectrometer. Figure 6 shows the selective ion monitoring chart for the elution process; about 0.6 ml of solvent was needed for the elution of the compound. This required 72 sec. The entire effluent stream was vaporized in continuous fashion through the ion source.

Figure 7 shows a composite record involving use of both UV detection and API detection. The compounds separated on the chromatographic column were progesterone (4-pregnen-3,20-dione) and 11-ketoprogesterone (4-pregnen-3,11,20-trione). Curve A shows the UV record indicating the separation of these steroid ketones. Curve B was obtained by single ion monitoring of the ion MH^+ from 11-ketoprogesterone. When the analogous ion from progesterone was monitored, only peak 1 was detected.

The ion profiles associated with peaks 1 and 2 are shown in Figure 8. These were observed during elution; the mode of operation was the same as that employed in GC-MS-COM systems. The UV monitor was employed to indicate the times at which scanning was required. The ions corresponded in each case to MH^+ and $(MHC_2H_5OH)^+$. The precise structure of the ions

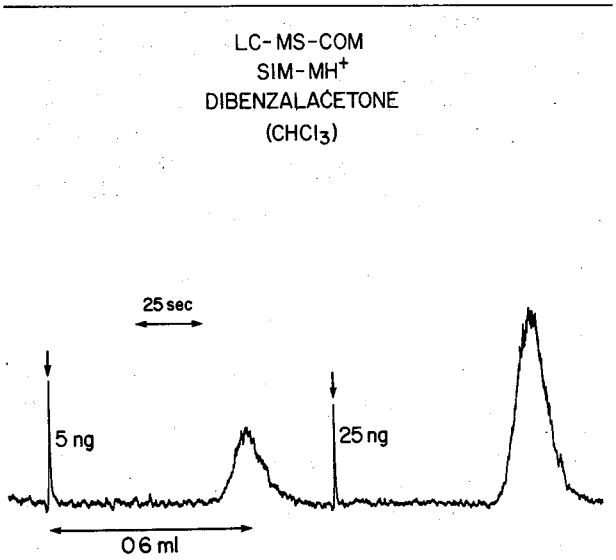

Figure 6. Chart record obtained by selective ion monitoring of MH^+ for dibenzalacetone after chromatography with a liquid chromatograph. Source: corona discharge; carrier gas; nitrogen; source temperature: 200°C; solvent: chloroform. The rate of flow was 0.5 ml/min. Two successive samples of 5 nanograms and 25 nanograms were injected at the points indicated by arrows. The entire effluent stream was vaporized through the API source.

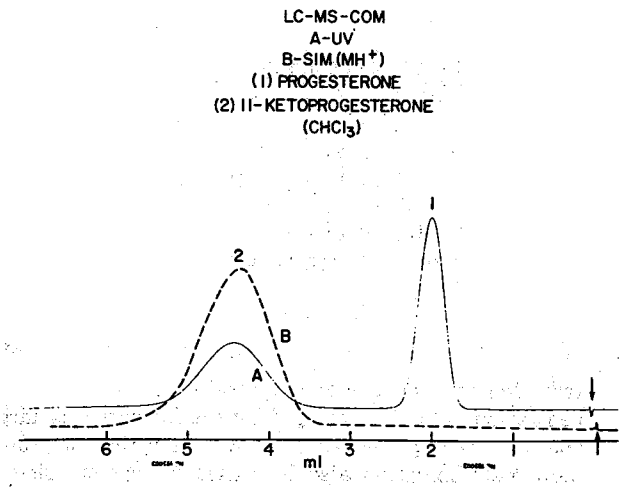

Figure 7. Separation of progesterone (1) and 11-ketoprogesterone (2) by liquid chromatography, with detection by (A) ultraviolet recorder and (B) API mass spectrometer with selective ion recording for MH^+ of 11-ketoprogesterone. Source: corona discharge; carrier gas: nitrogen; source temperature; 200°C; solvent: isooctane-chloroform, 85-15. The flow rate was the same as for Figure 6. Curve B was separately recorded and redrawn on the original chart showing curve A.

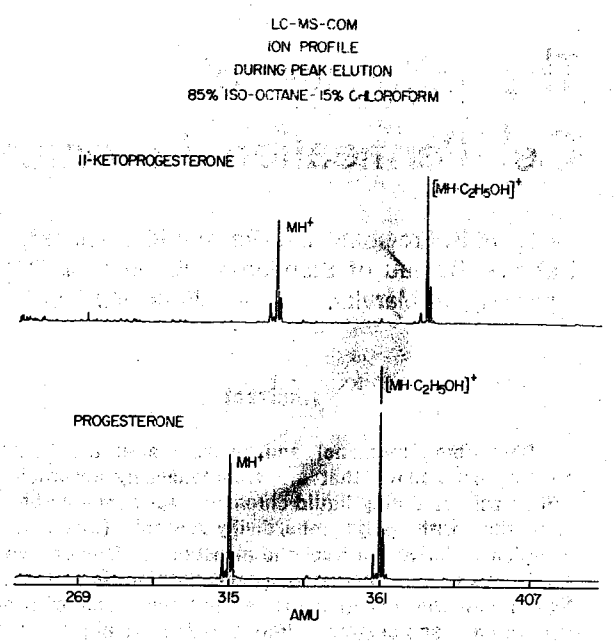

Figure 8. Positive ion profiles of 11-ketoprogesterone and progesterone obtained by mass scan procedure during elution of the steroids under the conditions indicated for Figure 7.

is not known with certainty, but the site of protonation is believed to be the 3-keto group.

Summary

These observations demonstrate that it is possible to vaporize the entire effluent stream of a liquid chromatograph in a vaporizer-reaction chamber assembly of an API mass spectrometer, and to obtain either ion profiles by wide range scanning techniques or data suitable for quantification by selective ion monitoring techniques. Solvent-mediated ionization reactions are employed. The limiting sensitivity of detection on a concentration basis should be the same as that attained in syringe injection procedures.

Acknowledgments

This work was supported by Grant HL-05435 of the National Heart and Lung Institute, Grants 13901 and 16216 of the National Institute of General Medical Sciences, and Grant Q-125 of the Robert A. Welch Foundation. ■