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# NEGATIVE CHEMICAL IONIZATION MASS SPECTRA OF MULTIFUNCTIONAL, POLAR, AND UNDERIVATIZED COMPOUNDS OF BIOLOGICAL INTEREST (1)

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*Intense pseudomolecular ions were observed for underivatized, multifunctional compounds of low volatilty (e.g., bile acid conjugates, alkaloids, antibiotics) by employing the direct probe technique of NCI MS; especially effective was the use of $CF_2CL_2$ as the reagent gas for producing chloride ion adduct negative ions.*

Many types of biologically interesting compounds (e.g., alkaloids, bile salts, glycosides, oligopeptides, oligosaccharides, prostaglandins, steroids) are multifunctional, polar, and "non-volatile" in the conventional sense of the term and are unsuitable for analysis by electron impact mass spectrometry or gas chromatography unless suitably derivatized. We(1) have found that for many compounds of biomedical interest present in biological fluids or other complex mixtures, minimal purification and little or no derivatization are adequate for obtaining useful spectra if some special techniques of chemical ionization mass spectrometry are empolyed. Thus, the CIMS ($NH_3$) technique has enabled us(2) to observe intense pseudomolecular positive ions for underivatized prostaglandins, alkaloids, penicillins, etc using a direct probe.

The recently developed(3) negative chemical ionization (NCI) technique(4) involves very low energy processes for producing negative ions. Consequently, fragmentation is minimal and the intensity of pseudomolecular ions is very high. Hunt and co-worders(5) have observed that the NCI technique can produce several hundred-fold higher sensitivity than the positive chemical ionization (PCI) technique.

We wish to report that direct probe NCI techniques are even more convenient than PCI techniques for many biomedical studies involving *underivatized* polar molecules. Currently bile acid analysis is of importance for studies on liver and gall bladder malfunctions. The use of chenodeoxy cholic acid for the non-surgical removal of gall stones has further increased the value of detailed bile acid analysis. In bile salts these acids are mostly present as amino acid conjugates which have to be autoclaved first to the component acids and then derivatized before analysis. We have found that using methane as the reagent gas the NCI technique can employed to obtain intense psuedomolecular ions from intact glycine conjugates of bile acids without any derivatization (Table 1). Even the sodium salt of glycolithocholic acid could by used for analysis via the direct probe provided ammonia was employed as the reagent gas: the peaks observed corresponded to $[M-1]^-$ and $[M-H_2O-1]^-$ of the *free* acid. Several penicillins in clinical use also produce strong pseudomolecular ions by the NCI ($CH_4$) technique.

The production of intense negative ions corresponding to $[M-1]^-$ from multifunctional compounds by the NCI ($CH_4$) technique is surprising. It is likely that some product of thermal decomposition is the true reagent for generating negative ions by resonance capture of electrons;

these ions in turn would produce anions from sample compounds. Even some trace impurities could be the source of negative ions formed by electron capture. Whatever be the exact mechanism of anion formation, the strong signals corresponding to pseudomolecular ions from multifunctional compunds are valuable for metabolic or biosynthetic studies or for rapid quantitation (6).

For some polyfunctional compounds that fail to produce pseudomolecular ions by the NCI ($CH_4$) technique, we have found a variation of another NCI technique to be suitable. This technique, described recently by Dougherty and coworkers (7) consists in ionizing molecules by the attachment of a chloride ion in a low energy process. Dougherty et al. have used dichloromethane vapor and observed chloride ion adduct pseudomolecular ions $(M+Cl)^-$ ions of high intensity from carboxylic acids and phenols. However, in the case of alcohols, aldehydes, ketones, ethers, and nitroaromatics, weak $(M+Cl)^-$ ions but strong $M^-$ or lower molecular weight peaks were observed by Dougherty and coworkers (7), who expressed the unnecessarily pessimistic view that it was unlikely "that chloride ion attachment spectra will have substantial analytical utility for direct characterization of these classes of materials".

We wish to report that dichlorodifluoromethane, readily available commercially as Freon 12, is a very convenient reagent gas for producing chloride ion adduct NCI spectra (1). Unlike $CH_2Cl_2$, Freon 12 produces only $Cl^-$ ions in the NCI spectrum under about 1 Torr pressure in the ionization chamber (8). We find that useful chloride attachment spectra are obtained by the Freon 12 technique from many polyfunctional molecules of biological importance. A partial list of compounds that gave strong molecular or pseudomolecular ions is provided in Table 2; some of these displayed $(M-1)^-$ rather than $(M+Cl)^-$ ions as the highest molecular weight ion. None of the compounds in Table 2 displayed $M^-$ ions expected from resonance electron capture.

The analysis of prostaglandins by conventional methods requires derivatization of one or more of the functional groups - often followed by gas chromatography for purification. The Freon 12 technique, however, was found to be very successsful with underivatized prostaglandins: 10-100 pg samples and source temperature of $200^0$C could be used; the base peak was due to the $(M+Cl)^-$ ion. (see Fig 1). Quantitative estimation of specific prostaglandins should present no problems as several deuterium-labeled prostaglandins are available (9) as internal standards.

On the basis of our experience, NCI ($CF_2Cl_2$) mass spectrometry appears to be a superior technique for simplifying studies involving trace amounts of multifuntional compounds. For many mixtures. the direct probe NCI technique in comparison to the GC/MS technique is faster, simpler,and more convenient.

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Table 1

NCI($CH_4$) of Conjugated Bile Acids

| Compound | MW | Ions Observed *(Rel. Intensity)* [Assignment] |
|---|---|---|
| Glycocholic acid | 465 | 464*(100)* $[(M-1)]^-$, 446*(50)* $[PM^*-H_2O]^-$, 428*(22)* $[PM-2H_2O]^-$, 410*(14)* $[PM-3H_2O]$, 375*(50)*[a] |
| Glycochenodeoxycholic acid | 449 | 448*(100)* $[M-1]^-$, 430*(47)* $[PM-H_2O]^-$ 412*(20)* $[PM-2H_2O]$ |
| Glycolithocholic acid | 433 | 432*(100)* $[M-1]^-$, 414*(20)* $[PM-H_2O]^-$ |

Table 2

NCI($CF_2Cl_2$) Mass Spectra

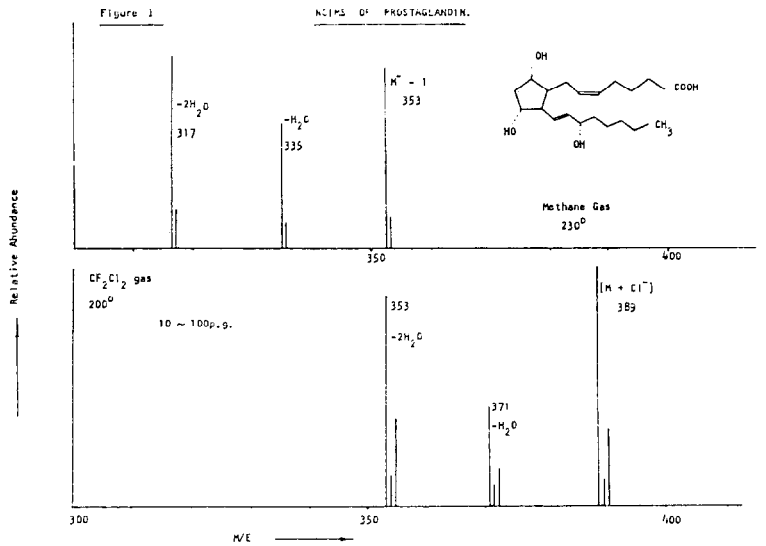
| Compound | MW | Ions Observed** *(Rel. Intensity)* [Assignment] |
|---|---|---|
| Testosterone | 288 | 323*(100)* $[M+Cl]^-$ |
| Reserpine | 608 | 643 $[M+Cl]^-$ |
| Lasolocid | 509 | 589*(100)* $[M-1]^-$ 571*(100)* $[PM-H_2O]^-$ 553*(40)* $[PM-2H_2O]^-$ |
| Tetracycline | 444 | 443*(50)* $[M-1]^-$, 425*(54)* $[PM-H_2O]^-$ |
| Prostaglandin $F_{2\alpha}$ | 354 | 389*(100)* $[M+Cl]^-$ 371*(60)* $[PM-H_2O]^-$ |
| 3-*t*-Bu-dimethyl silyl ether of methyl cholate | 536 | 571*(100)* $[M+Cl]^-$ 553*(5)* $[PM-H_2O]^-$ |

*PM = pseudomolecular ion

**The P+2 ions corresponding to $^{37}Cl$ have been omitted for the sake of clarity.

[a]Appears to be the $[M-1]^-$ peak of lithocholic acid present as a contaminant.

The NCI mass spectra were recorded with the ion source at 0.8-1.5 Torr pressure and at temperature of 190-280°C. Samples were introduced through a direct probe. The relative intensity of peaks varies with ion source temperature and pressure and other factors as yet unknown.




References and Notes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