Kozikowski has demonstrated other interesting uses for Diels–Alder adducts of 2[10] and analogues,[21] and many other uses can be envisioned.

Acknowledgment. We thank the National Institutes of Health for providing financial support (Grant No. GM 22760) and the National Science Foundation for funds to purchase the 300-MHz Bruker NMR instrument used in this study (Grant No. CHE 7905185). We also thank Dr. Alvin Marcus for determining the mass spectral data.

Registry No. 1, 60466-66-2; 2, 77003-92-0; 3, 77004-00-3; 4, 60466-71-9; 5, 77004-04-7; 6, 82798-92-3; 7, 82798-93-4; 8, 82798-94-5; 9, 82808-06-8; 10, 82798-96-7; 11, 82798-95-6; methyl vinyl ketone, 78-94-4; 2-cyclohexen-1-one, 930-68-7.

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Received June 3, 1982

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(26) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds"; Academic Press: New York, 1972; Vol. 2, pp 137–146. The assignment of configuration at the quarternary center of 11 is assumed on the basis of the mechanism of the [2,3]sigmatropic rearrangement.[27]

(27) Trost, B. M.; Melvin, L. S., Jr. "Sulfur Ylides"; Academic Press: New York, 1975; Chapter 7. Block, E. "Reactions of Organosulfur Compounds"; Academic Press, New York, 1978; Chapter 7.

Facile Ionization Induced by Ammonium Salts: Mass Spectra of Nonvolatile Compounds Using Unmodified Electron-Impact Mass Spectrometers[1]

Summary: A simple technique is described for obtaining meaningful spectra from nonvolatile and/or thermolabile compounds, using a direct probe in commercially available, low-resolution or high-resolution, electron-impact mass spectrometers.

Sir: Many biologically active compounds are nonvolatile and/or thermolabile and fail to give satisfactory mass spectra under electron-impact conditions.[2] We report here a simple technique for obtaining meaningful spectra from many such compounds using a direct probe in commercially available, low-resolution or high-resolution, electron-impact mass spectrometers (EIMS). Unlike other techniques[3] (field desorption, plasma desorption, laser desorption, flash desorption, fast atom bombardment, "in beam", etc.) our method does not require any modification of the conventional spectrometer or addition of expensive accessories.

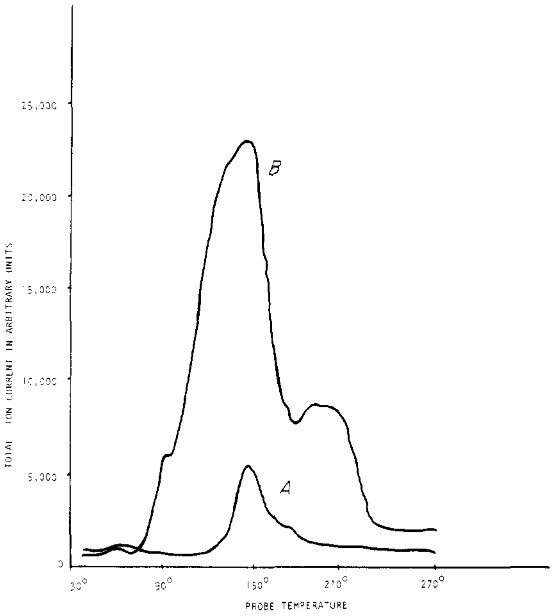

Figure 1. Total ion current profile of the potassium salt of penicillin G. A total of 40 scans was recorded while the temperature of the probe was raised from 50 to 300 °C over a period of 5 min. Since the mass spectrum of NH_4Cl by itself showed no ions at m/z >70, the region scanned was 70–500 amu. The shoulder appearing in the profile curves appears to indicate extensive thermal decomposition above 170 °C: (A) potassium salt of penicillin G, (B) potassium salt of penicillin G with NH_4Cl added.

This technique is based on our previous observation[4,5] that the addition of ammonium salts (NH_4X) to a solid sample in a chemical-ionization mass spectrometer produces abundant $(M + H)^+$ and/or $(M + NH_4)^+$ ions as well as $(M + X)^-$ and $(M - 1)^-$ ions.

We extended the ammonium chloride technique to electron-impact mass spectrometry and observed that ionization of sample compounds is remarkably facilitated (as shown by increase in the ion current in the higher molecular weight region of the spectrum; see Figure 1). Our technique for EI mass spectrometry involves using a conventional solids probe while adding a trace of ammonium chloride[6] as a solid (or a solution of ammonium iodide in methanol) to a solid sample, which may even be in the form of a salt.

To obtain some insight into the ionization enhancement in the presence of ammonium chloride we have studied the spectra of penicillin G in detail. Under electron-impact conditions, the potassium or sodium salt of penicillin G produces fragment ion that are much smaller than the whole molecule and therefore do not provide much structural information. Being thermolabile, even free penicillanic acid gave a poor spectrum—a weak molecular ion was seen very briefly.

By using the ammonium salt addition technique we observed much improved spectra: the molecular ion M^+ of the *free acid* corresponding to the salt was observed as the highest molecular weight peak (see Figure 2). That the observed molecular ion at m/z 334 was not $(M + NH_4^+ - H_2O)$[7] was established easily: the position of this peak remains unchanged upon the substitution of $^{15}NH_4Cl$ for

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(1) Mass Spectral Studies Part 12. For part 11, see Shefer, S.; Salen; G.; Cheng, F. W.; Dayal, B., Batta, A. K.; Tint, G. S.; Bose, A. K.; Pramanik, B. N. *Anal. Biochem.*, **1982**, *121*, 23.

(2) For recent reviews on mass spectrometry of nonvolatile and thermally unstable compounds, see: (a) Cotter, R. J. *Anal. Chem.* **1980**, 52, 1589A. (b) Daves, G. D. *Acc. Chem. Res.* **1979**, *12*, 359.

(3) For some recent publications, see below and references cited therein: (a) Zakett, D.; Schoen, E. E.; Cooks, R. G.; Hemberger, P. H. *J. Am. Chem. Soc.* **1981**, *102*, 1295. (b) Takeda, N.; Umemura, M.; Harada, K.; Suzuki, M.; Tatematsu, A. *J. Antibiot.* **1981**, *34*, 617. (c) Linscheid, M.; D'Angona, J.; Burlingame, A. L.; Dele, A., Ballou, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1471.

(4) Bose, A. K.; Fujiwara, H.; Pramanik, B. N.; Lazaro, E.; Spillert, C. R. *Anal. Biochem.* **1978**, *89*, 284.

(5) Bose, A. K.; Pramanik, B.; Tabei, K.; Bates, A. D. Presented at the 28th Annual Conference on Mass Spectrometry and Allied Topics, New York, May 25–30, 1980.

(6) Nonhalide salts such as $(NH_4)_2SO_4$ can also be used. Optimum results were obtained by adding 50–150 μg of NH_4Cl and about 50 μg of the sample compound. The lower limit of detection was 1–5 μg, depending on the sample.

(7) One of the reviewers had suggested that the apparent M^+ could be in reality be a CI-type ion $(M + NH_4^+ - H_2O)$; such, however, is not the case.

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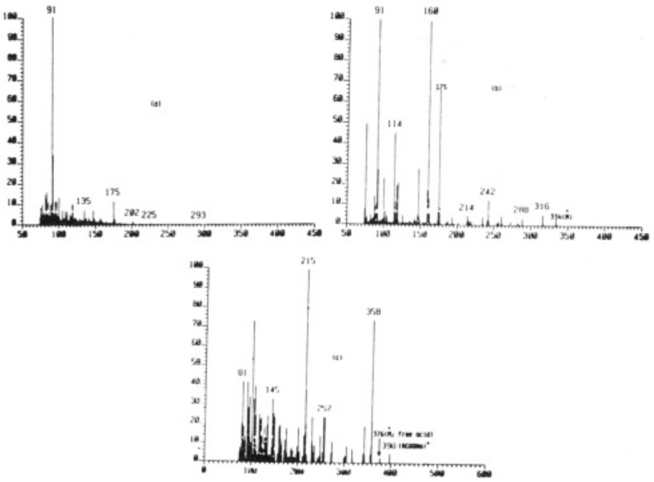
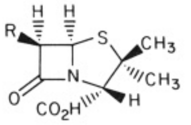
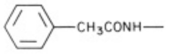


Figure 2. Spectra (relative intensity vs. m/z) of (a) potassium salt of penicillin G, (b) potassium salt of penicillin G with NH_4Cl added, and (c) sodium salt of lithocholic acid with NH_4Cl added.

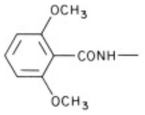
NH_4Cl; also, the exact elemental composition of the ion at m/z 334 was found to correspond to the free acid of penicillin G (**1**).



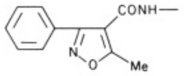
1, R =



2, R =



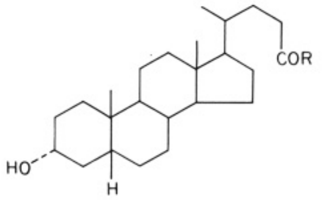
3, R =



The sodium salt of methicillin (**2**) displayed $(M - CO_2H)^+$ as the heaviest ion but the potassium salt of oxacillin (**3**) produced a fairly stable M^+ corresponding to the free acid. The base peak in the case of these antibiotics and the higher molecular weight fragment ions provided useful structural information.[8]

Cotter and Yergey[9] have recorded the spectra of quaternary ammonium salts placed at the tip of a probe specially designed for thermal ionization. An important point about their spectrometer was that the probe inlet was coaxial with the ion beam exit aperture of a quadruple EI mass spectrometer. When this probe was heated strongly with the electron beam switched off, quaternary ammonium ions were produced thermally. On the other hand, Ohashi et al.[10] have shown that if an extended probe is used in the "in beam" configuration even at a much lower temperature (350 °C), quaternary ammonium ions are produced from quaternary ammonium salts without an electron beam.

In our experiments the temperature was low (<300 °C) but the probe was *not* in the "in beam" configuration. When the temperature of the probe was allowed to rise to about 300 °C, the spectra of the sodium salt of bile acids showed some unusual features (see Table I). The $(RCO_2Na)^+$ ion was the heaviest observed—although briefly—in the case of the sodium salt of the bile acid (sodium lithocholate) as well as the glycine conjugate (glycolithocholate). It should be noted that the heaviest ions were not cationized species $(RCO_2H + Na)^+$ but the molecular ion corresponding to the sodium salt $(RCO_2Na)^+$ under study (see Figure 2).



lithocholic acid, R = OH
glycolithocholic acid, R = $NHCH_2CO_2H$

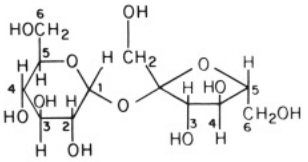
To gain information on the role of ammonium salts for enhancing ionization, we have studied the EI mass spectra of NH_4Cl, ND_4Cl, and $^{15}NH_4Cl$ (see Figure 3). Exact masses were measured by high-resolution techniques.

It is noteworthy that Cl^+ and $(NH_2)^+$ are formed in substantial abundance although Cl^- and $(NH_2)^-$ correspond to the preferred, lower energy, electronic configuration.[11] The most abundant ions are $(NH_3)^+$ and $(HCl)^+$, indicating that much of the NH_4Cl undergoes decomposition to $NH_3(g)$ and $HCl(g)$, which get ionized under electron-impact conditions.

The formation of significant quantities of high energy and presumably unstable Cl^+ and $(NH_2)^+$ ions from NH_4Cl raises the distinct possibility of ionization of some of the sample molecules to M^+ by electron transfer[12] to these ions.

Whatever be the exact mechanism(s) of ionization,[13] it is our observation that ammonium salts facilitate the ionization of nonvolatile/thermally unstable compounds and produce improved spectra in a standard EI mass spectrometer. Most of the ions are stable enough to permit high-resolution measurements to obtain exact elemental composition of the ions, thus providing valuable structural information.

In case of sucrose (**4**), which shows under standard EIMS



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[8] For the positive and negative ion desorption chemical ionization of some penicillins, see Gower, J. L.; Beaugrand, C.; Sattot, C. *Biomed. Mass Spectrom.* **1981**, *8*, 36.

[9] Cotter, R. J.; Yergey, A. L. *Anal. Chem.* **1981**, *53*, 1306.

[10] Ohashi, M.; Barron, R. P.; Benson, W. R. *J. Am. Chem. Soc.* **1981**, *103*, 3943.

[11] The Cl^+ ion was first observed in the EIMS of $COCl_2$; Thomson, J. J. *Philos. Mag.* **1912**, *24*, 209.

[12] (a) McLafferty, F. W. "Interpretation of Mass Spectra"; University Science Books: Mill Valley, CA, 1980; p 92. (b) In the course of our earlier CIMS work[16] using ammonia as a reagent gas or ammonium chloride added to the direct probe sample in the presence of methane or other reagent gases, we had observed pseudomolecular ions corresponding to the free acid of sample in the form of salts such as the sodium salt of penicillin G.

[13] We are thankful to one of the reviewers for drawing our attention to a publication[15] describing the addition of hydrated salts of Mg, Ni, and Al to solid samples in the probe tip of a CI mass spectrometer to obtain spectra of relatively nonvolatile compounds. Our experiments, however, were performed with standard EI mass spectrometers operating under 10^{-7}–10^{-6} torr pressure and with an open source configuration.

Table I. Ammonium Salt Enhanced EIMS of Sodium Lithocholate

	rel intensity of selected ions[a]			
scan no.	$(M^* - 1 + Na)^+$, m/z 398	M^{*+}, m/z 376	$(M^* - H_2O)^+$, m/z 358	$(A + H)^+$, m/z 215
15		14	100	84
28		3	66	100
39	7.9	2.9	66.4	100
42	8.6		65.7	100
47			57.4	98

[a] Probe temperature was raised from 150 to 300 °C over a period of about 5 min. M* corresponds to the molecular weight of the free acid corresponding to the salt. A corresponds to the tricyclic fragment ion formed by the loss of the D ring and the 3-hydroxyl group.

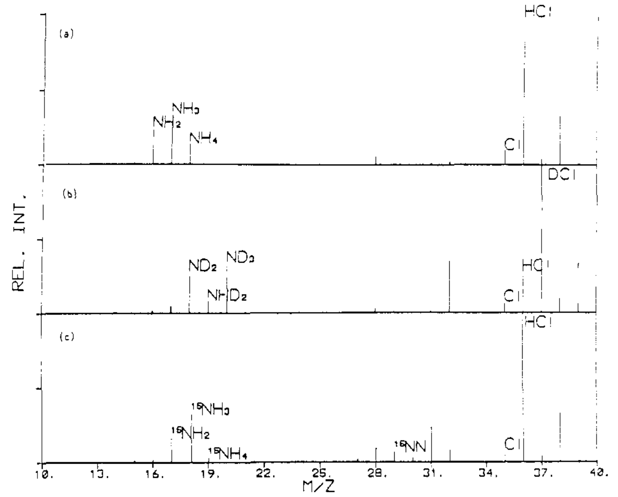

Figure 3. Spectra of (a) NH_4Cl, (b) ND_4Cl, and (c) $^{15}NH_4Cl$.

conditions only fragment ions of m/z <300, the heaviest stable ion corresponded to $(M - H_2O)^+$ at m/z 324. The spectrum was reproducible on two different mass spectrometers[14] and it was stable enough to permit exact mass determination of several major fragments, using high-resolution acessories. Additional structural information was obtained by rerecording the spectrum after substituting ND_4Cl for NH_4Cl and taking note of the extent of deuteration.

In summary, the ammonium chloride addition technique facilitates the recording of EI-type spectra (not proton or cation adduct type CI spectra) and permits the determination of the exact mass of the molecular ion and/or high molecular weight fragments.

Acknowledgment. We thank the Schering-Plough Corp. for research facilities and the National Institutes of Health (AI-16745) for partial support.

Registry No. 2 Na, 132-92-3; 3 K, 14217-64-2; 4, 57-50-1; NH_4Cl, 12125-02-9; ND_4Cl, 12015-14-4; penicillin G K salt, 113-98-4; penicillin G Na salt, 69-57-8; sodium lithocholate, 13284-86-1; sodium glycolithocholate, 24404-83-9.

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[14] A Finnigan MAT 312 double-focusing mass spectrometer was used to analyze the samples. Sucrose was also analyzed with a Finnigan Series 4000 quadruple mass spectrometer. For both instruments standard operating conditions for EIMS were employed; probe temperature 50–300 °C, source pressure 10^{-7}–10^{-6} torr. Pressure increased to 10^{-6} when the probe temperature reached 100 °C, presumably due to the decomposition of ammonium chloride.

(15) Buttrill, S. E.; Findeis, A. F. *Anal. Chem.* **1976**, *48*, 626.

(16) Bose, A. K.; Fujiwara, H.; Pramanik, B. N. *Tetrahedron Lett.* **1979**, 4017.