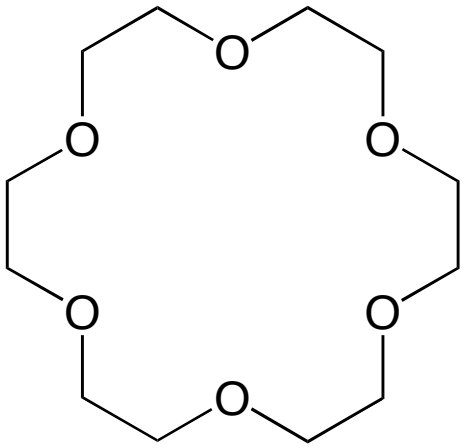
Chapter 5. Complexation of Tholins by 18-crown-6: Identification of Primary Amines

5.1. Introduction

Electrospray ionization (ESI) is an excellent technique for the ionization of complex mixtures, since it is soft enough that only molecular ions are formed, and the confusion caused by overlapping fragments can be avoided. In conjunction with ion traps [Julian, *et al.*, 2003a]; [Julian, *et al.*, 2003b] capable of MS^n experiments, the necessity for chromatography of mixtures before analysis can sometimes be avoided. ESI has been applied to the investigation of such analytically difficult substances as crude oil [Hughey, *et al.*, 2002] and humic acids [Cooper, *et al.*, 2002]; [Stenson, *et al.*, 2002].

To date, only Mark Smith's group at the University of Arizona has used ESI for the analysis of tholins. In combination with high-resolution mass spectrometry, empirical formulas for hundreds of tholin components have been obtained [Sarker, *et al.*, 2003]. MS^n and H/D exchange experiments have also been used to obtain structural information for a number of these components [Somogyi, *et al.*, 2005].

Electrospray is soft enough that even non-covalent complexes can be brought into the gas phase. For instance, 18-crown-6 (Scheme 5.1) forms strong non-covalent complexes with protonated amines that can be transferred into the gas phase. In our laboratories, 18-crown-6 has been used to tag protonated amines in proteins and peptides, as a way to identify lysine residues [Julian and Beauchamp, 2001], and to create gas phase biomimetic reagents that bind to lysine side chains.



Scheme 5.1. 18-crown-6.

Here, tholins are electrosprayed in the presence of 18-crown-6, and complexes are formed with the components of the tholins that contain amines. Two major ion series that contain primary amines are present. Both appear to the linear aliphatic molecules, one based on aminoacetonitrile, the other on an ion with mass 110 Da. The implications of these species for the formation of the tholins is discussed.

5.2. Experimental

All experiments were performed on a ThermoElectron LCQ Deca ion trap mass spectrometer. Tholin (sample CH154) was dissolved in dichloromethane at a concentration of 2 mg/mL. Assuming an average molecular weight of 250 amu, the concentration is approximately 8 mM. 18-crown-6 was then added to the solution at a concentration of 1-2 mM. The LCQ tuning parameters were adjusted to maximize the species of interest. It is important to note that a high electrospray needle voltage (6.5 kV) was necessary in order to obtain a sufficient abundance of tholin-crown ether complexes.

5.3. Complexation of 18-crown-6 with tholins

Figure 5.1 is the mass spectrum obtained by electrospray of a solution of tholins with 18-crown-6 in dichloromethane. The largest peaks in the spectrum are assigned to protonated 18-crown-6 (MH^+) and a cluster of 18-crown-6, a proton and water ($[M+H_3O^+]^+$). At m/z values below that of MH^+ (264), the normal distribution of tholins obtained by electrospray is apparent. This consists of a series of groups of peaks differing by 14 m/z, the mass of a methylene unit. At masses higher than that of crown ether, complexes of 18-crown-6 with other components of the solution are apparent. The series of peaks separated by 14 m/z must be tholins complexed with 18-crown-6. Two major ion series are present complexed to the crown ether. The first corresponds to the formula $CN(CH_2)_n(NH_3)^+$, where n = 1-6. This series, based on aminoacetonitrile, is marked with triangles in Figure 5.1. The regularity of the distribution in intensity of this series suggests that methylene units are added as a linear chain. Branching would likely lead to steric effects that would alter the ability of the crown ether to bind, and thus alter the intensity distribution.

The other major series is based on an ion with mass 110 Da, plus between zero and four methylene groups. This series is marked with circles in Figure 5.1. The structure of this ion is uncertain, but it also has a regular intensity distribution, and so is probably a linear, rather than branched, structure.

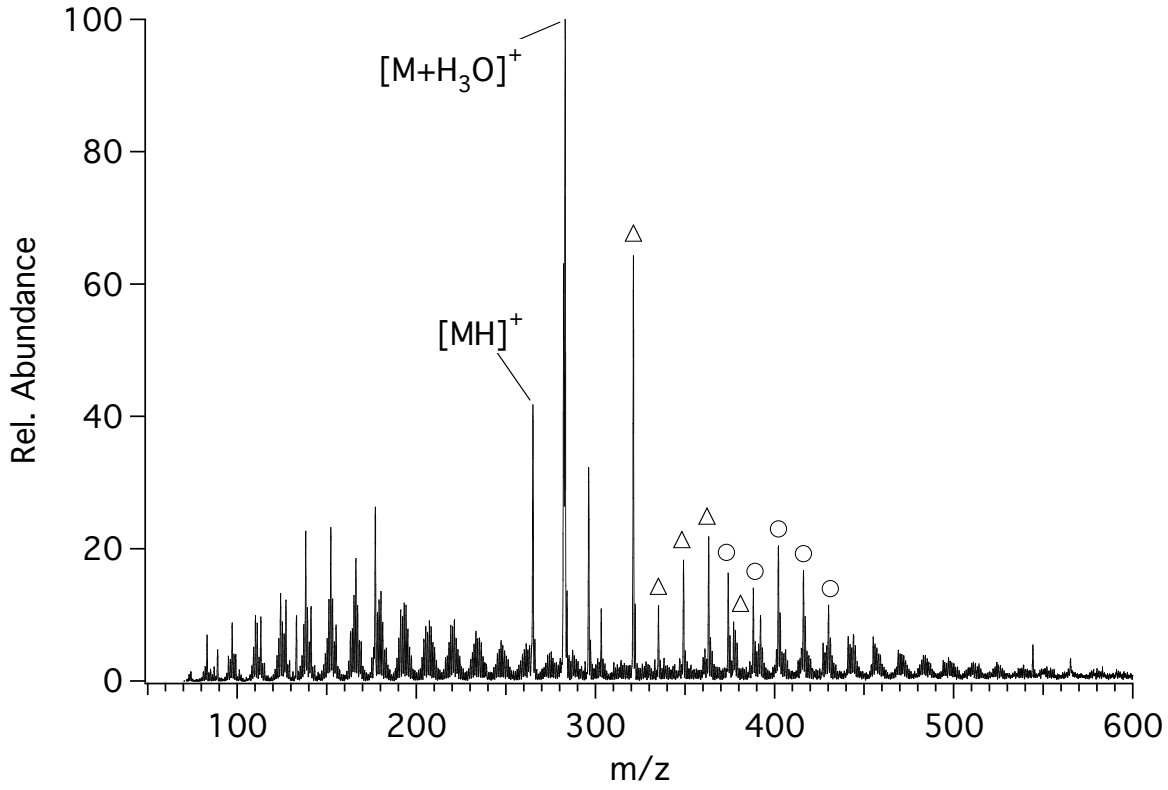


Figure 5.1. ESI mass spectrum of tholin complexed with 18-crown-6. Triangles, aminoacetonitrile series; Circles, 110 Da series.

We can confirm that the series are indeed crown ether adducts by isolating and fragmenting some of these ions. Figure 5.2a is the collisionally activated (CAD) mass spectrum of the ion at 416 m/z. The spectrum shows a mass loss of 264 Da, the mass of 18-crown-6, to give the ion at 152 m/z. The two other peaks in the spectrum, at 265 m/z and 283 m/z, correspond to protonated 18-crown-6 and 18-crown-6 complexed with hydronium ion. The hydronium ion peak is the result of protonated 18-crown-6 associating with background water vapor in the trap. The other peaks in the series have analogous CAD spectra. Figure 5.2b is the CAD spectrum of the ion at 153 m/z formed from the dissociation of 416 m/z. On activation, this ion loses small neutral molecules

such as HCN, NH_3, and acetonitrile. These kinds of losses are characteristic of the tholins.

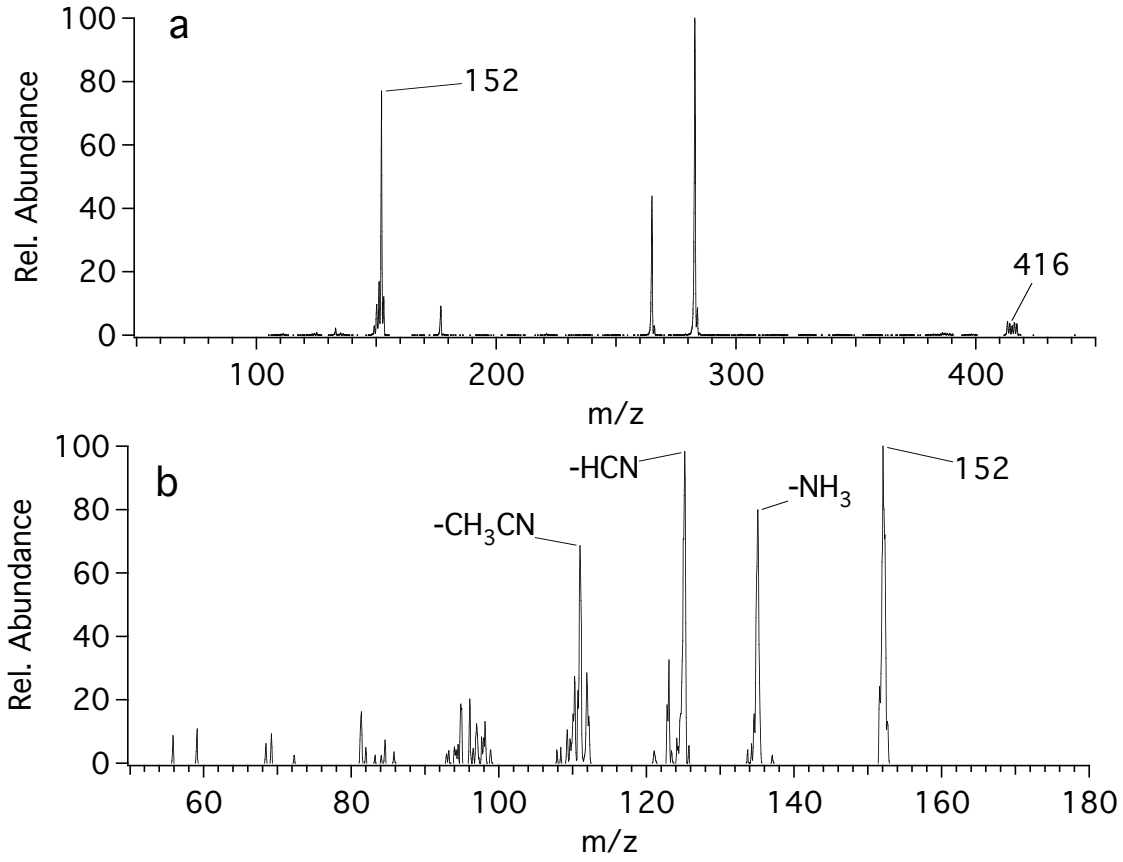


Figure 5.2. a) CAD spectrum of the ion at 416 m/z. b) CAD spectrum of the ion at 152 m/z from a).

The ion at mass 152 m/z has been extensively investigated by high resolution mass spectrometry and H/D exchange [Somogyi*, et al.*, 2005]. High resolution data reveals two peaks at a nominal m/z value of 152. The ions have the empirical formulae $C_8H_{14}N_3$ and $C_6H_{10}N_5$, and show seven and eight exchangeable protons, respectively. The 152 m/z ion is the fourth in the series we observe. Subtracting three methylene

groups from the above formulae leaves $C_5H_8N_3$ and $C_3H_4N_5$. Both ions may be capable of complexation with 18-crown-6.

To remove the possibility of non-specific binding of the crown ether to other positively charged groups that are present in the tholins, such as protonated imines, the source CAD capabilities of the LCQ were utilized. In this mode, voltage is applied to the octapole ion guide that feeds ions into the trap so as to provide a low level of collisional activation prior to trapping. This collisional activation should dissociate more weakly bound non-covalent complexes, leaving the more strongly bound complexes intact.

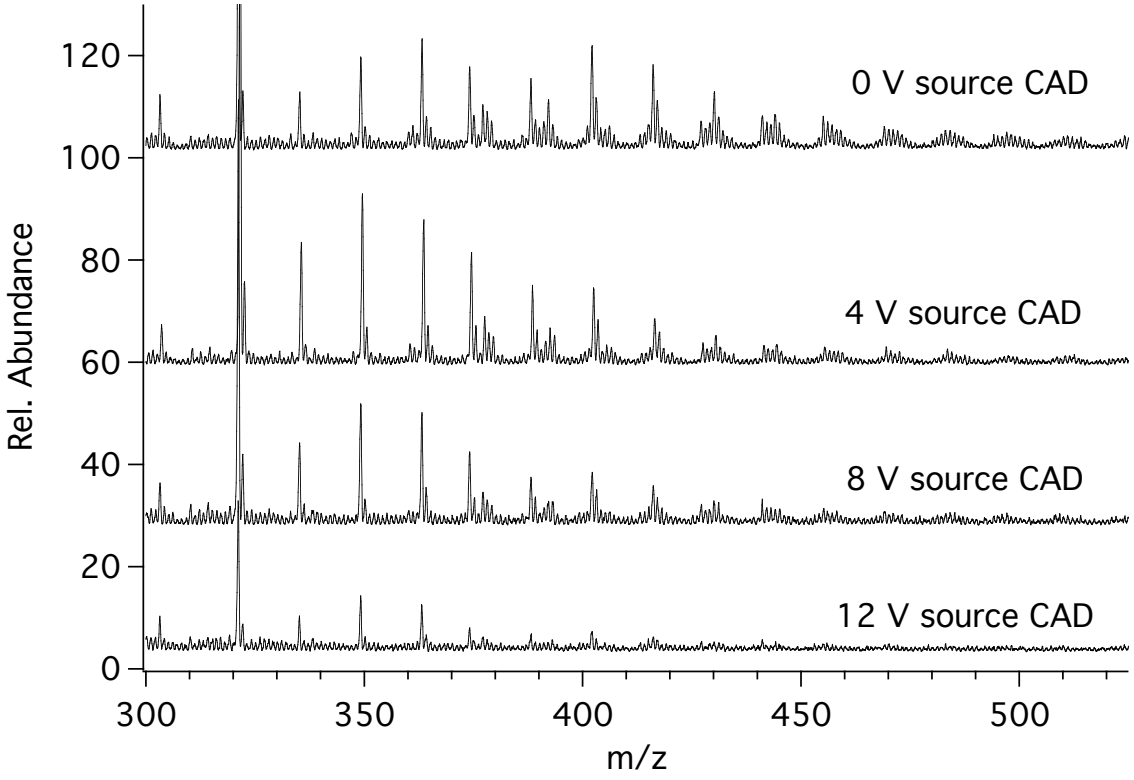


Figure 5.3. Source CAD of tholin-crown ether complexes.

Data from a range of CAD collision voltages is shown in Figure 5.3. None of the adducts in the two predominant ion series is particularly weakly bound, and so represent

protonated primary amines. The aminoacetonitrile series is more strongly bound to the crown ether than the $C_6H_{10}N_2$ series. For instance, the peak at 363 m/z (part of the aminoacetonitrile series) drops to half of its initial intensity at 12 V, while the ion at 402 m/z (part of the $C_6H_{10}N_2$) series has dropped to half its initial intensity by only 8 V.

No complexes are seen that contain more than one crown ether adduct. This suggests that most of the compounds that make up the tholins have either a single primary amine group or none at all. Perhaps primary amines are particularly reactive to the other components of the plasma in which the tholins are produced, and are converted into other functional groups, such as secondary or tertiary amines, during tholin production.

5.4. Conclusions

Electrospray ionization of tholins in the presence of 18-crown-6 enables the formation of complexes of 18-crown-6 with components of the tholins that possess primary amines. Two series of ions predominate in forming complexes with 18-crown-6. One series has the formula $CN(CH_2)_nNH_3^+$, where n = 1-6, which is assigned to aminoacetonitrile (n = 1) and analogs with additional methylene groups. The regularity of the distribution of this series suggests that methylene units are added as a linear chain. The other series is composed of ions of mass 110 + $(CH_2)_n$, where n = 0-4. The structure of this ion is uncertain. The aminoacetonitrile series is shown to be more strongly bound to the crown ether than the mass 110 series.

The use of 18-crown-6 to separate primary amines from a complex mixture is not limited to the analysis of tholins. The technique could also prove useful in analyzing the

amine content of crude oils, and in the tagging of lysine-containing peptides in tryptic digests.

5.5. References

Cooper, W. T., et al. (2002), Identification of individual humic molecules by electrospray ionization and ion cyclotron resonance mass spectrometry, *Abstracts of Papers of the American Chemical Society*, *223*, U514-U514.

Hughey, C. A., et al. (2002), Resolution of 11 000 compositionally distinct components in a single Electrospray ionization Fourier transform ion cyclotron resonance mass spectrum of crude oil, *Analytical Chemistry*, *74*, 4145-4149.

Julian, R. R., and J. L. Beauchamp (2001), Site specific sequestering and stabilization of charge in peptides by supramolecular adduct formation with 18-crown-6 ether by way of electrospray ionization, *International Journal of Mass Spectrometry*, *210*, 613-623.

Julian, R. R., et al. (2003a), Biomimetic approaches to gas phase peptide chemistry: combining selective binding motifs with reactive carbene precursors to form molecular mousetraps, *International Journal of Mass Spectrometry*, *228*, 851-864.

Julian, R. R., et al. (2003b), Molecular mousetraps: Gas-phase studies of the covalent coupling of noncovalent complexes initiated by reactive carbenes formed by controlled activation of diazo precursors, *Angewandte Chemie-International Edition*, *42*, 1012.

Sarker, N., et al. (2003), Titan aerosol analogues: Analysis of the nonvolatile tholins, *Astrobiology*, *3*, 719-726.

Somogyi, A., et al. (2005), Organic environments on Saturn's moon, Titan: Simulating chemical reactions and analyzing products by FT-ICR and ion-trap mass spectrometry, *Journal of the American Society for Mass Spectrometry*, *16*, 850-859.

Stenson, A. C., et al. (2002), Ionization and fragmentation of humic substances in electrospray ionization Fourier transform-ion cyclotron resonance mass spectrometry, *Analytical Chemistry*, *74*, 4397-4409.