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The effect of ruthenium(III) chloride on the formation of protonated parent ions in electrospray mass spectrometry

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We report the use of $RuCl_3$ as an "alkali metal sponge". This is a general and highly efficient method for generating protonated parent ions for a variety of compounds that usually do not show this ion in electrospray mass spectrometry. This technique is demonstrated to be highly useful in "cleaning up" spectra from multiply metallated ions, thereby substantially improving the signal-to-noise ratio.

Analysis of trace amounts of biologically, biosynthetically and synthetically derived compounds is routinely carried out by mass spectrometry. Detailed fragmentation pathways can usually be assigned by extensive sequential mass spectrometric (MS^n) analysis of different parent species such as the protonated parent ion $[M + H]^+$ as well as various metallated ions.[1] Analysis of different metallated ions can lead to complementary data, allowing the detailed determination of the analyte structure through exploitation of differing gas-phase chemistry.[2] Fragmentation of protonated ions can also produce additional structurally significant data. A number of compounds, however, evade such analysis due to their strong affinity for metal cations, limiting the opportunity to gain important structural information. Moreover, a wide range of complex natural products such as peptides and oligosaccharides tend to form multiply metallated adducts and cluster ions, thus reducing the intensity of the protonated ions and making detection and structural analysis somewhat cumbersome.

Recent studies on the ionophores monensin[3] and valinomycin[4] (Fig. 1) have demonstrated that this problem can be circumvented by addition of artificial ionophores, such as crown ethers, to compete for alkali metal cations. However, the protonated ions could still only be observed at low pH. This limits the use of the technique to robust compounds that show little acid sensitivity.

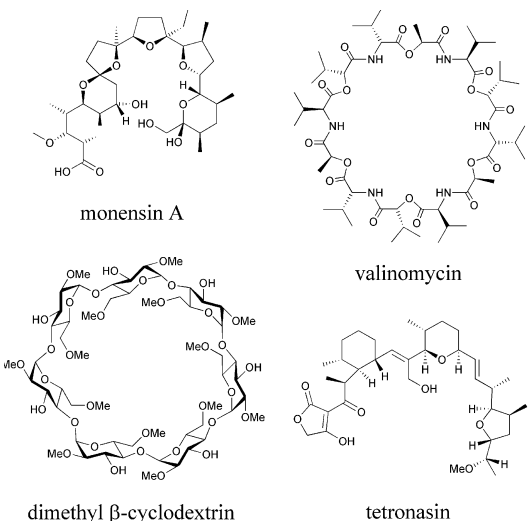

Fig. 1 Structures of the ionophores studied.

As part of our ongoing research, into the utility of MS^n for structural elucidation of trace amounts of natural products and biosynthetically derived analogs,[5] we were surprised to find that the addition of ruthenium(III) chloride to a solution of monensin A generated the protonated parent ion, $[M + H]^+$, at good intensity. This species had previously not been detectable under standard solution conditions. Reported here are the first findings of a systematic investigation of this phenomenon.

As a test compound, we studied tetronasin (Fig. 1) a naturally occurring ionophore that is well known to strongly bind alkali metal ions.[6] The mass spectrum of tetronasin (Fig. 2a) shows a very small $[M + H]^+$. Addition of 0.25 equivalents† of $RuCl_3$ leads to a slight increase of the intensity of the sodiated ion, $[M + Na]^+$ (*m/z* 625) and the $[M + H]^+$ (*m/z* 603) ion could be observed at approximately 5% relative intensity. A doubly sodiated ion (*m/z* 647) disappeared completely from the spectrum. At the same time, the total ion count (TIC) of all tetronasin monomer ions was boosted by over 150%. Further addition of $RuCl_3$ lead to a further increase in the intensity of the $[M + H]^+$ ion and a significant decrease in the $[M + Na]^+$ ion. At two equivalents of $RuCl_3$ the $[M + H]^+$ and $[M + Na]^+$ ions are at approximately equal intensity (Fig. 2). The data recorded on a triple quadrupole mass spectrometer are summarized in Fig. 3.

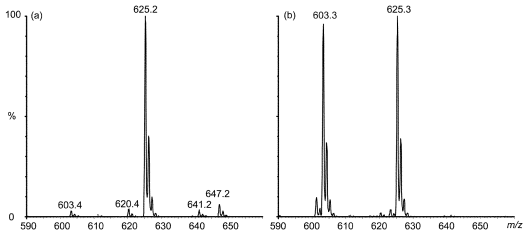

Fig. 2 The parent ion region of the mass spectrum of tetronasin. Spectrum (a) under normal conditions. The ions observed are double sodiated (*m/z* 647), potassiated (*m/z* 641), sodiated (*m/z* 625), ammoniated (*m/z* 620) and protonated (*m/z* 603). Spectrum (b) after addition of two equivalents of $RuCl_3$.

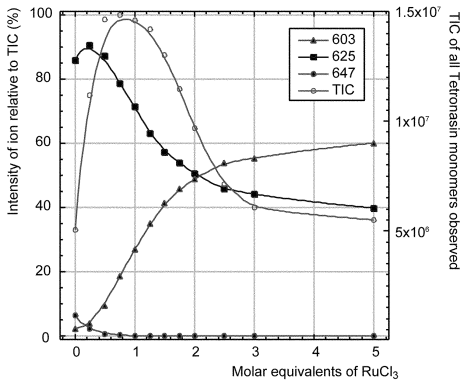

Fig. 3 Plot of the relative intensity of the double sodiated (*m/z* 647), sodiated (*m/z* 625) and protonated (*m/z* 603) ions and total ion count (TIC) *vs.* equivalents of $RuCl_3$ added. Each data point is the average of three repetitions of 10 combined scans.

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Similar results were achieved on a FTICR mass spectrometer thus showing independence of these results from the instrument and source design used. The initial small increase in the intensity of the $[M + Na]^+$ ion (Fig. 3) can be attributed to the removal of one sodium from the double sodiated ion (*m/z* 647).

Stimulated by these results we studied other natural ionophores like the polyether monensin and the cyclic *pseudo*-peptide valinomycin (Fig. 1). In both cases, comparable results to the ones described for tetronasin were observed. Upon addition of 0.5 equivalents of $RuCl_3$ for valinomycin and one equivalent for monensin the $[M + H]^+$ (which is normally not detectable) was observed at 100% relative intensity, along with a concomitant increase of the overall signal intensity.

Oligosaccharides are a class of compounds that are well known to form multiple metallated adduct ions in the gas-phase. We therefore chose to apply this methodology to poly-alkylated cyclodextrin[7] derivatives which are known to be even more susceptible to multiple metallation.[8] As expected, analysis of a sample of dimethyl β-cyclodextrin (Fig. 1) showed the $[M + H]^+$ along with a large number of metal ion and other adducts (Fig. 4a). Addition of one equivalent of $RuCl_3$ to this sample lead to a substantial reduction in these adducts thus significantly "cleaning up" the spectra and leaving essentially protonated species (Fig. 4b).‡ The overall signal intensity of $[M + H]^+$ showed up to a 40 fold increase in these spectra. This is due to a reduction in the number of *pseudo*-molecular ion species by (i) the removal of unwanted multiply cationated species and (ii) a substantial reduction in the number and intensity of analyte-cation cluster ions. These factors together cause a dramatic improvement in the signal-to-noise ratio (Fig. 4).

An investigation was also performed on the effect of $RuCl_3$ on the formation of cluster ions — often observed in ESI-MS. These are usually the result of analyte molecules forming gas-phase complexes. These unwanted ions have the effect of reducing the signal of the required ions. This is especially important, for example, in trace analysis or the analysis of complex mixtures from biosynthesis. Fig. 5 shows the wide mass-range ESI-MS spectrum of tetronasin recorded on the FTICR. Spectrum (a) is performed under normal conditions and shows considerable clustering up to *m/z* 2520. These cluster ions have the general formula $[(Tet_n + Na_n) + Na]^+$ (where Tet = tetronasin, and n ranges from 1 to 4). Spectrum (b) shows the ESI-MS spectrum of the same sample run at the same instrument conditions but after addition of 1 equivalent of $RuCl_3$. The higher mass clusters have been removed along with a considerable reduction in the multiple sodiated monomer peaks. The protonated ion is now observed (see previous).

The detailed nature of this highly efficient competition of $RuCl_3$ for alkali metal cations is currently not fully understood. The formation of the anions $RuCl_4^-$ and $RuCl_5^{2-}$ is a known property of $RuCl_3$ in solution.[9] It is believed that these anions form insoluble neutral complexes with alkali metals and are therefore not detectable under electrospray conditions in either positive or negative ionisation mode. Analysis of the insoluble residues by positive and negative ion MALDI-TOF shows the presence of the complexes $[RuCl_4Na]$ and $[RuCl_5Na_2]$. The formulae of these ions was confirmed by isotope distribution matching. This phenomenon is currently undergoing continued investigation in our group with the aim of increasing our understanding of $RuCl_3$ as an "alkali metal sponge".

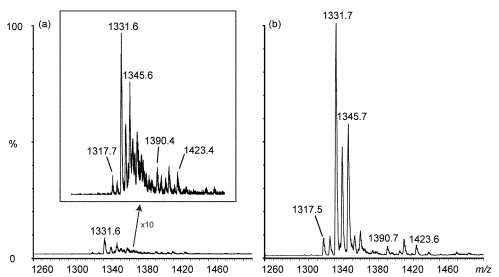

Fig. 4 The parent ion region of the mass spectrum of dimethyl β-cyclodextrin. Spectrum (a) under normal solution conditions, boxed is a ten times expansion. Spectrum (b) after addition of one equivalent of $RuCl_3$. The signal in spectrum (b) is approximately 40 times that in spectrum (a).

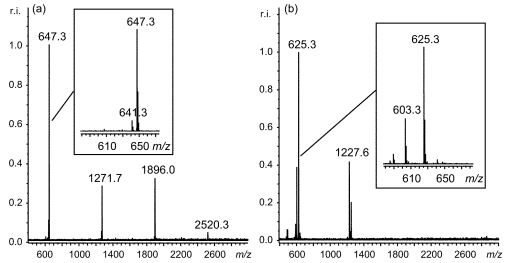

Fig. 5 Broad-band mass spectra of tetronasin. Spectrum (a) under normal solution conditions clearly showing cluster ion formation up to *m/z* 2520. Spectrum (b) after addition of one equivalent of $RuCl_3$ showing reduction in cluster ion formation and the generation of the protonated ion at *m/z* 603.

In conclusion, we have presented a general and highly efficient method for generating the protonated parent ion of a variety of compounds that usually do not show this ion in electrospray mass spectrometry. Moreover, this technique was shown to be highly useful in "cleaning up" spectra from multiply metallated ions (*i.e.* in the case of oligosaccharides) and thereby substantially improving the signal-to-noise ratio and enhancing the intensity of the protonated ion. This work represents the first study in this area. We believe this new methodology will find widespread application.

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Notes and references

† Solutions were made up in acetonitrile–water (80 : 20) or methanol–water (50 : 50) at a concentration of 0.62 mg ml^{-1} (1 μmol ml^{-1}). A suspension of ruthenium(III) chloride was made up at a concentration of 13 mg ml^{-1} (0.5 μmol ml^{-1}) in deionised water. After addition of the appropriate amount of this stock to the analyte solution, the solution was thoroughly mixed and spun down in a centrifuge at 13 000 rpm (10 min) to separate from insoluble residues.

‡ The species observed in addition to the expected protonated ion at *m/z* 1331 are due to the variable methylation of the sample analysed. There are also artifacts due to double charged dimers.

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