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Decomposition of *N*-hydroxylated compounds during atmospheric pressure chemical ionization

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***N*-Hydroxylated polyamine derivatives were found to decompose during the ionization process of liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) experiments. The phenomenon was studied with a model compound, a synthetic *N*-hydroxylated tetraamine derivative. It was found that reduction, oxidation and water elimination occurred during APCI to generate the corresponding amine, *N*-oxide, and imine. The investigation further revealed that decomposition of hydroxylamines during APCI depends upon the concentration of the analyte and on the acidity of the solution introduced into the ionization source. The pH-dependence of decomposition was utilized for the development of an MS method that allows for the unambiguous identification of N–OH functionalities. This method was applied for the study of natural products including polyamine toxins from the venom of the spider *Agelenopsis aperta* and mayfoline, a cyclic polyamine derivative of the shrub *Maytenus buxifolia*.** Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: high performance liquid chromatography; artifact; alkaloid; mass spectrometry; natural product; atmospheric pressure chemical ionization

Introduction

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are two of the most important ionization techniques used in mass spectrometry. These methods are of particular importance for analytical setups in which mass spectrometry is online coupled to liquid chromatography (LC-MS). Since ESI and APCI are mild ionization methods, they typically produce quasi-molecular ions of the analytes without fragmentation. Hence, they generate ions that usually provide direct and unequivocal information about the masses of the sample molecules. Such information, however, is convoluted by analytes that undergo fragmentations or decompositions prior to the MS analysis, e.g. before or during the ionization process. Formation of such artifacts, especially when their source is not known, might lead to misinterpretation of MS data. An understanding of potential decomposition reactions that might occur with analytes prior to their entry into the MS is particularly important for more complex investigations, e.g. for the investigation of natural products.

Artifact formation has already been observed with APCI, in which the analyte solutions are typically heated to 300–400 °C prior to ionization and therefore prone to thermally induced reactions. For example aromatic nitro compounds,[1,2] *N*-oxides[3–7] and imines[8], showed partial reduction to the corresponding amines upon APCI. An analogous reaction was also recently observed with *N*-hydroxylated compounds in the course of our investigations of spider venoms.[9–12] In this study we report that *N*-hydroxylated polyamine derivatives also undergo reduction to the respective amines too. This reaction has not previously been reported in literature. These results are significant because (1) *N*-hydroxylamines are well-known constituents of natural samples and also potential metabolites of drugs and (2) liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) is widely used for the study of natural products and drug metabolism. Thus, the lack of awareness of this APCI reduction could broadly lead to wrong conclusions. To obtain means to recognize and control artifact formation from *N*-hydroxylamines by APCI, we investigated this in-source reaction with a synthetic *N*-hydroxylated polyamine derivative in depth.

Experimental

Chemicals and sample preparation

High performance liquid chromatography (HPLC) supra grade acetonitrile (MeCN) from Scharlau (Barcelona, Spain), trifluoroacetic acid (TFA) and formic acid (HCOOH) from Fluka (Buchs, Switzerland) and aqueous solution of NH_3 (25%) from Merck (Darmstadt, Germany) were purchased in the respective highest qualities. HPLC grade H_2O ($\leq$5 ppb total organic content) was obtained by purification of deionized H_2O with a MilliQ gradient apparatus (Millipore, Milford, MA, USA). [4-Hydroxy-9-(2-nitrobenzenesulfonyl)-4,9-diazadodecane]-1,12-diphthalimide (**1**) was synthesized on solid support and purified by preparative HPLC.[13] Synthetic (−)-(2S)-9-hydroxy-2-phenyl-1,5,9-triazacyclotridecan-4-one (=mayfoline) was obtained from Hesse.[14] Lyophilized *Agalenopsis aperta* venom was purchased from Fauna Laboratories Ltd. (Almaty, Kazakhstan).

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High performance liquid chromatography and mass spectrometry

General

LC-MS analyses were performed on a Hewlett-Packard 1100 HPLC system (Hewlett-Packard Co., Palo Alto, CA, USA) fitted with a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), a Hewlett-Packard 1100 binary pump and a Hewlett-Packard 1100 diode array detector (DAD). The reversed-phase column used was an Interchim Uptisphere RP C18 column (UP3HDO-20QS, 3 µm, 2.3 × 200 mm, Interchim, Montluçon, France). Either a step gradient or isocratic conditions at flow rates between 150 and 180 µl/min were applied with solvents A and B (solvent A: H_2O + 0.1% TFA, solvent B: MeCN + 0.1% TFA).

The LC system was connected to an EsquireLC quadrupole ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), equipped with either an ESI or APCI Hewlett-Packard atmospheric pressure ion (API) source. Conditions for ESI were nebulizer gas (N_2, 40 psi), dry gas (N_2, 9 l/min), dry temperature (300 °C), HV capillary (4500 V) and HV EndPlate (−600 V). Conditions for APCI were nebulizer gas (N_2, 21 psi), dry gas (N_2, 7 l/min), dry temperature (300 °C), APCI temperature (300 °C), HV corona (2870 V), HV capillary (3713 V) and HV EndPlate (−600 V). The MS-parameters (target mass, compound stability and trap drive) were optimized for each measurement to obtain highest ion response and minimal in-source fragmentation. The MS acquisitions were performed in positive ion mode at normal resolution (0.6 u at half peak height) and under ion charge control conditions (ICC, target: 10 000). Full scan MS and MS/MS were averaged over 5–8 single spectra and acquired with a mass window between *m/z* 50 and 1000. For all MS/MS experiments, the isolation width was set to 1 Da, the fragmentation cut-off to 'fast calc', and the fragmentation amplitude to 1 in the 'SmartFrag' mode.

High-resolution Fourier transform (FT) mass spectral data were obtained with a LTQ-Orbitrap XL mass spectrometer (Thermo Electron, Bremen, Germany) equipped with a standard ESI source. The parameters were spray voltage (5 kV), tube lens voltage (120 V), capillary voltage (38 V) and temperature (275 °C). The mass spectrometer was calibrated for mass accuracy immediately before each measurement according to the manufacturer's instructions, the relative mass error being typically lower than 3 ppm (externally). The high-resolution FT-MS data were additionally calibrated internally during the measurements with established lock masses (*m/z* 429.088735 and 445.120025). Data was acquired within a mass range of *m/z* 150 to 1000. The automatic gain control (AGC) target setting for FT-MS experiments was set to 50 000. Spectra were acquired with a resolution of 60 000 (full width at half-maximum, FWHM) at *m/z* 400, and 10 spectra were averaged.

*Synthetic compound **1***

For LC-MS analyses, 5 µl of a stock solution of **1** (200 µg) in MeCN/H_2O (1 : 1, 1 ml) was injected at isocratic conditions with 40% of B and a flow rate of 0.18 ml/min. Direct infusion APCI experiments were carried out by pumping 200 µl/min of a 30-fold diluted stock solution of **1** into the mass spectrometer with a syringe infusion pump (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). For FT-MS experiments, a 10-fold diluted stock solution of **1** was introduced at 6 µl/min using the same syringe infusion pump.

Spider venom

Crude lyophilized *A. aperta* venom (100 µg) was dissolved in MeCN/H_2O (1 : 3, 50 µl) + 0.1% TFA, and an aliquot of 5 µl was injected into the LC-MS system. A linear gradient from 5 to 20% B over 40 min at a flow rate of 150 µl/min was applied. The post-column addition of NH_3 to the eluent was performed by the addition of an aqueous solution of NH_3 (10%) at a rate of 20 µl/min through a Tee located in-between the exit of the column and the entry of the APCI interface.

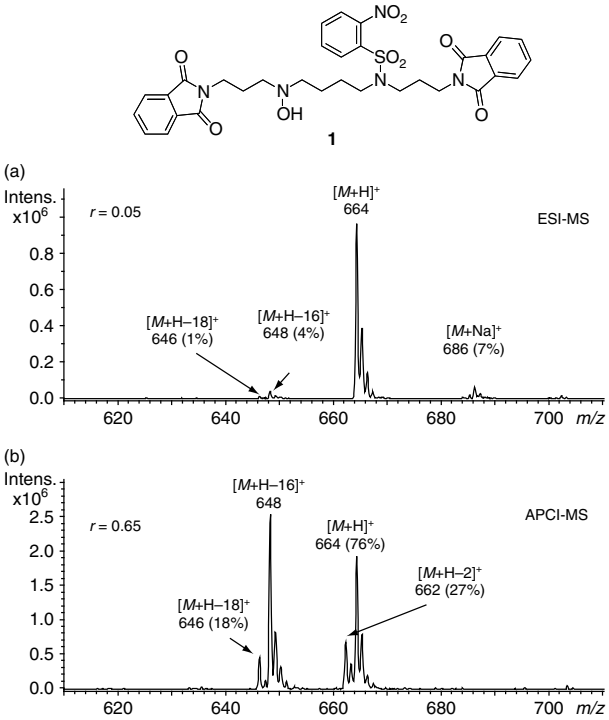

Figure 1. Structure and (a) LC-ESI-MS and (b) LC-APCI-MS of *N*-hydroxylated tetraamine derivative **1** in MeCN/H_2O (4 : 6) + 0.1% TFA.

Mayfoline

Mayfoline (6.34 µg) was dissolved in MeCN/H_2O (1 : 4, 1 ml), and an aliquot of 5 µl was injected into the LC-MS system under isocratic conditions with 20% B and a flow rate of 180 µl/min. The post-column addition of TFA to the sample was performed by the addition of a mixture of MeCN/H_2O/TFA (2 : 6 : 2) at a rate of 40 µl/min through a Tee located in-between the exit of the column and the entry of the APCI interface.

Results and Discussion

Investigations with synthetic *N*-hydroxylated compounds

Tetraamine derivative **1** (Fig. 1), which was prepared during our synthetic pursuit of *N*-hydroxylated polyamine spider toxins,[13] was chosen as the model compound for our study of the APCI behavior of *N*-hydroxylated secondary amines. The compound readily undergoes the investigated decomposition reactions and is also accessible in pure form. Compound **1** also contains an additional nitroaryl group, allowing the concurrent study of the decomposition of *N*-hydroxy and aromatic nitro[1] functionalities.

The investigation of the MS behavior of compound **1** started with two LC-MS runs performed with either an ESI or an APCI source under conditions previously applied for the analyses of polyamine spider toxins.[9,10,15,16] The LC-ESI-MS spectrum of the

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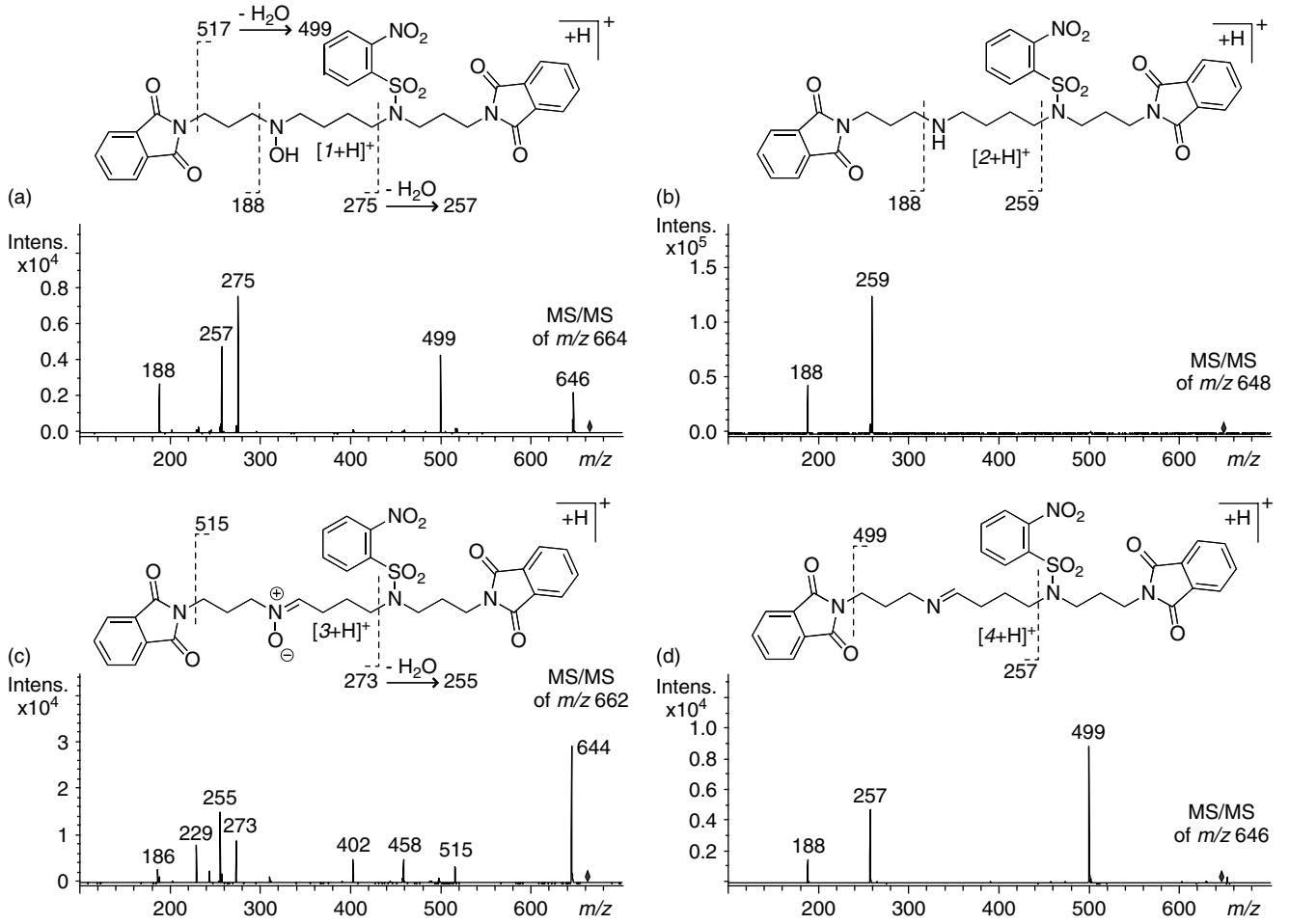

Figure 2. LC-APCI-MS/MS of (a) [***1***+H]$^+$, (b) [***1***+H−16]$^+$ = [***2***+H]$^+$, (c) [***1***+H−2]$^+$ = [***3***+H]$^+$ and (d) [***1***+H−18]$^+$ = [***4***+H]$^+$ ions with proposed structures and assignments of relevant fragment ions. For the nitrones **3** and the imines **4**, only one of the two isomeric forms is shown.

chromatographic peak of **1** (Fig. 1(a)) showed the expected signal for the protonated molecule [*M*+H]$^+$ at *m/z* 664 (base peak) and a weaker signal assigned to ions of the type [*M*+Na]$^+$ (*m/z* 686, 7%). Two additional signals at *m/z* 648 (4%) and *m/z* 646 (1%) were also observed corresponding to ions of the type [*M*+H−16]$^+$ and [*M*+H−18]$^+$, respectively. Often, ion signals with such low relative intensities are ignored. These signals, however, became relevant when APCI was used instead of ESI as the ionization method. Namely, the spectrum obtained by LC-APCI-MS (Fig. 1(b)) showed [*M*+H−16]$^+$ at *m/z* 648 (base peak) and [*M*+H−18]$^+$ at *m/z* 646 (18%) with significantly higher relative intensities. A third signal that cannot be ignored was found at *m/z* 662 (27%), corresponding to [*M*+H−2]$^+$ ions. Since it was shown by LC-UV (DAD) and by NMR that the sample compound was pure, these three additional ions had to be generated by in-source decomposition of **1,** and were not due to impurities.

A term *r* with the following equation is introduced to estimate the extent of the overall decomposition of **1** occurring in the different experiments.

$$r = \frac{\sum I_{\mathrm{DP_i}}}{I_{\mathrm{QMI}} + \sum I_{\mathrm{DP_i}}} = \frac{I_{[M+\mathrm{H}-2]^+} + I_{[M+\mathrm{H}-16]^+} + I_{[M+\mathrm{H}-18]^+}}{I_{[M+\mathrm{H}]^+} + I_{[M+\mathrm{H}-2]^+} + I_{[M+\mathrm{H}-16]^+} + I_{[M+\mathrm{H}-18]^+}} \quad (1)$$

In Eqn (1), $I_{\mathrm{DP_i}}$ corresponds to signal intensities of the decomposition products and I_{QMI} to signal intensity of the quasi-molecular ions. Although *r* is not the actual molecular ratio of the three decomposition products to the initial amount of **1**, it can still be regarded as a qualitative measure to describe the extent of decomposition of **1**, allowing, therefore, the characterization of different experiments.

The structures of the three artifactual ions at *m/z* 648, 662 and 646 were deduced from data acquired by LC-APCI-MS/MS and from their accurate masses measured by high-resolution ESI-MS of sample compound **1**. The exact masses of [*M*+H]$^+$, [*M*+H−16]$^+$, and [*M*+H−18]$^+$ revealed that the artifacts were generated by formal loss of O and H_2O from **1**, respectively (Table 1). No high-resolution ESI-MS data was available for the signal at *m/z* 662 ([*M*+H−2]$^+$) because of its intensity was too low. However, the loss of H_2 from the parent compound **1** appears to be the most reasonable process that could generate an artifact responsible for the respective signal.

The structures of the artifacts formed in the ion source were deduced from their MS/MS data as amine **2**, nitrones **3** and imines **4** (in protonated forms, Fig. 2). The loss of oxygen from **1** could have principally occurred either at the N–OH position or at the NO_2/SO_2 groups of the nosyl portion. The data revealed, however, that reduction only took place at the hydroxylamine functionality, thus forming product **2**. While fragment ions at *m/z* 275 were

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Table 1. Exact masses obtained from high-resolution ESI-FT-MS investigation of **1**

Ion signal	Elemental composition	*m/z* theoretical	*m/z* measured	Δ ppm
$[\mathbf{1}+H]^+$	$C_{32}H_{34}O_9N_5S$	664.20717	664.20727	0.1
$[\mathbf{1}+H-O]^+$	$C_{32}H_{34}O_8N_5S$	648.21226	648.21248	0.4
$[\mathbf{1}+H-H_2O]^+$	$C_{32}H_{32}O_8N_5S$	646.19661	646.19692	0.5

observed for compound **1**, the respective signal – which should be the same if deoxygenation would occur at the NO_2 or SO_2 groups – was not found in the MS/MS of **2** (Fig. 2(b)). Instead, a signal was registered at *m/z* 259, which is consistent with an amine instead of the hydroxylamine functionality. The fact that no ion response at *m/z* 275 was observed also revealed that deoxygenation of NO_2 to NO, as observed for aromatic nitro compounds,[1] was too slow to compete with the deoxygenation of the hydroxylamines. The eliminations of H_2 (formation of **3**) and of H_2O (formation of **4**) from sample compound **1** also occurred with the N-OH functional group rather than with the other groups contained in the molecule. Analogously to **2**, the MS/MS of artifacts **3** and **4** showed no ion signals at *m/z* 275 but signals at *m/z* 273 and *m/z* 257, respectively, which are diagnostic for decomposition located in the 'left-part' of the molecules (Fig. 2(c) and (d)).

Dependence of the APCI decomposition of hydroxylamines on the sample concentration

The online coupling of HPLC to MS allows fast acquisition of MS data of an analyte directly after column chromatography. Thus, mass spectra of differently concentrated analyte solutions can be measured when reasonably broad chromatographic peaks are obtained. LC-APCI-MS of **1** (1 μg) afforded a chromatographic peak sufficiently broad to allow its splitting into several segments of 0.1 min, in which each is characterized by a different analyte concentration. The averaged analyte concentration of the segments was estimated on the basis of the relative segment areas (Fig. 3). The MS of the two highlighted segments with low analyte concentration, at the beginning and at the end of the peak (~1.8 and 0.8 μM), showed rather high degrees of sample decomposition ($r = 0.86$ at t_R 20.6–20.7 min and $r = 0.90$ at 21.7–21.8 min, respectively). Still prominent but significantly less sample decomposition was observed at the time segment taken at the peak maximum ($r = 0.65$ at t_R 21.0–21.1 min). Thus, less decomposition was observed when more highly concentrated solutions were investigated. This effect is general as it was also observed in other measurements, e.g. in those performed with natural samples of polyamine spider toxins. These results suggest, in accordance with previously described studies, that APCI decompositions are surface-supported processes, which are controlled in their extent by the limited surface of the APCI interface.[8]

Dependence of the APCI decomposition of hydroxylamines on the solvent acidity

To study the pH-dependence of the APCI decomposition of *N*-hydroxylated secondary amines, compound **1** was dissolved either in pure MeCN/H_2O (1 : 1) or in MeCN/H_2O (1 : 1) admixed with TFA, HCOOH or TFA followed by NH_3 until neutralized and directly introduced into the APCI-MS. The results are shown with

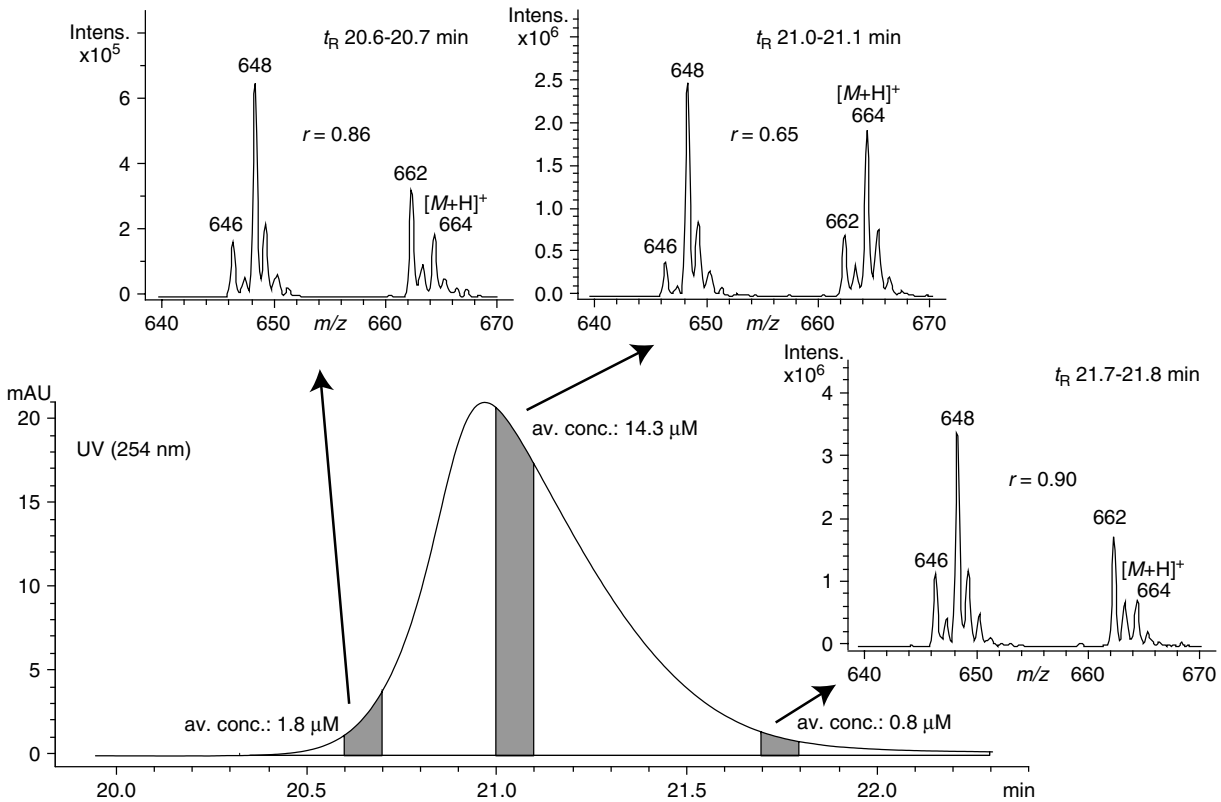

Figure 3. Concentration dependence of the APCI reduction of hydroxylamines shown with segments of a LC peak of **1**.

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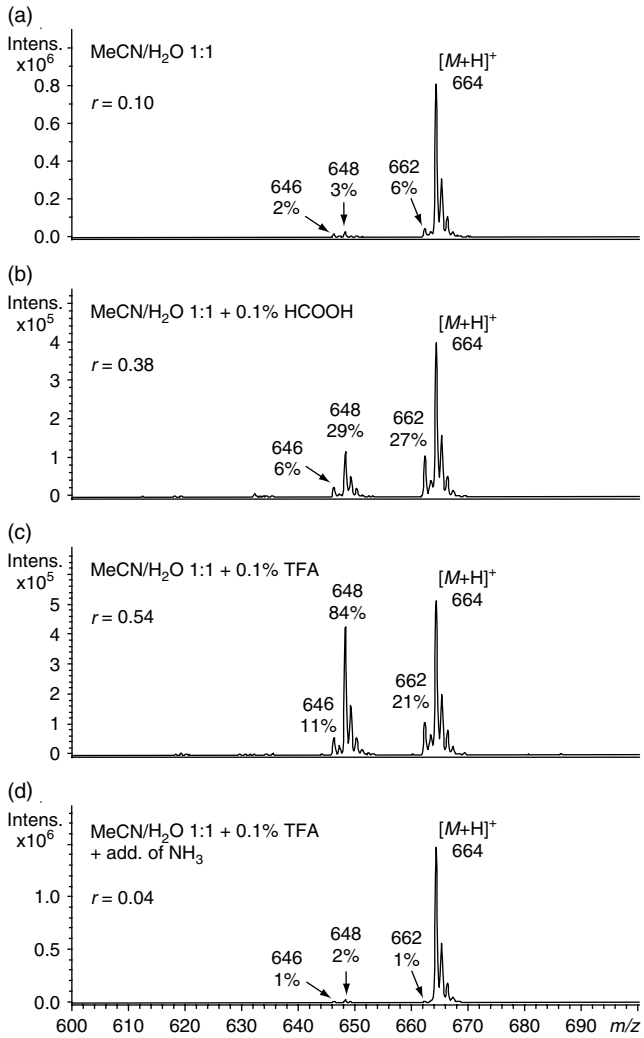

Figure 4. Direct infusion APCI-MS experiments performed with compound **1** dissolved (a) in pure MeCN/H_2O (1 : 1), or in MeCN/H_2O (1 : 1) admixed with (b) HCOOH, (c) TFA or (d) TFA followed by addition of NH_3 until neutralized.

the spectra in Fig. 4. Increased acidity of the sample solution led to more pronounced decomposition. While almost no decomposition of compound **1** was observed when the sample was introduced into the APCI-MS dissolved in the neutral mixture of MeCN/H_2O (1 : 1) (r = 0.1, Fig. 4(a)), the decomposition of **1** increased markedly in presence of HCOOH or TFA (r = 0.38 and 0.54, Fig. 4(b) and (c), respectively). Decomposition was highest when 0.1% TFA was used as the additive and therefore under the standard conditions used for the chromatographic separation of spider toxins. Decomposition of **1** was inhibited by the addition of aqueous NH_3 (to the initially acidic solution) as is recognized from spectrum c (r = 0.04, Fig. 4(d)). This latter result confirms the fact that the decomposition of **1** occurs in the ion source and not already before its entry into the HPLC.

The pH-dependence of the APCI decomposition of hydroxylamines can be used as a means to identify the N–OH functionality of a molecule. If a compound shows $[M+H-H_2]^+$ and, particularly, $[M+H-O]^+$ signals in APCI-MS spectra ($[M+H-H_2O]^+$ and if the formation of these ions can be enforced or inhibited by the addition of acid or base to the sample solution prior to its introduction into the instrument, the presence of the N–OH group in the molecule is rather likely. It should thus be possible to distinguish between artifacts and real N–OH-containing sample compounds by inhibition of the APCI decomposition. On the other hand, if no ions of the type $[M+H-H_2]^+$ and $[M+H-O]^+$ can be found for a compound – not even when the sample solution is acidified – an N–OH group is most likely not present in the sample molecules.

The pH-dependence of the APCI decomposition of hydroxylamines can thus be used for the unequivocal recognition of the N–OH functionality as is illustrated below with the analysis of native polyamine toxins found in spider venom and of mayfoline, a natural cyclic polyamine derivative.[17]

LC-APCI-MS Analysis of the Venom from the Spider *Agelenopsis aperta*

Some years ago, acylpolyamines of the venoms of *A. aperta*[10] and of *Paracoelotes birulai*[9] were characterized by means of LC-APCI-MS and -MS/MS, and both venoms were found to contain *N*-hydroxylated polyamine derivatives. Due to incomplete data, some of the constituents of *A. aperta* were not amenable to complete structural elucidation. They were regarded as structural isomers of other identified components, but now, with the knowledge about the in-source decomposition of *N*-hydroxylated secondary amines, they could also have represented artifacts. Thus, the venom of *A. aperta* was reinvestigated by LC-APCI-MS.

The 2D-plot in Fig. 5(a) summarizes the ion responses of all constituents of the acylpolyamine fraction of *A. aperta* obtained with the LC-MS setup already used previously. The spots in the chromatogram represent ion responses registered in dependence on retention times (abscissa) and *m/z* values (ordinate). Remarkable is the occurrence of signal doublets and triplets characterized by the same t_R and by *m/z* values differing by 16 u. Interpretation of the MS/MS data of the respective ions revealed that the triplets represent ions of di-, mono-, and non-*N*-hydroxylated polyamine derivatives and the doublets of mono- and non-*N*-hydroxylated polyamine derivatives, in each case sharing the polyamine backbones. Applying the acquired knowledge of the APCI reduction of hydroxylamines, the signals of the 'deoxygenated' structures were most likely due to APCI artifacts rather than the response of real sample compounds. For example, the two ion signals at *m/z* 449 and 433 of the doublet **B** at t_R = 25.9 min can be interpreted as the protonated molecules and the respective protonated 'deoxygenated' decomposition products $[M+H-O]^+$ of the co-eluting isomeric mono-*N*-hydroxylated toxins **8** + **9**.[10] The signal at *m/z* 433 could therefore be generated by partial reduction of **8** + **9** during the APCI process (Fig. 5(a)).

To prove that the doublets and triplets recorded in the 2D-chromatogram arise in fact from mono- or di-*N*-hydroxylated parent compounds, an LC-APCI-MS experiment of the toxin mixture was performed under the same optimized chromatographic conditions used before (MeCN/H_2O gradient + 0.1% TFA), but with post-column addition of NH_3 to inhibit the APCI decompositions. The respective 2D-plot is shown in Fig. 5(b). It is readily recognized that the doublets and triplets found in Fig. 5(a) largely disappeared, which allows the conclusion that the several vanished peaks arose from artifacts rather than from native compounds.

Hence, also *N*-hydroxylated acylpolyamines of spider venom underwent in-source decomposition during LC-APCI-MS experiments analogously to the synthetic *N*-hydroxylated compound **1**. The re-analysis of the venom of *A. aperta* revealed that compounds generated by APCI decomposition were previously misinterpreted as constituents of the venom.

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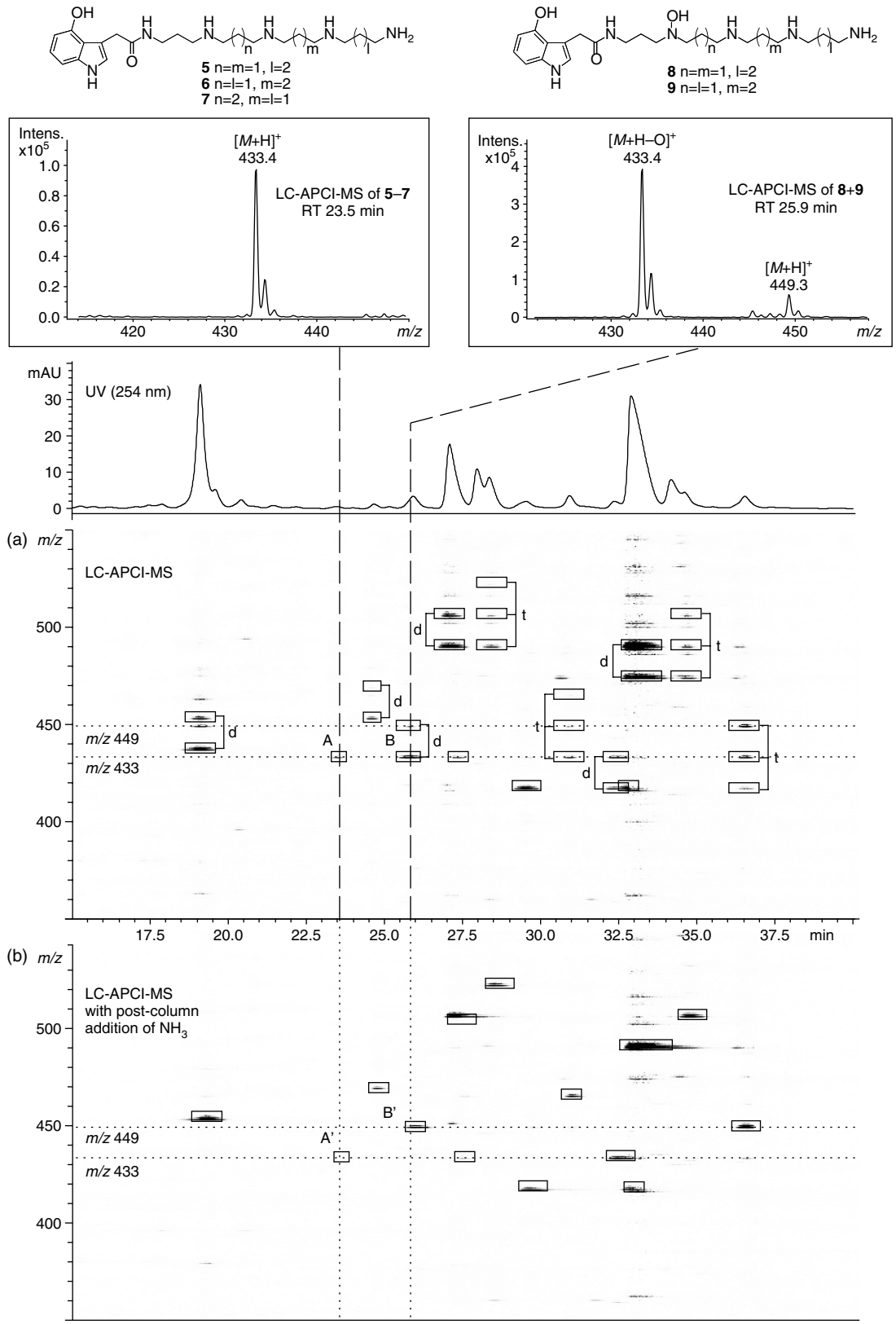

Figure 5. 2D-plot of an LC-APCI-MS run of *A. aperta* venom and the corresponding UV-chromatogram detected at $\lambda = 254$ nm using (a) MeCN/H_2O + 0.1% TFA as the mobile phase and (b) the same conditions but with post-column addition of NH_3. d and t designate a signal doublet and a signal triplet, respectively.

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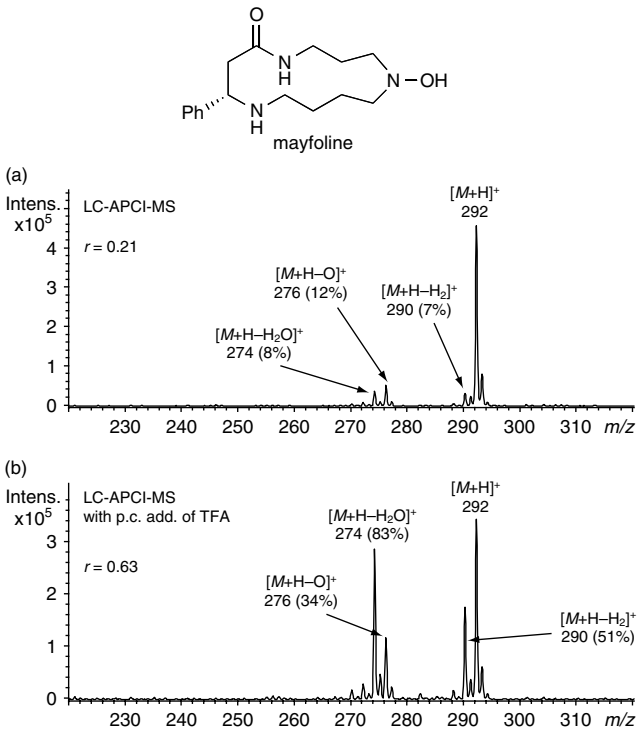

Figure 6. LC-APCI-MS of mayfoline (a) with MeCN/$H_2$0 gradient + 0.1% TFA at 180 µl/min and (b) with MeCN/$H_2$0 gradient + 0.1% TFA at 180 µl/min and post-column addition of MeCN/H_2O/TFA (2 : 6:2, 20 µl/min).

The eye-catching doublet- and triplet-patterns recognized in the 2D-chromatogram in Fig. 5(a) can be taken as a count for the number of N–OH groups in a molecule. Thus, two 2D-plots of *N*-hydroxylated (or potentially *N*-hydroxylated) compounds measured under acidic and basic APCI conditions can reveal the presence and number of N–OH groups in an unknown compound. Since *C*-hydroxy groups are not reduced during APCI-MS, this ionization mode can be used to distinguish *N*- from *C*-hydroxylation as shown, for instance, with the APCI-MS of the non-*N*-hydroxylated derivatives **5**–**7** that did not show signals for deoxygenated products. These results are in accordance with those obtained with the method reported to differentiate *N*-oxides from *C*-hydroxylated compounds.[3]

APCI-MS analysis of mayfoline

Mayfoline (Fig. 6) is a cyclic *N*-hydroxylated spermidine alkaloid isolated from the shrub *Maytenus buxifolia*,[17] and it was synthesized some years ago by Hesse *et al.*[14] Due to the N–OH functionality, mayfoline was expected to show the same type of APCI decomposition as the model compound **1** and the polyamine spider toxins described earlier. However, only a little decomposition was observed ($r = 0.21$, Fig. 6(a)) when a sample of the natural product was analyzed by LC-APCI-MS under the normal conditions applied for the separation of polyamine derivatives (MeCN/H_2O gradient + 0.1% TFA). The expected signals for the protonated decomposition products, $[M+H-H_2]^+$, $[M+H-O]^+$ and $[M+H-H_2O]^+$, were still observed but with unexpected low relative intensities of 7, 12, and 8% despite the high acidity of the solvent mixture used for the APCI-MS experiment. When a sample of mayfoline was brought into the ion source in neutral solvent (MeCN/H_2O 1 : 1), a spectrum with only a single signal, the $[M+H]^+$, was recorded. Thus, mayfoline represents an *N*-hydroxylated compound with little tendency to undergo APCI reduction.

Since APCI deoxygenation was intended to be taken as a conclusive argument for the identification of the N–OH functionality within a sample molecule, it was tested if APCI deoxygenation of mayfoline can be enforced so that the respective ions are unquestionably recognized. This, in fact, could be affected by the post-column addition of a mixture of MeCN/H_2O/TFA (2 : 6 : 2) to the analyte flow. Under these conditions, the MS revealed signals of the decomposition products at *m/z* 290 ($[M+H-H_2]^+$, 51%), *m/z* 276 ($[M+H-O]^+$, 34%) and *m/z* 274 ($[M+H-H_2O]^+$, 83%) with significantly higher intensities ($r = 0.63$, Fig. 6(b)). Decomposition was not at all observed with a sample of synthetic deoxymayfoline,[14] which has been treated the same way (data not shown).

Conclusions

The investigation of the various *N*-hydroxylated amines revealed that N–OH-containing compounds characteristically form artifacts upon APCI. The corresponding decomposition reactions are strongly pH-, and, to a lesser degree, concentration-dependent. The degree of decomposition also depends on the exact molecular structures of the analytes. For all compounds investigated, APCI decomposition could be enforced by the addition of sufficient acid to the analyte solution and suppressed by the addition of base.

Understanding in-source decomposition of *N*-hydroxylated amines can help avoid misinterpretation of MS data from samples that contain *N*-hydroxylated analytes (particularly of LC-MS data for which no additional analytic information is available). The pH-dependence of the APCI decomposition can be applied in two ways: it can be used (1) to distinguish unavoidable artifacts from native compounds, as shown with the investigation of the spider venom of *A. aperta,* and (2) for the conclusive identification of N–OH functionalities within a compound.

The insights gained with the MS investigation of hydroxylamines are of relevance for synthetic organic chemistry as well. For instance, decompositions of hydroxylamines, prepared by *Cope* elimination of N-oxides, were largely suppressed by the addition of base to the reaction medium.[13]

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