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Fragmentation Pathways of Deprotonated Phenanthroperylene Quinones from Fossil Sea Lilies by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

ThP 366

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Introduction

Phenanthroperylene quinones are prominent in the medicinal plant St. John's wort (*Hypericum perforatum*), and they are also known as dyes in fossil and recent sea lilies, marine animals belonging to the phylum Echinodermata like starfish and sea urchins. Particularly, they were detected in the fossil representatives *Carnallicrinus* (Triassic) and *Liliocrinus* (Jurassic). These findings go back to Blumer's work on Jurassic material [1] and have been verified or partially revised mainly by use of mass spectrometry [2].

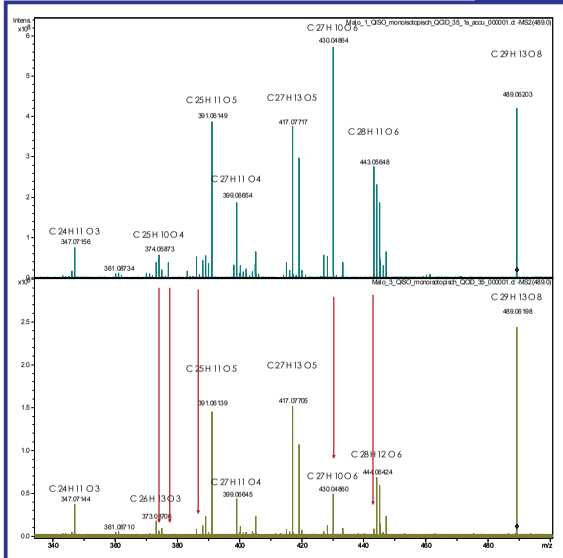
Nonetheless, the identification of these compounds has been based on comparison of electrospray triplequadrupole tandem mass spectra of their $[M-H]^-$ ions with those obtained from reference material. The fragmentation pathways of their $[M-H]^-$ ions however, were only rationalized on the basis of „reasonable neutral losses" [2,3].

Recently, we identified new phenanthroperylene quinone-type compounds in Jurassic sea lilies by the accurate masses of their $[M-H]^-$ ions. For their structure elucidation, a study of the fragmentation pathways of deprotonated phenanthroperylene quinones by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry was conducted.

The FT-ICR spectra exhibit peaks and relative intensities similar to those observed in previous triplequadrupole tandem mass spectra [2,3], thus simplifying direct comparison. In addition, FT-ICR-MS offered beyond 100.000 resolution and mass accuracies of less than 1 ppm for most fragment ions, thereby delivering unequivocal assignment of the molecular formulas of all fragment ions.

Here, we report on the first reliable formula assignment to all CID fragments of deprotonated fringelite F and hypericin plus fragmentation pathways derived therefrom.

Isomers of $[C_{29}H_{13}O_8]^-$



The tandem mass spectra of the $[M-H]^-$ ions of „demethylhypericin", *m/z* 489, $[C_{29}H_{13}O_8]^-$, as the second most abundant dye of two different, so far not fully characterized, fossils are compared here. Until recently, only one compound was assumed to be present in the fossil record.

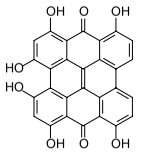
While the upper spectrum shows an abundant peak due to HCOOH loss, this is almost absent in the lower spectrum.

The $[M-H-CO_2]^-$ ion, *m/z* 445, in the upper spectrum undergoes loss of $CH_3\cdot$ to yield the base peak, whereas the same fragment is of low abundance in the second spectrum.

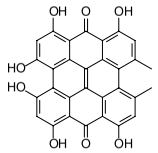
Additional differences are observed in the *m/z* 373–377 range of the spectra where the upper compound also produces more abundant signals. These are most probably due to subsequent fragmentations of the aforementioned ions at *m/z* 443 and 430.

What Do They Have In Common?

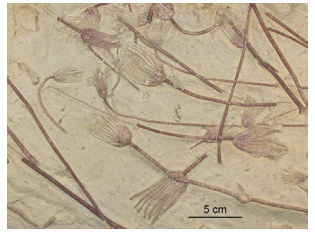
St. John's wort (*Hypericum perforatum*)



fringelite F
$C_{28}H_{12}O_8$
$[M-H]^-$ at *m/z* 475



hypericin
$C_{30}H_{16}O_8$
$[M-H]^-$ at *m/z* 503



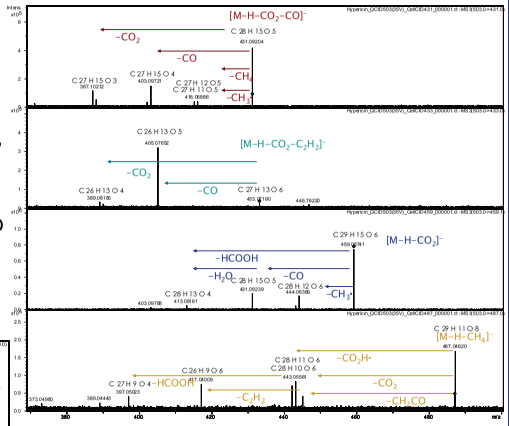
Fossil sea lilies (*Carnallicrinus carnalli*)

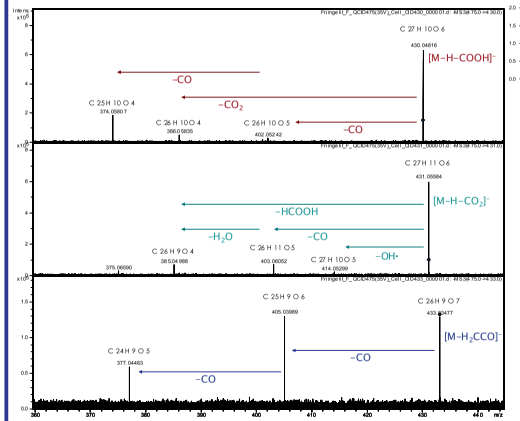
The plant (upper left) and the 240 million-year-old fossil (right) share the organic pigment hypericin. In addition, derivatives such as fringelite F and demethylhypericin were extracted from the fossil and pseudohypericin, a hypericinoid where one methyl is exchanged for a hydroxymethyl, was isolated from the plant.

MS^3 Experiments

MS^3 was employed to resolve the sequence of reactions of the $[M-H]^-$ ions. This was achieved by SORI-CID of the fragments at *m/z* 430, 431, and 433 formed upon dissociation of the $[M-H]^-$ ion of fringelite F in the hexapole collision cell (below) and by analogous treatment of the fragments at *m/z* 431, 433, 459, and 487 of the $[M-H]^-$ ion of hypericin (right).

The ions exhibit losses of small molecules, mainly CO and CO_2. They also eliminate C_2H_2, H_2O and HCOOH. Radical losses are rarely observed, but nonetheless present, e.g., OH· and $CH_3\cdot$ loss from $[M-H-CO_2]^-$.





The $[M-H-CO_2]^-$ ions generated from either precursor show elimination of CO followed by loss of H_2O. Alternatively, these findings can be interpreted in terms of competitive rather than consecutive reactions, i.e., by direct loss of HCOOH from the respective precursor ion. MS^4 would be required to distinguish between those pathways.

As long as a methyl group is present, it can easily be cleaved off. Again, it cannot be decided whether $CH_3\cdot$ loss preceedes H· loss to yield a formal CH_4 loss or whether elimination of the intact CH_4 molecule competes with the two-step process.

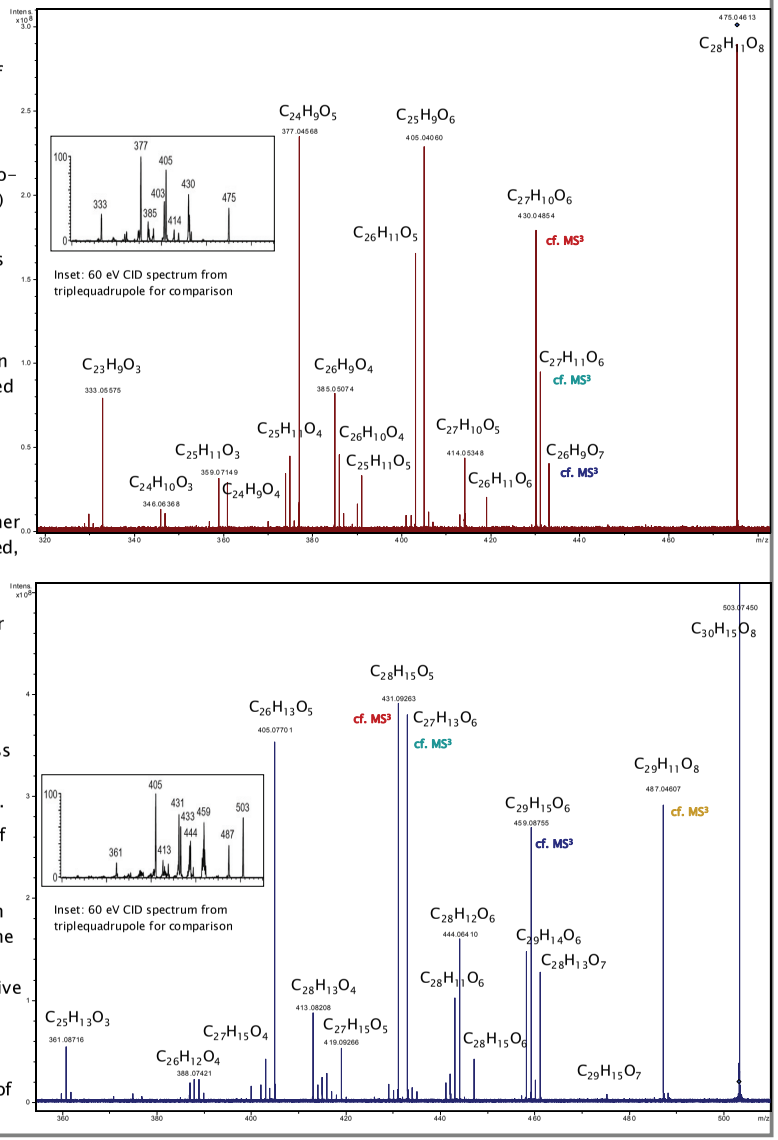
Tandem Mass Spectra of Deprotonated Molecules

The spectra resulting from CID inside the hexapole collision cell of the ApexQe correspond well to those previously obtained on our triple-quadrupole mass spectrometer (Finnigan TSQ700) at 60 V collision offset.

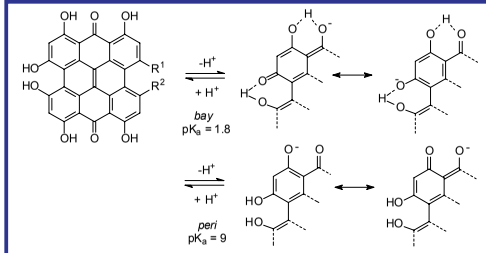
Now, molecular formulas can unequivocally be assigned to all these fragment ions, and for example, loss of C_2H_4, an isobar of CO, can be ruled out.

This will become important, when new phenanthroperylene quinones of masses higher than hypericin are studied, because side chain fragmentation will then become feasible for their $[M-H]^-$ ions.
This expectation is supported by the loss of CH_4 unique to the hypericin ion and the loss of $CH_3\cdot$ from its $[M-H-CO_2]^-$ fragment (see left).

Although the formulas of all fragment ions are indentified by now, their structure still remains an open question. Due to the fact that almost all reactions require extensive rearrangements, any structure assignment to fragments would be speculative at this level of research.



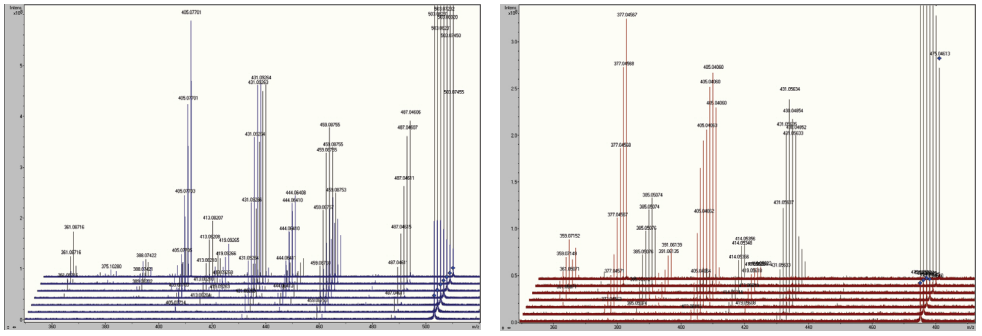
Deprotonation Site of the Precursor Ions



Deprotonation in the *bay* region (pKa = 1.8) is by far preferred over deprotonation in the *peri* region (pKa = 9) [4]. The reason for that is presented by the effective resonance stabilization of the $[M-H]^-$ ion of the vinylogous carbonic acid, whereas the other structure is less advantageous in this respect.

Therefore, the *bay*-deprotonated ion structure should be of highest relevance as the precursor for the fragmentations under study.

Energy Dependence of the Fragmentation Pathways



Deprotonated phenanthroperylene quinones are very stable ions. The onset of collision-induced dissociation is observed at about 20 V offset of the hexapole collision cell. The CID spectra of the $[M-H]^-$ ions of hypericin, *m/z* 503, (left) and fringelite F, *m/z* 475, (right) are acquired at 20, 23, 26, 29, 32, 35, and 38 V offset (from front to back). An offset of 35 V appears suitable for structure elucidation.

The energy dependence of the spectra is correlated to the observations from our MS^2 and MS^3 experiments. Stepwise losses of small molecules definitely are the preferred reactions, and there is no indication for rearrangements yielding any loss of major portions of the phenanthroperylene skeleton.

In other words, the spectra will be dominated by fragmentation of substituents as soon as they are available.

Experimental

The experiments were performed on 7.0 T (Bremen) and 9.4 T (Heidelberg) Bruker ApexQe (Qh-ICR hybrid) instruments with DualSource (ESI-to-MALDI switchable) in negative-ion electrospray mode. Tandem mass spectra were obtained by collision-induced dissociation (CID) of the ions in the hexapole collision cell. For MS^3 the first generation fragment ions from the hexapole cell were fragmented by SORI-CID in the ICR cell prior to mass analysis.
Electrospray parameters: liquid flow 2 µl min^{-1} at 4 kV spray voltage, desolvation gas flow 3.0 l min^{-1} at 250 °C, and nebulizer gas flow 1.5 l min^{-1}.
Ions were accumulated in the storage hexapole for 0.1-1.0 s and then transferred into the ICR cell. Trapping was achieved at sidekick potential of - 4.0 V and trapping potentials of slightly above 1 V. The mass spetra were acquired in the broadband mode with 1M or 2 M datapoints. The time-domain data were zerofilled and apodized by a sine-bell function prior to Fourier transformation. Depending on the abundance of the (precursor) ion 16 to 128 transients were accumulated for one magnitude spectrum.
Acquisition was controlled by Bruker ApexControl 2.0.0.beta software, data analysis was performed using the Bruker DataAnalysis 3.4 software.

Acknowledgment

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References

[1] M. Blumer, *Mikrochemie* 36/37, 1048-1055 (1951); M. Blumer, *Nature* 188, 1100-1101 (**1960**); M. Blumer, *Geochim. Cosmochim. Acta* 26, 225-227 (**1962**); M. Blumer, *Sci. Am.* 234(3), 34-45 (**1976**).

[2] K. Wolkenstein, J. H. Gross, H. Falk, H. F. Schöler, *Proc. R. Soc. B*, 273, 451-456 (**2006**).

[3] G. Piperopoulos, R. Lotz, A. Wixforth, T. Schmierer, K.-P. Zeller, *J. Chromatogr. B* 695, 309-316 (**1997**).

[4] H. Falk, *Angew. Chem. Int. Ed.* 38, 3134-3154 (**1999**).