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Mass Spectrometric Characterization of Fossil Pigments: Case Study of a 240 Million Year Old Sea Lily

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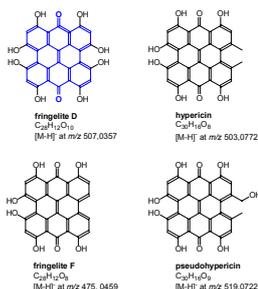
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Motivation

The Triassic crinoid *Chelocrinus carnalli* lived 240 million years ago on the bottom of the shallow sea flooding wide parts of Central Europe. *Chelocrinus* fossils can now be collected in the sediment layers of the Lower Muschelkalk. The intense brownish-violet color of some *Chelocrinus carnalli* fossils is in obvious contrast to the surrounding light brown limestone sediment. Pioneering work in the analysis of organic pigments of the related 140-150 million year old Jurassic crinoid *Millerocrinus* has been published by Blumer [1-4] who named these compounds fringelites. Recent crinoids and echinoderms possess related dyes. Blumer also detected some polycyclic aromatic hydrocarbons (PAHs) of the perylene type in those fossils [3]. However, using the analytical techniques of his time he never obtained a full spectral characterization of these pigments.

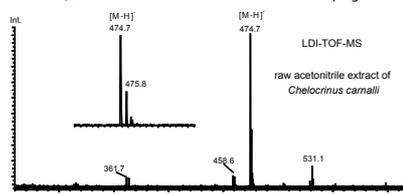
Structures

Fringelite as well as derivatives of hypericin that can be extracted from St. John's wort (*Hypericum perforatum*) belong to the group of phenanthroperylenequinones. Their basic structure is also known as meso-naphthodianthrone.



LDI-TOF Screening

LDI-TOF-MS presented the first mass spectral step to check extracts for the presence of UV absorbing dyes and accompanying compounds. In negative-ion LDI-TOF spectra, a signal at m/z 475 was obtained from every extract of *Chelocrinus carnalli* fossils in polar solvents. Depending on the actual fossil sample, additional peaks at m/z 489 and 503 could be detected at varying intensity.



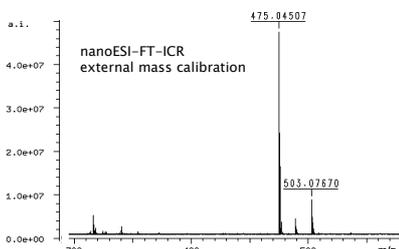
Experimental Conditions for LDI-TOF-MS

LDI-TOF experiments were performed on a Bruker Biflex TOF instrument operated in the reflector mode with pulsed ion extraction. LDI spectra were acquired in positive- and negative-ion mode. The extracts were supplied as dried solutions on chromium-plated targets and desorbed by irradiation with the 337 nm nitrogen laser.

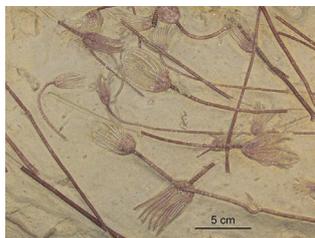
ESI-FT-ICR-MS

The empirical formulas of the dyes could be determined by ESI-FT-ICR-MS [6]. Measurements were performed on a Bruker Apex III instrument equipped with a 7 T magnet. Both conventional and nanoESI techniques were employed as required. The $[M-H]^-$ ion of the main component was identified as $[C_{28}H_{11}O_8]^-$ corresponding to fringelite F. The additional dye ions were assigned to $[C_{29}H_{13}O_8]^-$ and $[C_{30}H_{15}O_8]^-$ which can be related to desmethylhypericin and hypericin, respectively. The assignment of the hypericin structure was verified by its ESI-MS/MS spectrum.

exp. m/z	Formula	Error [mmu]
475.04507	$C_{28}H_{11}O_8$	+0.87
489.06121	$C_{29}H_{13}O_8$	+0.35
503.07670	$C_{30}H_{15}O_8$	+0.54



Chelocrinus carnalli



Crowns and stalks of *Chelocrinus carnalli* as presented in the light brown limestone sediment (Collection of Bergakademie Freiberg).

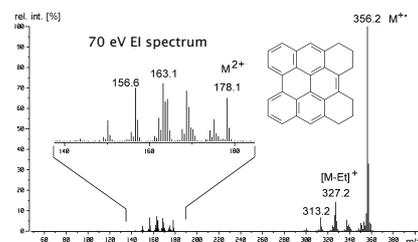
Procedure

Pieces of the stalk of *Chelocrinus carnalli* were separated from the limestone and dissolved in hydrochloric acid. The remaining crude pigments were subsequently extracted with toluene and methanol. Both extracts were subjected to positive- and negative-ion LDI-TOF-MS as a screening for light-absorbing species. After clean-up and separation by preparative HPLC the major components were characterized by combining various mass spectrometric techniques. In addition, ¹H-NMR spectra have been obtained in certain cases to get further proof of the structures. About 1 ml of a < 10⁻⁵ M solution of the dye could be obtained from 1 g of fossil material of the stalk of the sea lily. Thus, UV/Vis spectroscopy and mass spectrometry, in particular LDI-TOF-MS and ESI-MS were suited for their analysis. Solutions of the dye in polar solvents yielded consistent results in negative-ion ESI spectra. After optimization of the ESI conditions MS/MS and HR-ESI measurements could be performed.

EI-MS

The toluene extract revealed the presence of numerous PAHs. Hexahydrophenanthroperylene, the main component, was isolated by HPLC and subsequently identified by LR- and HR-EI-MS and by comparison to a synthetic reference [8]. Accompanying PAHs were characterized by their empirical formulas as obtained from HR-EI measurements.

EI spectra were acquired on a JEOL JMS-700 magnetic sector instrument at 70 eV. The samples were introduced via the direct probe. For accurate mass measurements from PAH mixtures, the resolution was set to $R = 5000$ and PFK was used for internal mass calibration.



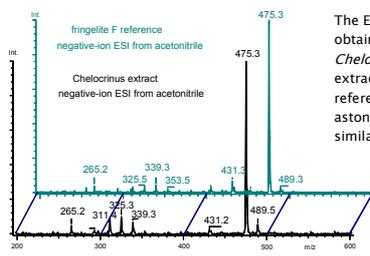
Additional PAHs Identified from HR-EI-MS

exp. m/z	Formula	Error [mmu]
356.1574	$C_{28}H_{20}$	+0.7
368.1572	$C_{29}H_{20}$	+0.9
370.1700	$C_{29}H_{22}$	-2.1
382.1741	$C_{30}H_{22}$	+2.0
384.1886	$C_{30}H_{24}$	+0.8
396.1884	$C_{31}H_{24}$	+0.6
408.1885	$C_{32}H_{24}$	+0.7

Further Reading

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- [6] FT-ICR-ESI-MS were performed at Bayer Industry Services, Leverkusen. We are indebted to Dr. S. Giesa, Dr. J. R. Wesener and H. Hamacher for performing these measurements.
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ESI-MS



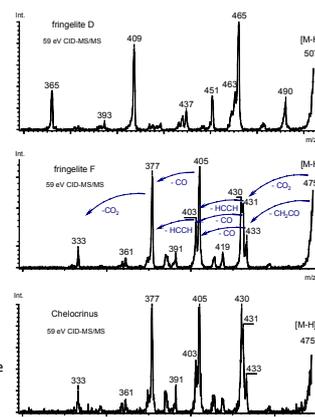
The ESI spectra obtained from *Chelocrinus* extracts and reference showed astonishing similarity.

Experimental of LR-ESI-MS and ESI-MS/MS

LR-ESI-MS and ESI-MS/MS measurement were performed on a Finnigan TSQ700 triple quadrupole mass spectrometer. The extracts in methanol, DMSO, ethanol, acetonitrile or THF were either introduced by means of a syringe pump into the conventional ESI interface (2-4 μl/min, fused silica capillary, 4,7-5,3 kV spray voltage, 35-40 psi nitrogen, desolvation capillary at 150-200 °C) or sprayed using a MasCom nanoESI interface (1.0 kV spray voltage, desolvation capillary at 100 °C). The spectra were obtained by averaging 10-20 scans at 10 s/scan in the m/z 50-1000 range. Argon at 0.9 mtorr was used as collision gas and a collision offset of 59 eV was set in MS/MS experiments

ESI-MS/MS

Fringelites as well as hypericin form $[M-H]^-$ ions of considerable stability. To achieve a sufficient degree of fragmentation, ESI-CID-MS/MS spectra were acquired at 59 eV collision offset. Even then, fragment ions below m/z 300 were not observed. The MS/MS spectra of fossil dyes and the respective reference compounds from



either synthetic or natural sources are virtually identical. Although all compounds exhibit losses of CO₂, CO, H₂CO and HCCH, their CID spectra exhibit sufficient differences to be well distinguished from each other. The constitution of fringelite F is well known from synthetic work [9,10]. In addition, negative-ion ESI-MS/MS spectra of the hypericin-type compounds are available from the literature. [5]

Summary

As expected from UV/Vis spectra, the methanol extracts of *Chelocrinus carnalli* contained fringelite-type dyes. Using ESI-MS, ESI-MS/MS [5], and FT-ICR-ESI-MS [6], the main component was identified as fringelite F ($[M-H]^-$ at m/z 475) which is accompanied by desmethylhypericin ($[M-H]^-$ at m/z 489). An additional signal indicates the presence of hypericin ($[M-H]^-$ at m/z 503) as a second minor component. Hypericin is usually known as a component of St. John's wort (*Hypericum perforatum*) and has found pharmaceutical application [7].

Extraction with toluene yielded PAHs, the most abundant of them being hexahydrophenanthroperylene. Its structure was assigned by comparison of the EI mass spectrum with that of a synthetic reference and its empirical formula was obtained from HR-EI-MS. In addition, numerous related PAHs were identified from their empirical formula in HR-EI-MS.

Gratefully

We are grateful for the donation of fossil samples by N. Hauschke (Universität Halle) and C. Neumann (Museum für Naturkunde, Berlin), synthetic references of fringelite-D by H. Falk (Universität Linz), hypericin and pseudohypericin references by M. Diehm (Phytoplan, Heidelberg), and fringelite-F references by W. Steglich (LMU, München).

