Electronic Supplementary Information

Determination of the naturally occurring vanadium-complex amavadin in *Amanita muscaria* with HPLC-ICPMS

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Content

1. Chemicals and instruments ..................................................................................................................2

2. NMR spectra ..........................................................................................................................................4

3. IR spectra .............................................................................................................................................6

4. HPLC-ESMS settings .............................................................................................................................8

5. Mass spectrum of amavadin .................................................................................................................9

6. Chromatograms ......................................................................................................................................10
1. Chemicals and instruments

Ultrapure water (18.2 MΩ*cm) was used throughout the study. The following chemicals were bought from Sigma Aldrich (St. Louis, USA): Sodium hydride (60% dispersion in mineral oil), trifluoromethanesulfonic anhydride (triflic anhydride, ≥99%), (-)-Methyl L-lactate (98%, optical purity ee: 97% (GLC)), 2,6-Lutidine (ReagentPlus, 98%), ethyl acetate (for HPLC, ≥99.7%), formic acid (puriss. p.a., ACS reagent, ≥98%), Dowex® 50WX2 hydrogen form (200-400 mesh), Vanadium(V) oxide (≥98%), ammonia solution (25% for analysis, Emsure). Zinc acetate dihydrate (puriss. p.a.) was purchased from Merck KGaA (Darmstadt, Germany).

Methanol (HiPervSolv Chromanorm for HPLC – Gradient grade), ethanol (HiPerSolv Chromanorm for HPLC) and citric acid (ACS) were obtained from VWR International (Darmstadt, Germany).

Vanadium single element standard (ICP-Standard-Solution Roti®Star), potassium hydroxide (≥85%, p.a.), trichloromethan (Rotisolv® HPLC), disodium ethylenediaminetetraacetate dihydrate (Na₂EDTA, ≥99%, USP) and nitric acid (≥ 65 % p.a., further purified inhouse via sub-boiling) were obtained from Carl Roth GmbH + Co.KG, Karlsruhe, Germany.

Acetic anhydride (puriss p.a., ACS reagent, ≥99%) was purchased from Fluka (Buchs, Switzerland)

Dry methylene chloride was purified via a Pure Solv Solvent Purification System and stored over molecular sieves.

Hydroxylamine hydrochloride (1.74 g, 25 mmol, pro analysis, min. 99%, Merck KGaA, Darmstadt, Germany) was added to sodium hydride (60 % in mineral oil, 1000 mg, 25 mmol) in methanol (25 mL) to obtain a hydroxylamine solution.

The certified reference materials (CRMs) SRM 1573a (Tomato Leaves) and SRM 1640a (Trace Elements in Natural Water) were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, USA).
Air- and moisture sensitive reactions were carried out following standard Schlenk technique with Ar as inert atmosphere. NMR spectra were measured on a Bruker (Rheinstetten, Germany) Advance III 300 MHz NMR spectrometer in deuterated solvents. $^1$H NMR spectra were recorded at 300.13 MHz and $^{13}$C NMR at 75.48 MHz and were referenced to the respective solvent peak. The chemical shifts $\delta$ are given in ppm. The multiplicity of peaks is denoted as singlet (s), doublet (d), triplet (t), quadruplet (q), and multiplet (m). All samples were prepared in air in standard 5 mm NMR tubes.

IR-spectra were measured on an Alpha F-TIR spectrometer (Bruker, Ettlingen, Germany). Samples were measured in air in the solid state at 24 scans and 4 cm$^{-1}$ resolution in a spectral range of 400-4000 cm$^{-1}$.

Electrospray ionization mass spectrometry (ESMS) was performed with a 6120 Quadrupole LC/MS (Agilent Technologies, Waldbronn, Germany). A 1260 Infinity HPLC (Agilent Technologies, equipped with da degasser, a binary pump, a thermostatted autosampler and a thermostatted column compartment) and a Nucleodur C18 Pyramid column (4.6*250 mm 5 µm, Macherey-Nagel, Düren, Germany) were used to separate the compounds in front of the ESMS.

Total V concentrations were determined on a 7700x ICPMS (Agilent Technologies). For speciation analysis, a 1260 Infinity HPLC (Agilent Technologies) was coupled to a 7700x ICPMS or a tandem ICPMS (ICPMS/MS, 8900, Agilent Technologies). A silica-based strong anion-exchange column (Zorbax SAX, 4.6 * 150 mm, 5 µm, Agilent Technologies) was used for separation of the V species. The outlet of the column was connected to the nebulizer of the ICPMS via polyether ether ketone (PEEK) tubing, with an inner diameter of 0.125 mm.

The water content of the mushroom samples was determined with a SMART Trac II Moisture & Fat Analyzer (CEM, Kamp-Lintfort, Germany). An ultraClave IV (MLS GmbH, Leutkirch, Germany) was used for microwave assisted digestions. Synthesis intermediates and extracts were centrifuged at 3300*g on a Rotina 420 R (Hettich Lab Technology, Tuttingen, Germany). Nylon syringe filters (0.22 µm, Simplepure, BGB, Lörrach, Germany) were used for filtering the extracts prior to analysis.
2. NMR spectra

Figure S1. 1H NMR spectrum of (1)
Figure S2. $^{13}$C NMR spectrum of (1)

Figure S3. $^1$H NMR spectrum of (2)
Figure S4. $^{13}$C NMR spectrum of (2)

3. IR spectra

Figure S5. IR spectrum of (2)
**Figure S6.** IR spectrum of vanadyl acetate

![Vanadyl acetate IR spectrum](image)

**Figure S7.** IR spectrum of amavadin

![Amavadin IR spectrum](image)
4. HPLC-ESMS settings

- Column: Nucleodur C18 Pyramid, 4.6*250 mm, 5 µm, Macherey-Nagel
- Flow rate: 0.6 mL min\(^{-1}\)
- Mobile phase: 70 % A (0.1 % formic acid in water), 30 % B (methanol)
- Injection volume: 10 µL
- Column Temperature: 20°C
- Runtime: 30 min
- Spray chamber method: API-ES
- Nebulizer pressure: 2.14*10\(^5\) Pa
- Drying gas flow rate: 12 L min\(^{-1}\)
- Drying gas temperature: 250°C
- Capillary voltage: 3000 V

Scan mode:
- Polarity: Positive
- Mass range: m/z 120 – 600
- Fragmentor: 55 V

SIM mode:
- Polarity: Positive
- Ions: m/z 158, 176, 186, 402
- Fragmentor: 70 V
5. Mass spectrum of amavadin

![Mass spectrum of amavadin](image)

**Figure S8.** Mass spectrum of amavadin, full scan range (m/z 120 – 600)

![Mass spectrum of amavadin: [M+3H]^+ at m/z 402 (100 % relative abundance), [M+2H]^+ at m/z 401 (60% rel. abundance), [M(13C_1)+3H]^+ at m/z 403 (16.5% rel. abundance).](image)

**Figure S9.** Mass spectrum of amavadin: [M+3H]^+ at m/z 402 (100 % relative abundance), [M+2H]^+ at m/z 401 (60% rel. abundance), [M(13C_1)+3H]^+ at m/z 403 (16.5% rel. abundance). The respective Na adducts [M+Na]^+ can be seen at m/z 423, 424 and 425.
6. Chromatograms

Figure S10. HPLC-ICPMS chromatograms (V at m/z 51) of the different parts of *Amanita muscaria*, sample A. Left: 0-20 minutes. Right: 0-60 minutes, zoomed in to see peaks of less abundant V species.
Figure S11. HPLC-ICPMS chromatograms (V at m/z 51) of an extract of *Amanita muscaria* gills. Black: Pure extract. Blue: Pure extract, spiked online (*via* the HPLC autosampler) with a standard containing 100 µg V L⁻¹ of amavadin (and also vanadyl acetate). The injection volumes were: 2 µL extract + 20 µL standard.