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# Red Pyrroloquinoline Alkaloids from the Mushroom *Mycena haematopus*

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Four so far unknown red alkaloid pigments, haematopodin B (**1**) and the mycenarubins D (**3**), E (**5**) and F (**6**), were isolated from fruiting bodies of *Mycena haematopus*. The structures of these pyrroloquinoline alkaloids were established by 2D NMR spectroscopic and MS (ESI) methods. Their absolute configurations were determined by comparison of their CD spectra with that of haematopodin (**2**) or mycenarubin A (**4**). Metabolic profiling of the red pigments of intact and injured fruiting bodies revealed that the known degradation product haematopodin (**2**) originates from haematopodin B (**1**), which is the native main pigment of *M. haematopus*.



## Introduction

The bleeding mycena, *Mycena haematopus* (Pers.: Fr.) P. Kumm. (German name: Großer Bluthelmling), is a small mushroom, which is widespread in Europe and Northern America on decaying logs and stumps of beech trees.[1] The fruiting bodies of the bleeding mycena can easily be recognised, as they contain a characteristic red blood-like fluid that is exuded if they are cut or bruised. The pigments responsible for the red colour of the latex and the fruiting bodies of *M. haematopus* were already investigated in 1993.[2] However, at that time the structure of the native main pigment **1** of intact fruiting bodies remained obscure on account of its low stability, although it was assumed that it is closely related to the stable degradation product haematopodin (**2**). Recently, we isolated red pyrroloquinoline alkaloids from the fruiting bodies of the related species *M. rosea*[3] and *M. sanguinolenta*.[4] These results inspired us to reinvestigate the structure of the precursor of **2** and to investigate whether other pyrroloquinoline pigments occur in *M. haematopus*. Comparative metabolic profiling by HPLC–UV of fresh fruiting bodies of *M. haematopus* with those of *M. rosea* and *M. sanguinolenta* pointed to the presence of mycenarubin A (**4**), sanguinolentaquinone (**7**) and of different, but structurally related alkaloids in *M. haematopus*. In this paper we describe the isolation and structural elucidation of four new red pyrroloquinoline alkaloids along with known alkaloids **2**, **4** and **7** from *M. haematopus* (Figure 1). We named the major red pigment haematopodin B (**1**), as it turned out to be the direct precursor of the degradation product haematopodin (**2**), whereas the minor new red pigments were named mycenarubins D (**3**), E (**5**) and F (**6**), because they are closely related to **4**.

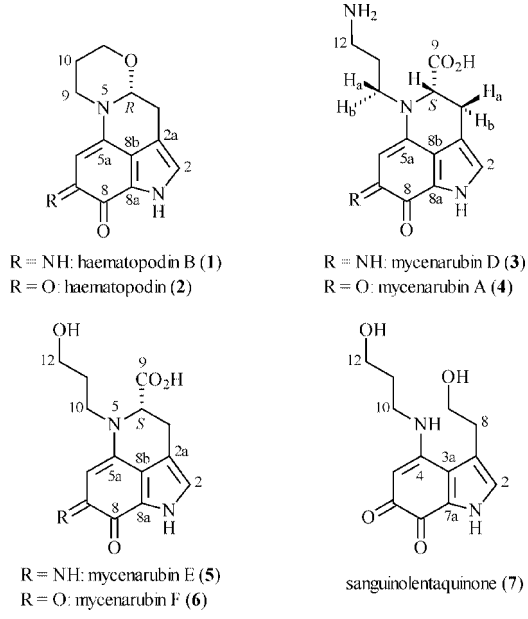


Figure 1. Pyrroloquinoline alkaloids from *M. haematopus*.

## Results and Discussion

Haematopodin B (**1**) (33.0 mg), haematopodin (**2**) (1.5 mg), mycenarubin D (**3**) (12.5 mg), mycenarubin A (**4**) (8.7 mg), mycenarubin E (**5**) (4.9 mg), mycenarubin F (**6**) (5.6 mg) and sanguinolentaquinone (**7**) (0.5 mg) were extracted from a sample of frozen fruiting bodies (100 g) of *M. haematopus* with methanol at 25 °C and purified immediately by HPLC on a preparative RP-18 column. To avoid

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Table 1. NMR spectroscopic data of **1** and **2**.

| Proton | Haematopodin B (**1**) | | Haematopodin (**2**) | |
|---|---|---|---|---|
| | $\delta_C$[a] [ppm] | $\delta_H$ [ppm] (mult., $J$ in Hz)[b] | $\delta_C$[c] [ppm] | $\delta_H$ [ppm] (mult., $J$ in Hz)[d] |
| 2 | 128.8 | 7.18 (s) | 126.1 | 7.05 (s) |
| 2a | 117.1 | | 115.9 | |
| 3 | 26.4 | 3.04 (dd, 17.5, 3.4) $H_{eq}$ | 27.1 | 2.93 (dd, 17.4, 2.8) $H_{eq}$ |
| | | 3.35 (dd, 17.5, 6.1) $H_{ax}$ | | 3.21 (dd, 17.4, 5.5) $H_{ax}$ |
| 4 | 90.5 | 5.40 (dd, 6.1, 3.4) $H_{ax}$ | 90.1 | 5.18 (dd, 5.5, 2.8) $H_{ax}$ |
| 5a | 155.9 | | 155.0 | |
| 6 | 87.2 | 5.82 (s) | 94.9 | 5.46 (s) |
| 7 | 159.2 | | 180.3 | |
| 8 | 167.9 | | 171.1 | |
| 8a | 123.8 | | 125.2 | |
| 8b | 123.4 | | 124.6 | |
| 9 | 49.2 | 3.79 (ddm, 13.3, 12.8) $H_{ax}$ | 47.7 | 3.53 (ddd, 13.4, 13.1, 2.5) $H_{ax}$ |
| | | 4.34 (dm, 13.3) $H_{eq}$ | | 4.14 (dm, 13.1) $H_{eq}$ |
| 10 | 27.9 | 1.87 (dm, 13.8) $H_{eq}$ | 26.4 | 1.66 (dm, 13.7) $H_{eq}$ |
| | | 2.19 (dddm, 13.8, 12.8, 12.5) $H_{ax}$ | | 2.12 (dddm, 13.7, 13.4, 12.9) $H_{ax}$ |
| 11 | 69.7 | 4.04 (ddm, 12.5, 11.6) $H_{ax}$ | 69.1 | 3.97 (ddd, 12.9, 11.2, 2.0) $H_{ax}$ |
| | | 4.14 (dd, 11.6, 3.7) $H_{eq}$ | | 4.09 (dd, 11.2, 3.8) $H_{eq}$ |

[a] 151 MHz, $D_2O$, 300 K. [b] 600 MHz, $CD_3OD$, 300 K. [c] Derived from the HMBC, 151 MHz, $CD_3OD$, 280 K. [d] 600 MHz, $CD_3OD$, 280 K.

degradation of the isolated compounds, especially that of **1** to **2**, the solvents were removed by lyophilisation and the natural products were stored at –35 °C.

The UV/Vis spectrum of the red main alkaloid haematopodin B (**1**) exhibited absorption maxima at $\lambda$ = 242, 357 and 507 nm. The absorption maxima were hypsochromically shifted with respect to those reported for haematopodin (**2**),[2] but they exhibited similar extinction values, which points to the presence of a pyrroloquinoline alkaloid core structure similar to that of **2**. The HRMS (ESI) of **1** showed an $[M + H]^+$ ion at $m/z$ = 244.1081, which is in agreement with the molecular formula $C_{13}H_{13}N_3O_2$. In comparison to the molecular formula $C_{13}H_{12}N_2O_3$ of **2**, an oxygen atom is formally replaced by NH. The $^1H$ NMR spectrum, recorded at 300 K in $CD_3OD$, exhibited 11 nonexchangeable protons. According to HSQC correlations, the 13 signals in the $^{13}C$ NMR were assigned to 4 $CH_2$ groups, 3 CH groups and 6 quaternary carbon atoms. The COSY spectrum revealed the presence of a $CH_2CH_2CH_2$ moiety, a $CH_2CH$ fragment and two aromatic protons that are also present in **2**. Moreover, the $CH_2CH$ fragment, the $CH_2CH_2CH_2$ moiety and the aromatic protons exhibited chemical shifts similar to the corresponding protons in **2**. Likewise, it was evident from the HMBC that all protons except 6-H at $\delta_H$ = 5.82 ppm and 2-H at $\delta_H$ = 7.18 ppm were coupled to carbon atoms with chemical shifts similar to those of the corresponding carbon atoms in **2** (Table 1 and Figure 2).

However, the C-6 resonance at $\delta_C$ = 87.2 ppm and the C-7 resonance at $\delta_C$ = 159.2 ppm differed considerably from the corresponding ones at $\delta_C$ = 94.9 and 180.3 ppm in **2**. The chemical shift of C-7 indicated that a nitrogen atom adjacent to the carbon atom at $\delta_C$ = 159.2 ppm replaced the corresponding carbonyl oxygen of **2**. The three-dimensional structure and the position of the diastereotopic protons of **1** were established by analysis of the $J_{H,H}$ coupling constants and by NOE measurements (Figure 2).

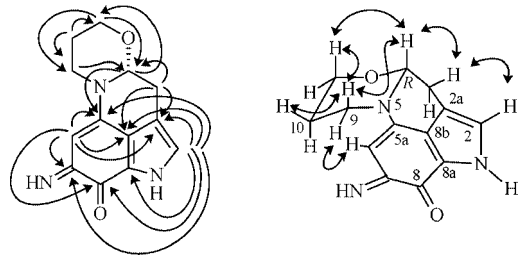


Figure 2. HMBC (→) and NOE (↔) correlations of **1**.

The presence of an *ortho* iminoquinone moiety is in agreement with the molecular formula and the corresponding chemical shifts of sanguinone A[4] and makaluvamine H,[5] which also contain an iminoquinone moiety. The CD spectra of **1** and **2** were almost identical, which established for **1** the *R* configuration at C-4. Therefore, the native main pigment haematopodin B (**1**) only differs from its degradation product haematopodin (**2**) by the presence of an iminoquinone instead of a quinone moiety.

The minor pigment mycenarubin D (**3**) exhibited absorption maxima at $\lambda$ = 242, 368 and 544 nm and similar extinction values to those of mycenarubin A (**4**), which points to the presence of a similar structure as that of **4**. The HRMS (ESI) of **3** revealed an $[M + H]^+$ ion at $m/z$ = 289.1291, which corresponds to the molecular formula $C_{14}H_{16}N_4O_3$ for **3**. Hence, in comparison to **4**, in **3** an oxygen atom is exchanged by NH. The NMR spectra exhibited similar shift values for all carbon atoms and protons and correlations in the COSY, HSQC and HMBC as those of **4** (Table 2). The only significant differences concerned 6-H, C-6 and C-7. The C-7 resonance at $\delta_C$ = 158.6 ppm indicated the presence of an iminoquinone instead of a quinone moiety. This result is in agreement with the molecular formula. The CD spectrum of **3** resembled that of **4** and established the 4*S*

configuration for **3**. Hence, as with **1** and **2**, **3** is the iminoquinone analogue of **4**. However, **3** appears to be more stable than **1**.

Table 2. $^{13}C$ NMR spectroscopic data of mycenarubins D (**3**), A (**4**), E (**5**) and F (**6**).

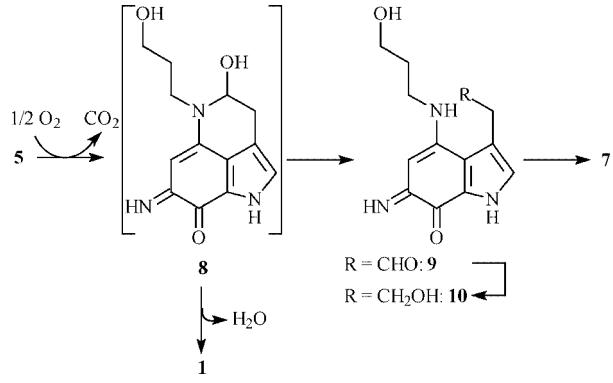
| Proton | **3** $\delta_C$[a] [ppm] | **4** $\delta_C$[a] [ppm] | **5** $\delta_C$[b] [ppm] | **6** $\delta_C$ [c] [ppm] |
|---|---|---|---|---|
| 2 | 128.8 | 127.4 | 127.2 | 125.2 |
| 2a | 117.2 | 117.5 | 117.1 | 117.0 |
| 3 | 25.5 | 25.5 | 24.0 | 25.3 |
| 4 | 67.5 | 67.1 | 66.0 | 66.7 |
| 5a | 158.6 | 157.7 | 156.7 | 157.2 |
| 6 | 87.4 | 93.9 | 86.1 | 93.3 |
| 7 | 158.6 | 180.8 | 156.6 | –[d] |
| 8 | 169.0 | 172.6 | 167.7 | –[d] |
| 8a | 124.5 | 125.5 | 123.0 | 125.5 |
| 8b | 125.8 | 126.2 | 124.3 | 126.1 |
| 9 | 176.6 | 178.0 | 175.2 | 176.2 |
| 10 | 50.6 | 49.9 | 49.3 | 49.4 |
| 11 | 27.0 | 26.7 | 30.0 | 31.4 |
| 12 | 38.3 | 38.5 | 58.5 | 59.6 |

[a] 226 MHz, $D_2O$, 300 K. [b] 151 MHz, $D_2O$, 300 K. [c] Derived from the HMBC, 151 MHz, $CD_3OD$, 280 K. [d] Signals missing due to low intensity.

The minor red alkaloid mycenarubin E (**5**) is also closely related to **4**, as it exhibited absorption maxima at $\lambda$ = 243, 368 and 548 nm and similar extinction values to those of **4**. According to the HRMS (ESI) of **5** with an $[M + H]^+$ ion at *m*/*z* = 290.1131 it has the same molecular formula $C_{14}H_{15}N_3O_4$ as **4**. The NMR spectra of **5**, however, closely resembled those of **3**, except for the chemical shifts of the carbon atom ($\delta_C$ = 58.5 ppm) and the protons ($\delta_H$ = 3.68 ppm) at C-12, which are typical for a $CH_2OH$ group. Therefore, in comparison to **3**, a hydroxy group is present instead of an amino group at C-12 in iminoquinone **5**. Again, the CD spectrum of **5** resembled that of **4**, which thus established the 4*S* configuration for **5**.

The minor alkaloid mycenarubin F (**6**) exhibited an $[M + H]^+$ ion at *m*/*z* = 291.0979 in the HRMS (ESI), which corresponds to a molecular formula of $C_{14}H_{14}N_2O_5$. Mycenarubin F eluted at nearly the same retention time as that of **2** in the preparative HPLC, which prevented the collection of pure mycenarubin F (**6**). Therefore, the NMR spectra of **6** were contaminated with haematopodin (**2**). Nevertheless, all signals of **2** and **6** exhibited different chemical shifts in the proton NMR, which enabled unambiguous assignment of **6** in the presence of **2**. In the NMR spectra of mycenarubin F (**6**) exhibited signals closely resembling those of **4** except for the chemical shifts of the carbon atom ($\delta_C$ = 59.6 ppm) and the protons ($\delta_H$ = 3.66 ppm) at C-12, which are typical for a $CH_2OH$ group (Table 2). Therefore, quinone **6** has the same structure as that of **4**, except that a hydroxy group replaces the amino group at C-12. The CD spectrum was recorded from a 3:1 mixture of **6** and **2**. Nevertheless, the data obtained point to the 4*S* configuration for **6**.

The presence of sanguinolentaquinone (**7**) in fruiting bodies of *M. haematopus* points to the hypothetical biosynthesis of haematopodin B (**1**) from **5**, as **1** and **10**, the so far unknown iminoquinone analogue of **7**, might both originate from **5**. Mycenarubin E (**5**) might be converted enzymatically to hypothetical intermediate **8** by Baeyer–Villiger-type oxidative decarboxylation.[6] Ring closure with loss of one molecule of $H_2O$ would lead to **1**, whereas ring opening of aminal **8** and subsequent reduction of aldehyde **9** would lead to **10** (Scheme 1). Mycenarubin E (**5**) itself is probably derived from tryptophan and (*S*)-adenosylmethionine.




Scheme 1. Hypothetical biosynthesis of **1** and **7**.

In comparison to the stable pyrroloquinoline alkaloid mycenarubin A (**4**), haematopodin B (**1**) was less stable. For instance, if **1** was stored for several days in methanol at 25 °C, it was partially converted into **2**. This result explains why only **2** could be isolated when the pyrroloquinoline alkaloids of *M. haematopus* were first investigated in 1993.[2] At that time, the alkaloids were separated in a time-consuming procedure by gel chromatography on a Sephadex LH-20 column by using methanol as the solvent, which led to the degradation of **1** into **2**. Even when we separated the compounds present in a crude methanol extract of *M. haematopus* immediately by HPLC, some haematopodin (**2**) was detected (Figure 3). However, it can be concluded that intact fruiting bodies either contain **2** only in traces or not at all. In contrast, haematopodin (**2**) was generated almost quantitatively from **1** (Figure 3) if the fruiting bodies of *M. haematopus* were injured mechanically by homogenisation, extracted after several hours with methanol and analysed by HPLC.

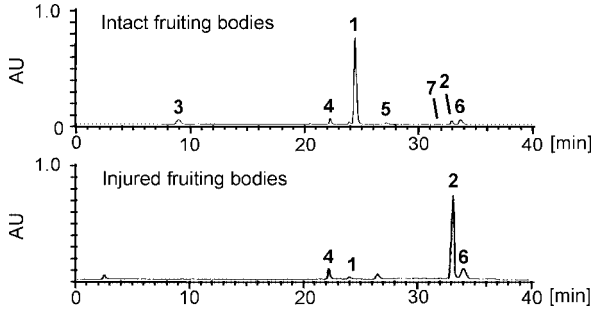
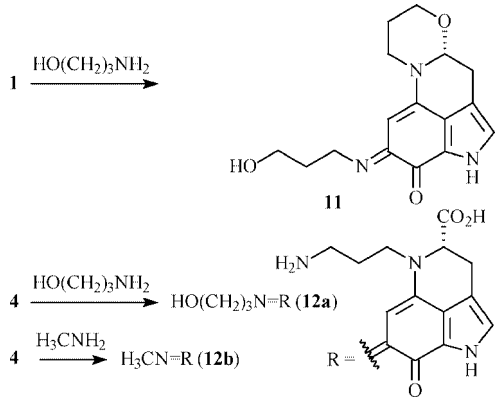



Figure 3. HPLC chromatogram (analytical HPLC, $\lambda$ = 360 nm) of a methanol extract of intact and injured fruiting bodies of *M. haematopus*.

Obviously, the iminoquinone moiety of **1** is quite reactive towards nucleophiles. Hence, **2** is generated from **1** in the presence of water. Similarly, in bacterial methylamine dehydrogenase, which uses tryptophan tryptophylquinone (TTQ) as a cofactor, the iminoquinone moiety of the cofactor is converted into the corresponding quinone moiety during the transamination of methylamine into formaldehyde.[7] Moreover, it is known that TTQ and related quinones are able to react at C-7 with primary amines to yield the corresponding imines.[7] The reaction with amines has also been used for the biomimetic synthesis of some marine pyrroloquinoline alkaloids, such as makaluvamin C.[8] Accordingly, if a tenfold excess of 3-aminopropanol was added to an aqueous solution of **1** or **4**, molecular ions corresponding to **11** or **12a**, respectively, could be detected in the MS (Scheme 2). Therefore, upon injury, the liberated pyrroloquinoline alkaloids also might play a role in the chemical defence of *M. haematopus*, as they might, for instance, react with the ε-amino group of lysine residues in proteins.




Scheme 2. Condensation products of **1** and **4** with amines.

It is also noteworthy that mycenarubins **3** and **5** are more stable towards hydrolysis of the iminoquinone moiety than **1**. Interestingly, in mycenarubins A (**4**), B[3] and F (**6**), the 6-H proton exchanges within a few hours with the NMR solvent $D_2O$, whereas 6-H is not exchanged in mycenarubins D (**3**) and E (**5**). Mycenarubins containing an iminoquinone at C-7/C-8 can therefore be distinguished from those containing a quinone moiety at C-7/C-8 by $^1H$ NMR spectroscopy.

Until recently, pyrroloquinoline alkaloids were considered to be rare in terrestrial sources,[9] whereas many representatives are known from the marine environment.[9] However, the presence of a variety of such alkaloids in *M. rosea*,[3] *M. sanguinolenta*[4] and in *M. haematopus* confirms that the occurrence of pyrroloquinoline alkaloids is not restricted to marine sources, but they also appear to be common, in the very least, in some *Mycena* species.

## Experimental Section

**General Procedures:** Evaporation of the solvents was performed under reduced pressure by using a rotary evaporator. Preparative HPLC separations were performed by using two Waters 590EF pumps equipped with an automated gradient controller 680 and a Kratos Spectroflow 783 UV/Vis detector. The samples were separated on a Luna C-18 (2), 5 μm, 15 × 250 mm column (Phenomenex) by using the following gradient program: 10 min at 99.9% $H_2O$/0.1% AcOH, then within 40 min linear to 100% MeOH; flow rate: 12 mL min$^{-1}$; detection: UV at 360 nm. Analytical HPLC separations were performed by using two Waters 510 pumps equipped with a Waters 717 autosampler and a Waters 996 photodiode array detector running under the software package Waters Millenium 2.10. The samples were separated on an RP-18 column (5 μm, 4.6 × 250 mm, Macherey–Nagel) by using the following gradient program: 10 min at 99.9% $H_2O$/0.1% AcOH, then within 40 min linear to 100% MeOH; flow rate: 1.0 mL min$^{-1}$; detection: UV at 360 nm. UV spectra were recorded with a Varian Cary 100 Bio UV/Vis spectrometer. Optical rotation values were measured with a Jasco P-1030 polarimeter. CD spectra were obtained with a Jasco J-715 spectropolarimeter. NMR spectra were recorded with a Bruker DMX 600 spectrometer equipped with a TXI cryo probe ($^1H$ at 600.13, $^{13}C$ at 150.9 MHz) and a Bruker DMX 900 spectrometer equipped with a TXI cryo probe ($^1H$ at 900.13, $^{13}C$ at 226.3 MHz). Chemical shifts were determined relative to the solvents $D_2O$ ($\delta_H$ = 4.65 ppm) or $CD_3OD$ ($\delta_H$ = 3.31, $\delta_C$ = 49.0 ppm) as internal standards. MS spectra were recorded with a Finnigan TSQ 7000 mass spectrometer, equipped with a Finnigan ESI ion source interface operating in the positive ESI mode [ionisation 4.5 kV, capillary temperature 200 °C, mass range 50–800 mu, multiplier 1000 V (scan modus), MS–MS: argon collision gas 2.0 mbar, sheath gas ($N_2$) 2.9 bar, multiplier 1400 V, collision energy automatically rotated at –20, –30, –40 eV]. HRMS (ESI) and HRMS–MS (ESI) spectra were obtained with a Thermo Scientific LTQ-Orbitrap mass spectrometer. The spectrometer was operated in positive mode (1 spectrum s$^{-1}$; mass range: 50–1000, with nominal mass resolving power of 60 000 at $m/z$ = 400 with a scan rate of 1 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation by using polydimethylcyclosiloxane {$[(CH_3)_2SiO]_6$, $m/z$ = 445.120025} as internal lock mass.

**Mushrooms:** Fruiting bodies of *M. haematopus* (leg. et det. S. Peters and P. Spiteller) were collected in September and October 2004, 2005 and 2006 in beech forests near Kelheim. Voucher samples of *M. haematopus* are deposited at the Institut für Organische Chemie und Biochemie II der Technischen University of München, Germany. The mushrooms were frozen and stored at –35 °C after collecting.

**Extraction and Isolation:** Frozen fruiting bodies (5 g) were crushed after addition of MeOH (25 mL) and extracted with MeOH (2 × 25 mL) at 25 °C for 10 min. The red-coloured extract was then concentrated in vacuo at 40 °C. The resulting residue was dissolved in $H_2O$ (5 mL), prepurified with an RP-18 cartridge and separated by preparative HPLC (UV detection at 380 nm). The pigments were obtained as red solids. The frozen fruiting bodies (100 g) of *M. haematopus* yielded haematopodin B (**1**) (33.0 mg), haematopodin (**2**) (1.5 mg), mycenarubin D (**3**) (12.5 mg), mycenarubin A (**4**) (8.7 mg), mycenarubin E (**5**) (4.9 mg), mycenarubin F (**6**) (5.6 mg) and sanguinolentaquinone (**7**) (0.5 mg).

**Haematopodin B (1):** Red solid. $HPLC_{prep}$: $R_t$ = 22.4 min. $HPLC_{anal}$: $R_t$ = 24.8 min. $[\alpha]_D^{25}$ = +490 ($c$ = 0.00101, $H_2O$). CD ($H_2O$): $\lambda$ ($\Delta\varepsilon$) = 240 (–4.5), 266 (+0.8), 316 (–0.1), 362 (–0.9), 541 (+1.7). $^1H$ NMR: see Table 1. $^{13}C$ NMR: see Table 1. UV/Vis ($H_2O$): $\lambda_{max}$ (log $\varepsilon$) = 242 (4.32), 357 (4.06), 507 (3.37) nm. MS (ESI): $m/z$ = 244 $[M + H]^+$. HRMS (ESI): calcd. for $C_{13}H_{14}N_3O_2$

$[M + H]^+$ 244.1081; found 244.1081. MS–MS (ESI, parent ion *m/z* = 244, 30 eV) *m/z* (%) = 244 (86) $[M + H]^+$, 186 (100) $[M + H – CH_2=CHCH_2OH]^+$.

**Mycenarubin D (3):** Red solid. $HPLC_{prep}$: $R_t$ = 10.0 min. $HPLC_{anal}$: $R_t$ = 9.1 min. $[\alpha]_D^{25}$ = +630 (*c* = 0.0071, $H_2O$). CD ($H_2O$): $\lambda$ ($\Delta\varepsilon$) = 241 (–5.0), 265 (+1.2), 297 (+0.2), 337 (+0.8), 386 (–3.0), 549 (+1.5). $^1H$ NMR (600 MHz, $D_2O$, 300 K): $\delta$ = 1.97 (m, 2 H, 11-H), 2.97 (m, 2 H, 12-H), 3.07 (dd, *J* = 16.6, 7.3 Hz, 1 H, 3-$H_b$), 3.19 (dd, *J* = 16.6, 1.2 Hz, 1 H, 3-$H_a$), 3.39 (ddd, *J* ≈ 14, 7, 7 Hz, 1 H, 10-$H_a$), 3.77 (ddd, *J* ≈ 14, 7, 7 Hz, 1 H, 10-$H_b$), 4.32 (dd, *J* = 7.3, 1.2 Hz, 1 H, 4-H), 5.61 (s, 1 H, 6-H), 6.97 (s, 1 H, 2-H) ppm. $^{13}C$ NMR: see Table 2. UV/Vis ($H_2O$): $\lambda_{max}$ (log $\varepsilon$) = 242 (4.06), 368 (3.96), 544 (2.85) nm. MS (ESI): *m/z* = 289 $[M + H]^+$. HRMS (ESI): calcd. for $C_{14}H_{17}N_4O_3$ $[M + H]^+$ 289.1295; found 289.1291. MS–MS (ESI, parent ion *m/z* = 289, 20 eV): *m/z* (%) = 289 (86) $[M + H]^+$, 245 (100) $[M – CO_2 + H]^+$, 232 (9), 202 (9), 188 (22) $[M + H – CO_2 – CH_2=CHCH_2NH_2]^+$, 85 (8), 58 (3).

**Mycenarubin E (5):** Red solid. $HPLC_{prep}$: $R_t$ = 25.0 min. $HPLC_{anal}$: $R_t$ = 27.2 min. $[\alpha]_D^{25}$ = +1010 (*c* = 0.0046, $H_2O$). CD ($H_2O$) $\lambda$ ($\Delta\varepsilon$) 240 (–6.4), 266 (+1.7), 297 (+0.3), 337 (+1.5), 385 (–4.4), 542 (+2.2). $^1H$ NMR (600 MHz, $CD_3OD$, 300 K): $\delta$ = 1.96 (dm, *J* ≈ 13 Hz, 1 H, 11-H), 2.01 (dm, *J* ≈ 13 Hz, 1 H, 11-H), 3.20 (dd, *J* = 16.5, 7.5 Hz, 1 H, 3-$H_b$), 3.51 (d, *J* = 16.5 Hz, 1 H, 3-$H_a$), 3.52 (dm, *J* ≈ 14 Hz, 1 H, 10-$H_a$), 3.68 (m, 2 H, 12-H), 4.07 (ddd, *J* ≈ 14, 7, 7 Hz, 1 H, 10-$H_b$), 4.44 (d, *J* = 7.5 Hz, 1 H, 4-H), 5.88 (s, 1 H, 6-H), 7.08 (s, 1 H, 2-H) ppm. $^1H$ NMR (600 MHz, $D_2O$, 300 K): $\delta$ = 1.87 (m, 2 H, 11-H), 3.13 (dd, *J* = 16.8, 7.4 Hz, 1 H, 3-$H_b$), 3.21 (dd, *J* = 16.8, 1.3 Hz, 1 H, 3-$H_a$), 3.34 (ddd, *J* ≈ 14, 7, 7 Hz, 1 H, 10-$H_a$), 3.56 (m, 2 H, 12-H), 3.88 (ddd, *J* ≈ 14, 7, 7 Hz, 1 H, 10-$H_b$), 4.35 (dd, *J* = 7.4, 1.3 Hz, 1 H, 4-H), 5.74 (s, 1 H, 6-H), 7.02 (s, 1 H, 2-H) ppm. $^{13}C$ NMR: see Table 2. UV/Vis ($H_2O$): $\lambda_{max}$ (log $\varepsilon$) = 243 (4.04), 368 (3.94), 548 (2.86) nm. MS (ESI): *m/z* (%) = 579 (15) $[2M + H]^+$, 290 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{14}H_{16}N_3O_4$ $[M + H]^+$ 290.1135; found 290.1131. MS–MS (ESI, parent ion *m/z* = 290, 40 eV): *m/z* (%) = 290 (4) $[M + H]^+$, 244 (57) $[M – HCO_2H + H]^+$, 201 (51), 186 (100) $[M – HCO_2H – CH_2=CHCH_2OH + H]^+$. HRMS–MS (ESI, parent ion *m/z* = 290): *m/z* = 244.1090 $[M – HCO_2H + H]^+$, 186.0661 $[M – HCO_2H – CH_2=CHCH_2OH + H]^+$.

**Mycenarubin F (6):** Red solid. $HPLC_{prep}$: $R_t$ = 32.6 min. $HPLC_{anal}$: $R_t$ = 33.8 min. CD ($H_2O$): $\lambda$ ($\Delta\varepsilon$) = 242 (–1.3), 264 (+0.5), 274 (+0.5), 303 (+0.8), 364 (–1.7), 540 (+0.7) nm. $^1H$ NMR (600 MHz, $CD_3OD$, 280 K): $\delta$ = 1.91 (dm, *J* ≈ 13 Hz, 1 H, 11-H), 1.96 (dm, *J* ≈ 13 Hz, 1 H, 11-H), 3.15 (dd, *J* = 15.6, 7.8 Hz, 1 H, 3-$H_b$), 3.33 (dm, *J* ≈ 14 Hz, 1 H, 10-$H_a$), 3.40 (d, *J* = 15.6 Hz, 1 H, 3-$H_a$), 3.66 (m, 2 H, 12-H), 3.88 (dm, *J* ≈ 14 Hz, 1 H, 10-$H_b$), 4.27 (d, *J* = 7.8 Hz, 1 H, 4-H), 5.48 (s, 1 H, 6-H), 6.95 (s, 1 H, 2-H) ppm. $^{13}C$ NMR: see Table 2. MS (ESI): *m/z* = 291 $[M + H]^+$. HRMS (ESI): calcd. for $C_{14}H_{15}N_2O_5$ $[M + H]^+$ 291.0976; found 291.0978. MS–MS (ESI, parent ion *m/z* = 291, 45 eV): *m/z* (%) = 291 (4) $[M + H]^+$, 245 (17) $[M – HCO_2H + H]^+$, 202 (42), 187 (100) $[M – HCO_2H – CH_2=CHCH_2OH + H]^+$. HRMS–MS (ESI, parent ion *m/z* = 291): *m/z* = 245.0921 $[M – HCO_2H + H]$.

**Generation of 11, 12a and 12b:** Haematopodin B (**1**, 0.5 mg) or mycenarubin A (**4**, 0.5 mg) was dissolved in degassed $H_2O$ (1 mL) and an aqueous solution of 3-aminopropanol (1%, 10 equiv.) or an aqueous solution of methylamine (1%, 10 equiv.) was added, and the solution was stirred under an argon atmosphere for 2 h. Then, the solvent was removed, and the samples were subjected to analysis by MS (ESI). According to the MS, **1** and **4** were converted quantitatively into **11**, **12a** and **12b**. Data for **11**: MS (ESI): *m/z* = 302 $[M + H]^+$. Data for **12a**: MS (ESI): *m/z* = 347 $[M + H]^+$. Data for **12b**: MS (ESI): *m/z* = 303 $[M + H]^+$.

**Supporting Information** (see footnote on the first page of this article): UV/Vis, CD, NMR and mass spectra of **1**, **3**, **5** and **6**.

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[1] G. Robich, *Mycena d'Europa*, Associazione Micologica Bresadola, Trento, **2003**, pp. 457–462.
[2] a) C. Baumann, M. Bröckelmann, B. Fugmann, B. Steffan, W. Steglich, W. S. Sheldrick, *Angew. Chem.* **1993**, *105*, 1120–1121; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1087–1089; b) C. Hopmann, W. Steglich, *Liebigs Ann.* **1996**, 1117–1120.
[3] S. Peters, P. Spiteller, *Eur. J. Org. Chem.* **2007**, 1571–1576.
[4] S. Peters, P. Spiteller, *J. Nat. Prod.* **2007**, *70*, 1274–1277.
[5] E. W. Schmidt, M. K. Harper, D. J. Faulkner, *J. Nat. Prod.* **1995**, *58*, 1861–1867.
[6] a) L. Prado, E. Fernández, U. Weißbach, G. Blanco, L. M. Quirós, A. F. Braña, C. Méndez, J. Rohr, J. A. Salas, *Chem. Biol.* **1999**, *6*, 19–30; b) F. Pojer, R. Kahlich, B. Kammerer, S.-M. Li, L. Heide, *J. Biol. Chem.* **2003**, *278*, 30661–30668.
[7] S. Itoh, N. Takada, S. Haranou, T. Ando, M. Komatsu, Y. Ohshiro, S. Fukuzumi, *J. Org. Chem.* **1996**, *61*, 8967–8974.
[8] S. Nishiyama, J.-F. Cheng, X. L. Tao, S. Yamamura, *Tetrahedron Lett.* **1991**, *32*, 4151–4154.
[9] E. M. Antunes, B. R. Copp, M. T. Davies-Coleman, T. Samaai, *Nat. Prod. Rep.* **2005**, *22*, 62–72.

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