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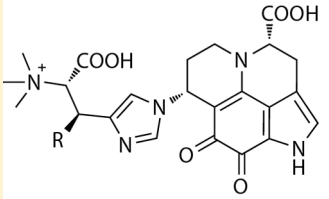
Pelianthinarubins A and B, Red Pyrroloquinoline Alkaloids from the Fruiting Bodies of the Mushroom *Mycena pelianthina*

Anna Pulte, Silke Wagner, Herbert Kogler, and Peter Spiteller*

Institut für Organische und Analytische Chemie, Universität Bremen, Leobener Straße NW2C, D-28359 Bremen, Germany

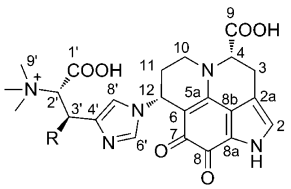
Supporting Information

ABSTRACT: Pelianthinarubin A (**1**) and pelianthinarubin B (**2**), two previously unknown pyrroloquinoline alkaloids, have been isolated from fruiting bodies of *Mycena pelianthina*. The structures of these alkaloids have been deduced from their HR-(+)-ESIMS and 2D NMR data. The absolute configurations of the pelianthinarubins A (**1**) and B (**2**) were assigned by analysis of the NOE correlations and coupling constants and by comparison of the CD spectra of **1** and **2** and of hercynine obtained by degradation of **1** with suitable compounds of known absolute configuration. The pelianthinarubins A (**1**) and B (**2**), which contain an *S*-hercynine moiety, differ considerably from the known pyrroloquinoline alkaloids from marine organisms and other *Mycena* species, such as the mycenarubins, the haematopodins, and the sanguinones.



1: Pelianthinarubin A: R = H
2: Pelianthinarubin B: R = OH

Mycena pelianthina (Fr.) Quél., the black-edged bonnet (German name: Schwarzgezähnelter Rettichhelmling), is a relatively small mushroom possessing a purplish-colored cap and a typical radish-like odor. It is widely distributed in hardwood or mixed hardwood−conifer forests in Europe and Northern America.[1,2] Its common name is derived from its dark-edged gills. So far there are no reports on the chemical structures of the pigments of *M. pelianthina*. A comparative metabolic profiling of fruiting bodies of *M. pelianthina* by HPLC-UV with those of *Mycena rosea*,[3] *Mycena sanguinolenta*,[4] and *Mycena haematopus*[5] revealed the presence of mycenarubin A (**3**) and mycenarubin D (**4**) together with previously unknown pyrroloquinoline alkaloids. In this article we describe the isolation and structural elucidation of two new red pyrroloquinoline alkaloids, which we have named pelianthinarubins A (**1**) and B (**2**) and the identification of **3** and **4** in *M. pelianthina*.



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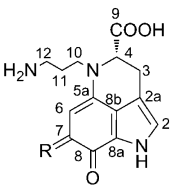

3: Mycenarubin A: R= O
4: Mycenarubin D: R= NH

RESULTS AND DISCUSSION

The pelianthinarubins A (**1**) and B (**2**) as well as the mycenarubins A (**3**) and D (**4**) were extracted from frozen fruiting bodies of *M. pelianthina* with MeOH at 25 °C. The alkaloids were purified by HPLC, using an RP-18ec column for the initial and an RP-8 column for the final purification step. A 100 g amount of frozen fruiting bodies yielded 1.7 mg of pelianthinarubin A (**1**), 0.5 mg of pelianthinarubin B (**2**), 2.5 mg of mycenarubin A (**3**), and 12.1 mg of mycenarubin D (**4**).

The UV/vis spectra of the two red pelianthinarubins A (**1**) and B (**2**) are almost identical. Pelianthinarubin A (**1**) shows four absorption maxima at λ 243, 281, 358, and 517 nm, while **2** exhibits absorption maxima at λ 243, 285, 355, and 513 nm. The UV/vis spectra of **1** and **2** are closely related to the UV/vis spectra of mycenarubin A (**3**),[3] suggesting the presence of a pyrroloquinoline core structure.

The HR-(+)-ESIMS spectrum of pelianthinarubin A (**1**) exhibits an $[M]^+$ ion at m/z 468.1879, corresponding to the molecular formula $C_{23}H_{26}N_5O_6^+$ (calculated: m/z 468.1878). The odd number of nitrogen atoms in combination with an even molecular mass indicates that **1** is a permanent cationic compound. When compared with **3**, pelianthinarubin A (**1**) contains an additional $C_9H_{11}N_2O_2$ fragment.

The ^{1}H NMR spectrum, recorded at 283 K in D_2O, exhibits 23 nonexchangeable protons in total. Consequently, **1** contains three exchangeable protons. The HSQC spectrum shows signals accounting for three equivalent CH_3, four CH_2, and six CH groups. Thus, **1** must contain 10 carbon atoms with no hydrogens attached. The COSY spectrum in combination with the ^{1}H NMR spectrum reveals the presence of an X-Me_3 group, two CH_2CH fragments, a CH_2CH_2CH fragment, and three aromatic CH singlets. In the COSY spectrum, two of the aromatic CH groups exhibit weak couplings to each other, while the third CH group at δ_H 6.94 ppm is isolated. In the

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Table 1. NMR Spectroscopic Data of Pelianthinarubin A (1) (600 MHz, D_2O, 283 K), Pelianthinarubin B (2) (700 MHz, D_2O, 283 K), and Mycenarubin A (3) (900 MHz, D_2O, 300 K)

	pelianthinarubin A (1)		pelianthinarubin B (2)		mycenarubin A (3)[3]	
no.	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C[a]	δ_H[a]
2	128.6, CH	6.94 (s)	128.7, CH	7.04 (s)	128.7, CH	6.95
2a	118.4, qC		118.6, qC		118.8, qC	
3	26.7, CH_2	3.17[b] (m)	26.7, CH_2	3.24 (m)	26.8, CH_2	3.20 (H_{eq}) 3.15 (H_{ax})
4	68.6, CH	4.15 (m)	68.7, CH	4.23 (m)	68.4, CH	4.25
5a	156.6, qC		156.9, qC		159.0, qC	
6	101.0, qC		101.3, qC		95.2, CH	5.36
7	178.1, qC		178.3, qC		182.1, qC	
8	173.5, qC		173.7, qC		173.9, qC	
8a	127.0, qC		127.2, qC		126.8, qC	
8b	126.0, qC		126.2, qC		127.5, qC	
9	178.8, qC		179.0, qC		179.3, qC	
10	46.3, CH_2	3.32 (dm, 14.2, H_{eq}) 3.18[b] (ddm, 16.0, 14.2, H_{ax})	46.4, CH_2	3.40 (dm, 14.1, H_{eq}) 3.29[b] (m, H_{ax})	51.2, CH_2	3.75 (H_b) 3.32 (H_a)
11	31.3, CH_2	2.27 (ddm, 16.0, 13.6, H_{ax}) 2.13 (dm, 13.6, H_{eq})	31.5, CH_2	2.35 (ddm, 14.7, 13.9, H_{ax}) 2.21 (dm, 13.9, H_{eq})	28.0, CH_2	2.06
12	49.5, CH	5.18 (m)	49.5, CH	5.30 (m)	39.8, CH_2	3.11
1′	173.8, qC		172.3, qC			
2′	81.1, CH	3.80 (dm, 11.2)	83.8, CH	4.05 (d, 10.3)		
3′	28.5, CH_2	3.04 (m) 3.12[b] (dd, 13.4, 11.2)	68.8, CH	5.19 (d, 10.3)		
4′	136.5, qC		140.6, qC			
6′	139.8, CH	7.70 (s)[c]	140.3, CH	7.79 (s)[c]		
8′	119.9 CH	7.12 (s)[c]	120.1, CH	7.34 (s)[c]		
9′	54.5, 3 × CH_3	3.15 (s)	56.1, 3 × CH_3	3.34 (s)		

[a]NMR chemical shifts were adjusted to HDO δ = 4.75 at 300 K,[6] and ^{13}C values have been recalibrated using IUPAC recommendations,[7] which changed the chemical shifts by 1.3 ppm low field. [b]The chemical shift value was obtained from the HSQC spectrum. [c]Weak correlations are present in the COSY spectrum between H-6′ and H-8′. Chemical shifts have been checked using N. Haider, W. Robien; http.://nmrpredict.orc.univie.ac.at/c13robot/robot.php.

HSQC spectrum this aromatic proton is directly connected with the carbon resonance at δ_C 128.6 ppm; this ^{13}C chemical shift resembles that of C-2 in compound **3** (Table 1). Moreover, the proton at δ_H 6.94 ppm exhibits HMBC correlations to three aromatic carbons (Figure 2), whose

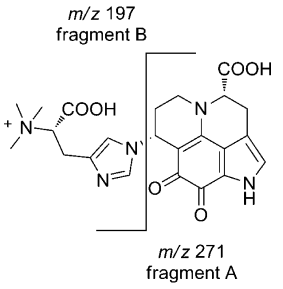

Figure 1. Fragments A and B of pelianthinarubin A (**1**).

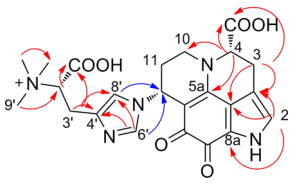

Figure 2. Pelianthinarubin A (**1**) with selected HMBC correlations (red arrows). Additional HMBC correlations displayed by pelianthinarubin B (**2**) are shown in blue.

chemical shifts are similar to those of C-2a, C-8a, and C-8b of **3** (Table 1). Likewise, the CH_2CH fragment with δ_H 3.17 (δ_C 26.7) and 4.15 (δ_C 65.5) ppm in **1** exhibits similar chemical shift values compared to those of the CH_2CH fragment consisting of C-3 and C-4 of mycenarubin A (**3**). The HMBC correlations of H-3 and H-4 of **1** (Figure 2) resemble those of the corresponding protons in **3**. Therefore, δ_C 156.6 ppm was assigned to C-5a, δ_C 46.3 ppm to C-10, and δ_C 178.8 ppm to C-9, indicating that **1** contains the same pyrroloquinoline core structure as mycenarubin A (**3**). Instead of a $CH_2CH_2CH_2$ moiety as in **3**, pelianthinarubin A (**1**) contains a CH_2CH_2CH fragment. According to the HMBC correlation from H-4 to C-10, this residue (δ_C 46.3 (C-10); δ_C 31.3 (C-11); δ_C 49.5 (C-12)) is attached via the CH_2 group (C-10) to the nitrogen N-5 (Figure 2). Consequently, C-12 is attached to C-11 and two additional substituents. Its chemical shift (δ_C 49.5 ppm) is approximately 10 ppm downfield-shifted when compared with C-12 of **3**, indicating that the two other substituents of C-12 are a nitrogen, as evident in comparison with **3**, and a quaternary carbon. By comparing the carbon chemical shifts of **1** with **3**, δ_C 101.0 ppm in **1** was assigned to C-6, δ_C 178.1 ppm to C-7, and δ_C 173.5 ppm to C-8. In contrast to C-6 in **3**, C-6 in **1** has no hydrogen attached to it, indicating that pelianthinarubin A (**1**) carries one additional substituent at C-6. Due to the presence of a characteristic fragment ion at m/z 271.0711 in the HR-(+)-ESIMS/MS, the CH_2CH_2CH residue is connected via C-12 to the carbon at C-6, thus forming an additional ring, when compared to mycenarubin A (**3**). Consequently, pelianthinar-

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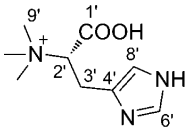
ubin A (**1**) consists of partial structure A and a fragment B (Figure 1) attached at C-12.

According to the HR-(+)-ESIMS/MS data, fragment B is equivalent to $C_9H_{15}N_3O_2$. By analysis of the ^{1}H NMR and the COSY spectrum the hydrogens of fragment B are assigned to the three chemically equivalent CH_3 groups (δ_H 3.15, δ_C 54.5 ppm), a CH_2CH fragment (δ_H 3.04 and 3.12, δ_C 28.5; δ_H 3.80, δ_C 81.1 ppm), two different aromatic CH groups (δ_H 7.12, δ_C 119.9 and δ_H 7.70, δ_C 139.8 ppm), and one exchangeable proton. The chemical shifts of the CH_3 groups (δ_H 3.15, δ_C 54.5 ppm) and an HMBC correlation from the H-9′ protons to C-9′ (Figure 2) indicate the presence of a trimethylammonium moiety, explaining the cationic nature of **1**. This residue is attached to the CH of the CH_2CH fragment on account of a $^3J_{CH}$ correlation from H-9′ to C-2′ (δ_C 81.1 ppm). H-2′ (δ_H 3.80 ppm) shows a characteristic HMBC correlation to the carbon atom C-1′ (δ_C 173.8 ppm), which is, due to its chemical shift, part of a carboxyl moiety. Likewise, the diastereotopic protons H-3′ (δ_H 3.04 and 3.12, δ_C 28.5 ppm) show a correlation to C-1′. Moreover, HMBC correlations from H-3′ to the carbon C-4′ (δ_C 136.5 ppm) and to C-8′ (δ_H 7.12, δ_C 119.9 ppm) indicate that C-3′ is connected via C-4′ to an aromatic moiety (Figure 2). Within the COSY spectrum H-8′ displays a weak coupling to H-6′ (δ_H 7.70, δ_C 139.8 ppm). Since all but two nitrogen atoms are now assigned, C-6′, C-8′, and C-4′ must be part of an imidazole residue. Hence, fragment B is hercynine (**5**), a common natural product.



5: *S*-Hercynine

In the ROESY data correlations are observed between H-12 (δ_H 5.18 ppm) and H-6′ (δ_H 7.70 ppm) as well as between H-12 and H-8′ (δ_H 7.12 ppm), which suggest a bond between N-7′ and C-12, leading to the constitution formula of pelianthinarubin A (**1**) (Figure 4).

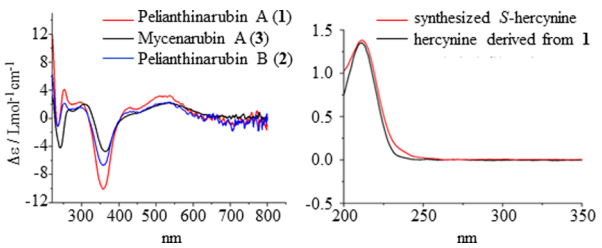


Figure 3. CD spectra in H_2O of **1**, **2**, and **3** as well as *S*-hercynine and hercynine derived from **1**.

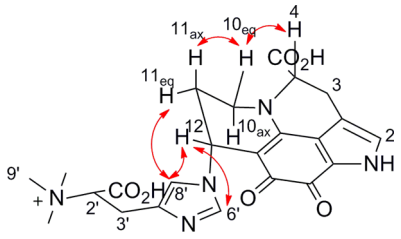


Figure 4. Pelianthinarubin A (**1**) with selected ROESY correlations.

The absolute configuration of pelianthinarubin A (**1**) with its three stereocenters (at positions 4, 12, and 2′) was established by CD spectroscopy, NOE measurements, and analysis of the coupling constants in the ^{1}H NMR data. In order to determine the stereochemistry at position 2′, the hercynine moiety of pelianthinarubin A (**1**) was cleaved off under mild acidic conditions and the resulting hercynine (**5**) was purified via HPLC. Then, the CD spectrum of hercynine derived from the natural product **1** was recorded and compared to the CD spectra of freshly synthesized *R*- and *S*-hercynine,[8] revealing the stereocenter at position 2′ to be *S* configured (Figure 3). To assign the absolute configuration at position 4, the CD spectrum of pelianthinarubin A (**1**) was compared with that of mycenarubin A (**3**), which is known to be *S* configured at C-4.[3] Since the CD spectra of **1** and **3** exhibit nearly the same Cotton effects, the absolute configuration of C-4 of pelianthinarubin A (**1**) is also *S* (Figure 3).

The absolute configuration of the stereocenter at C-12 was deduced indirectly from the spatial orientation of H-4 and H-12 to each other. The orientation was assigned by analysis of the NOEs and coupling constants of the protons H-4, H-10_{ax}, H-10_{eq}, H-11_{ax}, H-11_{eq}, H-12, H-6′, and H-8′. The coupling constants in the ^{1}H NMR spectrum of **1** are not well-resolved, but coupling constants larger than 10 Hz are visible. H-12 does not exhibit any large coupling constants, while H-11_{ax} displays two and H-11_{eq} and H-10_{eq} one large coupling constant. For pelianthinarubin A (**1**) H-10_{ax} is overlapping with other resonances. However, the phase-sensitive COSY recorded for pelianthinarubin B (**2**), which has the same core structure as pelianthinarubin A (**1**), delivers more information. The cross-peak of H-10_{ax} to H-11_{ax} exhibits one large active coupling constant of at least 10 Hz, indicating a stable and preferred conformation of this six-membered ring with bis-axially orientated protons (pseudochair; see Figure 4). H-12 is equatorially oriented because this resonance is lacking large coupling constants and is correlated via NOEs to both protons at C-11 (δ_H 2.13, 2.27 ppm). The proton H-11 at δ_H 2.27 ppm exhibits two large coupling constants, while the proton H-11 at δ_H 2.13 ppm exhibits only one large coupling constant. Consequently, the proton H-11 at δ_H 2.27 ppm is axially oriented, and the proton H-11 at δ_H 2.13 ppm is in the equatorial position. The NOE correlation between H-11_{ax} at δ_H 2.27 ppm and H-10_{eq} at δ_H 3.32 ppm and the absence of a NOE between H-11_{ax} and H-10_{ax} at δ_H 3.18 ppm support the axial/equatorial assignment based on the coupling constants. This assignment is in agreement with the NOE correlations between H-11_{eq} and both protons at H-10. Moreover, H-4 is located in the axial position; this is supported by the presence of a strong NOE correlation between H-4_{ax} and H-10_{eq} and the absence of an NOE correlation between H-4_{ax} and H-10_{ax} (Figure 4), thus leading to the 4*S*, 12*R*, 2′*S* configuration for **1**.

The HR-(+)-ESIMS spectrum of pelianthinarubin B (**2**) shows an $[M]^+$ ion at *m*/*z* 484.1827 corresponding to the molecular formula $C_{23}H_{26}N_5O_7^+$ (calculated: *m*/*z* 484.1827), differing from that of **1** only by the presence of one additional oxygen atom. Comparing the ^{1}H NMR spectra of **1** and **2**, recorded in D_2O, the spectrum of **2** shows only 22 nonexchangeable protons—one less than the spectrum of **1**—and a higher chemical shift value for H-3′ with an integral of 1 instead of 2 (Table 1). Hence, pelianthinarubin B (**2**) contains an additional OH group at C-3′ in comparison with **1**.

Pelianthinarubin B (**2**) carries one additional stereocenter at C-3′. In order to deduce its stereochemistry, the spatial

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orientation of H-2′ and H-3′ to each other was analyzed by ROESY experiments and determination of the coupling constants in the ^{1}H NMR. Since H-2′ (δ_H 4.05 ppm) and H-3′ (δ_H 5.19 ppm) are lacking an NOE to each other but display a large coupling constant ^3J(H-2′, H-3′) of 10.3 Hz, these protons are antiperiplanar to each other. The nine protons of the NMe_3 group and H-8′ of the imidazole ring both show NOEs to H-2′ and H-3′ but not to each other. Therefore, these two residues are located opposite to each other (see Figure 5).

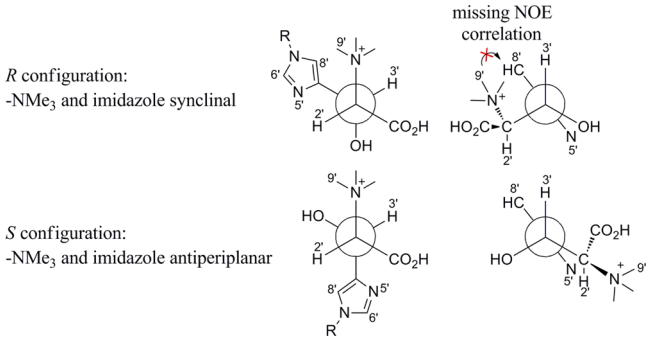

Figure 5. Newman projections of the hydroxyhercynine moiety.

Since the stereocenter at C-2′ is *S* configured, C-3′ also has to be *S* configured. This is in agreement with the stereochemistry of *β*-hydroxyergothioneine, which is closely related to *β*-hydroxyhercynine, exibiting similar chemical shifts and coupling constants in the proton NMR.[9]

Until some years ago pyrroloquinoline alkaloids have been considered to be typical marine alkaloids, since nearly all known representatives, for instance the batzellines,[10] damirones,[11] isobatzellines,[12] makaluvamines,[13] and discorhabdins,[14] were isolated from marine sources. However, from 2006 to 2008 our group was able to isolate a considerable number of new pyrroloquinoline alkaloids from fruiting bodies of terrestrial *Mycena* species, such as *M. rosea*,[3] *M. haematopus*,[5] and *M. sanguinolenta*.[4]

In contrast to the pyrroloquinoline alkaloids isolated from marine sources, the pyrroloquinoline alkaloids from *Mycena* species often feature a carboxylic group at C-4.[3−5] The presence of this carboxylic group fits well to the hypothetical biosynthesis of **1** and **2** from L-tryptophan, *S*-adenosylmethionine, and histidine.

Pyrroloquinoline alkaloids have attracted considerable interest, since pyrroloquinoline alkaloids containing a *para*-iminoquinone moiety often exhibit wide-ranging bioactivities. For instance, the discorhabdins and isobatzellines display strong activity toward a large panel of tumor cell lines.[12,15] Pelianthinarubins A (**1**) and B (**2**) were not active against *Escherichia coli*, *Bacillus brevis*, *Bacillus subtilis*, or *Cladosporium cucumerinum*. Although **1** and **2** possess no activity toward the springtail *Folsomia candida* or herbicidal activity toward garden cress (*Lepidium sativum*), they might play an ecological role in the chemical defense[16,17] of *M. pelianthina*.

EXPERIMENTAL SECTION

General Experimental Procedures. Evaporation of the organic solvents was performed under reduced pressure using a rotary evaporator or in the case of H_2O by freezing the samples and lyophilizing them. Preparative HPLC was performed on Waters 590EF pumps equipped with an automated gradient controller 680 and a Knauer UV/vis detector. Extracts from *M. pelianthina* were separated first on a C_{18} ec column (Nucleodur, 100 Å, 5 μm, 21 × 250 mm, Machery-Nagel) with the following gradient: Within 45 min linear from 100% H_2O (+ 0.01% HOAc) to MeOH−H_2O (75:25), then within 5 min linear to 100% MeOH; flow rate 12 mL/min; UV detection at 360 nm. A second separation step was performed on a C_8 column (Nucleosil, 100 Å, 8 × 250 mm) with the following gradient: Starting from MeOH−H_2O (5:95) linear to MeOH−H_2O (75:25) within 45 min, then to 100% MeOH within 5 min (flow rate 4 mL/min; UV detection at 360 nm). Hercynine samples were purified using a C_{18} ec column (Nucleodur, 100 Å, 5 μm, 21 × 250 mm (Machery-Nagel)) and the following isocratic separation conditions: 100% H_2O (+ 0.2% HOAc); flow rate 12 mL/min; UV detection at 220 nm; UV: Thermo Scientific Genesys 10S UV/vis spectrometer operated by the software VISIONlite; CD: J-715 spectropolarimeter (Jasco); NMR: Bruker Avance DRX-600 (^{1}H at 600.22, ^{13}C at 150.91 MHz), Bruker Avance II spectrometer equipped with a TXI cryo probe (^{1}H at 700.20, ^{13}C at 176.08 MHz), Bruker Avance III spectrometer equipped with a TCI cryo probe (^{1}H at 500.30, ^{13}C at 125.81 MHz). ^{1}H chemical shifts were calibrated using the chemical shift of the HDO solvent signal corrected for the well-known temperature dependency of water[6] (δ_H(HDO) = 4.94 ppm at 283 K). The ^{13}C chemical shifts were calibrated using the IUPAC recommendations[7] using the conversion factor of 0.251 449 53 for calculating the absolute frequency of ^{13}C (0.0 ppm). LC-ESIMS spectra were obtained on an LCQ DecaXP Plus ESIMS spectrometer (Thermo Fisher Scientific Inc., USA). The spectrometer was operated in positive mode (0.625 spectrum s^{-1}; mass range 50−1000). Nitrogen was used as sheath gas (80 arbitrary units) and helium served as collision gas. The spectrometer was equipped with a Hewlett-Packard HPLC system (Series 1100) consisting of degasser, pump, DAD detector, and autosampler (injection volume 5 μL). The separations were performed with a C_{18} ec column (Nucleodur, 100 Å, 5 μm, 3 × 250 mm, Machery-Nagel) using the following gradient program: Within the first 45 min linear from 100% H_2O (+ 0.01% HOAc) to MeOH−H_2O (75:25), within the next 5 min linear to 100% MeOH, flow rate 0.66 mL/min. HR-ESIMS spectra were obtained on an LTQ Orbitrap ESIMS spectrometer (Thermo Fisher Scientific Inc., USA). The spectrometer was operated in the positive mode (1 spectrum s^{-1}; mass range 50−1000) with a 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 using polydimethylcyclosiloxane ($[(CH_3)_2SiO]_6$, m/z 445.120025) as internal lock mass. Nitrogen was used as sheath gas (6 arbitrary units), and helium served as collision gas.

Mushrooms. Fruiting bodies of *M. pelianthina* (Fr.) Quél. (leg. et det. P. Spiteller) were collected in September and October of 2004 to 2014 in beech forests near Mühltal or Wellenburg in Bavaria, Germany, and immediately frozen and stored at −35 °C after their collection. Voucher samples of *M. pelianthina* are deposited at the Institut für Organische und Analytische Chemie, Universität Bremen, Germany.

Biological Tests. For plate diffusion assays 0.1 mg (0.21 μmol) or 0.5 mg (1.07 μmol) of **1** (respectively 0.1 mg (0.21 μmol) or 0.5 mg (1.03 μmol) of **2**) was dissolved in distilled water and dropped onto paper discs (Φ 6 mm, thickness 0.5 mm), which then were dried under sterile conditions. The dry paper discs were placed on agar plates inoculated with the test organism (*Escherichia coli*, *Bacillus brevis*, *Bacillus subtilis*, and *Cladosporium cucumerinum*). The plates were incubated at 37 °C for 24 h (bacteria) and at 18 °C for 48 h (*C. cucumerinum*). In order to test herbicidal acitivity 1 mg (2.13 μmol) of **1** (respectively 0.5 mg (1.03 μmol) of **2**) was dissolved in 1 mL of distilled and sterilized water, and the solution was used to drench a cotton ball (with a weight of approximately 0.4 g) within a sterile glass container with a lid. Then, 10 *Lepidium sativum* seeds were placed on the cotton ball, and their growth at 25 °C was monitored for the next 7 days in comparison to 10 *L. sativum* seeds growing under the same conditions but on a cotton ball drenchend only in water. After 7 days the weight and length of the seedlings were measured. For activity tests against springtails (Collembola) 0.5 mg (1.07 μmol) of **1** (respectively 0.3 mg (0.62 μmol) of **2**) was dissolved in 250 μL of

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distilled and sterilized water, and this solution was transferred in a sterile glass container with a lid. Then, seven specimens of *Folsomia candida* were placed in this container, also. After 5 days at 25 °C their survival rate was compared to seven specimens being in a container with only water.

Extraction and Isolation. Frozen fruiting bodies (100 g) were extracted with 500 mL of MeOH for 10 min at 200 rpm and 25 °C. The solvent was evaporated, and the resulting residue dissolved in 7 mL of H_2O. After centrifugation at 15 000 rpm and 25 °C for 5 min, the supernatant was prepurified with an RP18ec cartridge by using first H_2O and then H_2O−MeOH (50/50 v/v) as eluent to obtain two fractions. The H_2O−MeOH fraction contained **1** and **2** and was further purified by two subsequent preparative HPLC separations first on RP18ec and then on an RP8 column. A 100 g amount of fruiting bodies yielded 1.7 mg of pelianthinarubin A (**1**) and 0.5 mg of pelianthinarubin B (**2**).

*Pelianthinarubin A (**1**):* red solid; $HPLC_{prep}$, t_R = 14.8 min (step 1), 12.5 min (step 2); LC-(+)-ESIMS t_R = 14.9 min; UV/vis (H_2O) λ_{max} 245, 357, 529; CD (H_2O) λ ($\Delta\varepsilon$) 235 (−1.1), 252 (+4.2), 272 (+2.0), 295 (+2.3), 357 (−10.0), 429 (+1.6), 518 (+3.1) nm; ^{1}H NMR (600 MHz, D_2O, 283 K, pH 9.0), see Table 1; ^{13}C NMR (151 MHz, D_2O, 283 K, pH 9.0), see Table 1; HR-(+)-ESIMS *m/z* 490.1697 (55) $[M - H^+ + Na^+]^+$, 468.1879 (100) $[M]^+$ (calculated for $C_{23}H_{26}O_6N_5^+$, 468.1878), 424.1981 (78) $[M - CO_2H]^+$; HR-(+)-ESIMS/MS (parent ion *m/z* 468.1879, 35 eV) *m/z* 424.1970 (28) $[M - CO_2H]^+$, 271.0711 (100) [M − hercynine]$^+$.

*Pelianthinarubin B (**2**):* red solid; HPLC, t_R = 15.5 min (step 1), 10.8 min (step 2); LC-(+)-ESIMS t_R = 15.4 min; UV/vis (H_2O) λ_{max} 245, 357, 527; CD (H_2O) λ ($\Delta\varepsilon$) 234 (−1.1), 251 (+2.1), 271 (+1.3), 298 (+1.6), 355 (−6.6), 429 (+1.0), 514 (+2.2) nm; ^{1}H NMR (700 MHz, D_2O, 283 K), see Table 1; ^{13}C NMR (126 MHz, D_2O, 280 K), see Table 1; HR-(+)-ESIMS *m/z* 506.1647 (66) $[M - H^+ + Na^+]^+$, 484.1827 (100) $[M]^+$ (calculated for $C_{23}H_{26}O_7N_5^+$, 484.1827), 440.1929 (62) $[M - CO_2H]^+$; HR-(+)-ESIMS/MS (parent ion *m/z* 440.1929, 35 eV) *m/z* 440.1919 (100) $[M - CO_2H]^+$, 271.0710 (100) [M − hydroxyhercynine]$^+$.

*Mycenarubin A (**3**):* red solid; LC-(+)-ESIMS t_R = 11.1 min; *m/z* 290 (100) $[M + H]^+$; (+)-ESIMS/MS (parent ion *m/z* 290, CID collision energy of 30%) *m/z* 246 (100) $[M + H - CO_2]^+$, 217 (9) $[M + H - CO_2 - (CH_2{=}NH)]^+$, 203 (17) $[M + H - CO_2 - (C_2H_5N)]^+$, 189 (5) $[M + H - CO_2 - (C_3H_7N)]^+$.

*Mycenarubin D (**4**):* red solid; LC-(+)-ESIMS t_R = 5.1 min; *m/z* 289 (100) $[M + H]^+$; (+)-ESIMS/MS (parent ion *m/z* 289, CID collision energy of 30%) *m/z* 245 (100) $[M + H - CO_2]^+$, 202 (10) $[M + H - CO_2 - (C_2H_5N)]^+$, 188 (12) $[M + H - CO_2 - (C_3H_7N)]^+$.

Synthesis of *R*- and *S*-Hercynine.[8] For the synthesis of *S*-hercynine a 0.28 mL portion of a 37% aqueous CH_2O solution was added to 350 mg (1.67 mmol) of L-histidine monohydrochloride monohydrate in 5 mL of H_2O. For the synthesis of *R*-hercynine D-histidine monohydrochloride monohydrate was used instead of L-histidine monohydrochloride monohydrate. After the catalyst (Pd/C) was added, the mixture was hydrogenated at 12 bar for 50 min. The catalyst was separated by filtration and washed with MeOH. Afterward, the solvent was removed under reduced pressure. The colorless, thick liquid was dissolved in 3 mL of MeOH, and 8 mL of Et_2O was added portionwise. The solution turned milky and was removed after 10 min by decantation. The remaining colorless oil was *N*,*N*-dimethylhistidine. Without further purification 287 mg (1.57 mmol) of *N*,*N*-dimethylhistidine was dissolved in 2 mL of MeOH, and with the addition of aqueous 25% NH_4OH solution the pH was adjusted to 9. Then 120 μL of MeI (1.93 mmol) was added, and the reaction mixture was heated under reflux at 60 °C for 2 h. The solvent was then removed under reduced pressure, and the product was purified via HPLC. A total of 119 mg (36%) of hercynine was isolated as a white solid.

*Hercynine (**5**):* colorless solid; HPLC, t_R = 2.8 min; CD (H_2O) *S*-hercynine λ ($\Delta\varepsilon$) 211 (+1.4) nm, *R*-hercynine λ ($\Delta\varepsilon$) 211 (−1.3) nm; ^{1}H NMR (600 MHz, D_2O, 277 K) δ 8.27 (1H, s, H-6′), 6.94 (1H, s, H-8′), 3.58 (1H, dd, *J* = 12.4, 3.5 Hz, H-2′), 3.12 (1H, dd, *J* = 14.0, 3.5 Hz, H-3′a), 3.01 (1H, dd, *J* = 14.0, 12.4 Hz, H-3′b), 2.92 (9H, s, H-9′); ^{13}C NMR (151 MHz, D_2O, 277 K) δ 169.1 (C, C-1′), 133.3 (CH, C-6′), 125.9 (C, C-4′), 116.9 (CH, C-8′), 76.0 (CH, C-2′), 51.3 (3 × CH_3, C-9′), 21.8 (CH_2, C-3′); (+)-ESIMS *m/z* 198 $[M]^+$.

Hercynine (5) Obtained by Degradation of Pelianthinarubin A (1). A 2 mg amount of pelianthinarubin A (**1**) was dissolved in 5 mL of H_2O, and 0.5 mL of concentrated AcOH was added. The solution was stirred for 1 h at 50 °C. The solvent was then removed under reduced pressure. The residue was purified by preparative HPLC, yielding 0.5 mg of hercynine.

*Hercynine (**5**) Derived from **1**:* colorless solid; $HPLC_{prep}$, t_R = 2.8 min; CD (H_2O) *S*-hercynine λ ($\Delta\varepsilon$) 211 (+1.4) nm; (+)-ESIMS *m/z* 198 $[M]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00942.

Selected UV/vis, CD, NMR, and mass spectra of compounds **1**, **2**, and **5** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +49-421-218-63100. Fax: +49-421-218-63102. E-mail: peter.spiteller@uni-bremen.de.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Robich, G. *Mycena d'Europa*; Associazione Micologica Bresadola: Trento, 2003; pp 91−96.

(2) Arora, D. *Mushrooms Demystified*; Ten Speed Press: Berkeley, 1986; p 231.

(3) Peters, S.; Spiteller, P. *Eur. J. Org. Chem.* **2007**, 1571−1576.

(4) Peters, S.; Spiteller, P. *J. Nat. Prod.* **2007**, *70*, 1274−1277.

(5) Peters, S.; Jaeger, R. J. R.; Spiteller, P. *Eur. J. Org. Chem.* **2008**, 319−323.

(6) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512−7515.

(7) Harris, R. K.; Becker, E. D.; Cabral de Menezes, S. M.; Granger, P.; Hoffman, R. E. *Pure Appl. Chem.* **2008**, *80*, 59−84.

(8) Reinhold, V. N.; Ishikawa, Y.; Melville, D. B. *J. Med. Chem.* **1968**, *11*, 258−260.

(9) Kimura, C.; Nukina, M.; Igarashi, K.; Sugawara, Y. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 357−363.

(10) Sakemi, S.; Sun, H. H.; Jefford, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1989**, *30*, 2517−2520.

(11) Stierle, D. B.; Faulkner, D. J. *J. Nat. Prod.* **1991**, *54*, 1131−1133.

(12) Sun, H. H.; Sakemi, S.; Burres, N.; McCarthy, P. *J. Org. Chem.* **1990**, *55*, 4964−4966.

(13) Radisky, D. C.; Radisky, E. S.; Barrows, L. R.; Copp, B. R.; Kramer, R. A.; Ireland, C. M. *J. Am. Chem. Soc.* **1993**, *115*, 1632−1638.

(14) Perry, N. B.; Blunt, J. W.; McCombs, J. D.; Munro, M. H. G. *J. Org. Chem.* **1986**, *51*, 5476−5478.

DOI: 10.1021/acs.jnatprod.5b00942
J. Nat. Prod. XXXX, XXX, XXX−XXX

(15) Antunes, E. M.; Copp, B. R.; Davies-Coleman, M. T.; Samaai, T. *Nat. Prod. Rep.* **2005**, *22*, 62−72.
(16) Spiteller, P. *Nat. Prod. Rep.* **2015**, *32*, 971−993.
(17) Spiteller, P. *Chem. - Eur. J.* **2008**, *14*, 9100−9110.

DOI: 10.1021/acs.jnatprod.5b00942
J. Nat. Prod. XXXX, XXX, XXX−XXX