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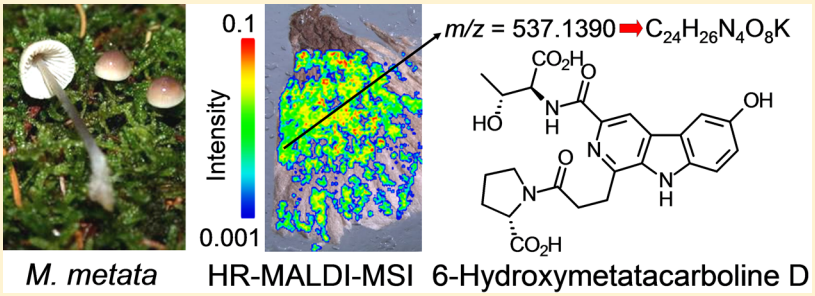
HR-MALDI-MS Imaging Assisted Screening of β-Carboline Alkaloids Discovered from *Mycena metata*

Robert J. R. Jaeger,[†] Marc Lamshöft,[‡] Sebastian Gottfried,[‡] Michael Spiteller,[‡] and Peter Spiteller*[,†]

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

[‡]Institut für Umweltforschung (INFU) der Fakultät Chemie, Technische Universität Dortmund, Otto-Hahn-Straße 6, D-44221 Dortmund, Germany

Supporting Information



ABSTRACT: Fruiting bodies of *Mycena metata* were screened for the presence of new secondary metabolites by means of HPLC-UV, LC-HR-ESIMS, and high-resolution matrix-assisted laser desorption/ionization mass spectrometry imaging (HR-MALDI-MS imaging). Thus, a new β-carboline alkaloid, 6-hydroxymetatacarboline D (**1d**), was isolated from fruiting bodies of *M. metata*. 6-Hydroxymetatacarboline D consists of a highly substituted β-carboline skeleton, which is likely to be derived biosynthetically from L-tryptophan, 2-oxoglutaric acid, L-threonine, and L-proline. The structure of the alkaloid was established by 2D NMR spectroscopic methods and HR-ESIMS. Moreover, by extensive application of LC-HR-ESIMS, LC-HR-ESIMS/MS, and LC-HR-ESIMS^3 techniques we were able to elucidate the structures of a number of accompanying β-carboline alkaloids, **1a**−**1c**, **1e**−**1i**, and **2a**−**2g**, structurally closely related to 6-hydroxymetatacarboline D, which are present in *M. metata* in minor amounts. The absolute configuration of the stereogenic centers of the β-carboline alkaloids was determined by GC-MS comparison with authentic synthetic samples after hydrolytic cleavage and derivatization of the resulting amino acids.

The isolation of new secondary metabolites is becoming increasingly difficult. This is due to the fact that readily available organisms have often already been well investigated. Consequently, natural product chemists are looking for unexplored source organisms to discover new natural products. From 2006 onward, we have concentrated our research on fruiting bodies of the genus *Mycena*, since it turned out to be a rich source of secondary metabolites. For instance, red *Mycena* species such as *Mycena sanguinolenta*,[1] *Mycena haematopus*,[2] and *Mycena rosea*[3] contain red and blue pyrroloquinoline alkaloids, while the orange color of *Mycena aurantiomarginata* is caused by a polyene.[4] Besides pigments, both the mycelia and the fruiting bodies of *Mycena* species contain bioactive compounds that play a role in their constitutive and wound-activated chemical defense.[5] For example, the mycelia of *Mycena galopus*[6,7] and *Mycena tintinnabulum*,[8] contain fungicidal strobilurins, while alcalinaphenols are present in the mycelia of *Mycena alcalina*[9] and the fruiting bodies of *M. galopus* contain benzoxepins.[10]

Until now, no secondary metabolites have been reported from the fruiting bodies of the basidiomycete *Mycena metata* (Fr.) Kumm. (German name: Fleischbraunkegeliger Helmling), small mushrooms that are often found in coniferous and deciduous forests. They are characterized by a flesh-colored, bell-shaped cap 0.6 to 1.8 cm in diameter,[11] a thin stipe 4.5 to 9 cm in length,[11] and the intensive iodoform-like odor[12] that occurs when the fruiting bodies are kept in a closed box. Therefore, we decided to screen this mushroom for the presence of new natural products.

Since there is a need not only for new source organisms but also for effective methods for the detection of new natural products, we decided to use not only HPLC-UV and LC-HR-ESIMS for the screening for new compounds in *M. metata* but also high-resolution MALDI mass spectrometry imaging (HR-MALDI-MS imaging). So far, MALDI-MS imaging has mainly been applied to the analysis of mammalian tissues[13,14] to investigate the spatial distribution of compounds such as proteins,[13,14] peptides,[13] lipids,[13,15] or drugs.[13,16] In histology this method is a valuable tool for localizing and discovering disease-related biomarkers.[13,14,17] For instance, the spatial distribution of phosphocholine and choline, which are increased in metastatic breast tumors, has been investigated by MALDI-MS imaging.[18] Furthermore, the uptake, bioavail-

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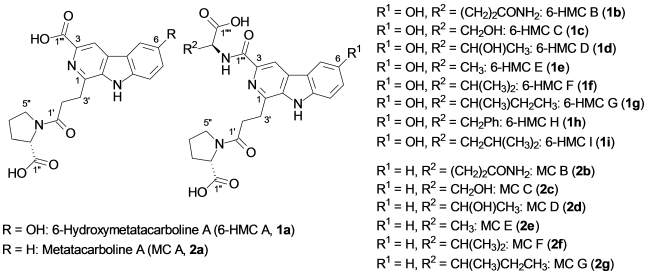
ability, and metabolism of therapeutic agents have been studied by MALDI-MS imaging.[13,16] Recently, this technique has been applied to investigate the spatial distribution of natural products,[19] for instance in bacteria,[20] plants,[21,22] or insects such as ants.[23] There are also a few studies on the function of certain natural products, for instance in chemical defense[23,24] and in metabolic exchange.[20] While MALDI-MS imaging delivers only spatially resolved low-resolution mass data of tissues, HR-MALDI-MS imaging yields spatially resolved high-resolution mass data, enabling determination of the molecular formula of compounds present in a distinct tissue sample. This piece of information is useful for evaluating the potential novelty of natural products directly from tissues. However, HR-MALDI-MS imaging has not been applied yet in screening for new natural products. In this paper we evaluate the effectiveness of HPLC-UV, LC-HR-ESIMS, and HR-MALDI-MS imaging in the screening for new natural products in fruiting bodies of *M. metata* and describe the isolation and structural elucidation of a series of *β*-carboline alkaloids, **1a**−**1i** and **2a**−**2g**, that we have named metatacarbolines.



RESULTS AND DISCUSSION

Frozen fruiting bodies of *M. metata* were extracted with methanol, and the resulting crude extract was separated on an analytical C_{18} column. For the detection of natural products a photo diode array detector was used, recording UV/vis spectra in the range *λ* 200−500 nm. By this means a series of compounds exhibiting strong absorptions at *λ* 280 nm was detected (Figure 1). The compound exhibiting the strongest

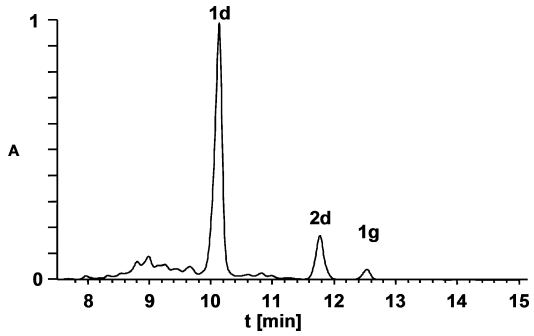


Figure 1. HPLC profile of the methanolic crude extract of *M. metata* at *λ* 280 nm.

absorption at *λ* 280 nm showed an $[M + H]^+$ ion in the LC-HR-ESIMS corresponding to a molecular formula of $C_{24}H_{26}N_4O_8$. Since this molecular formula has not been reported before for a natural product, it originated from a novel compound.

The same molecular formula was found by HR-MALDI-MS imaging. A spray-coated sample of the upper surface of a cap fragment exhibited intensive mass spectral peaks at *m/z* 537.1390 and 499.1832, representing the $[M + K]^+$ and the $[M + H]^+$ ion of $C_{24}H_{26}N_4O_8$, respectively (Figure 2). The

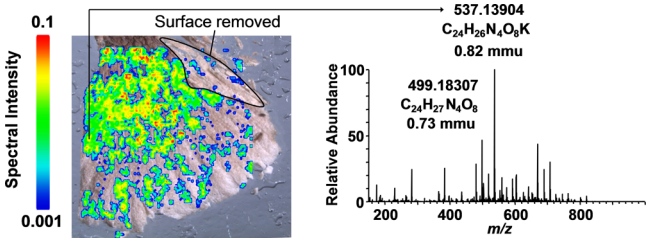


Figure 2. Left side: HR-MALDI-MS imaging at *m/z* 537.138 ± 0.002 from a cap fragment of *M. metata*. In the background a photo of the cap fragment is shown. Right side: High-resolution mass spectrum of the area highlighted by the black arrow.

intensity of these ions rises apparently from the rim to the center of the cap surface (Figure 2). In contrast, the ions at *m/z* 537.1390 and 499.1832 are absent on the part of the cap where the upper tissue layers were removed with the aid of a scalpel (Figure 2). Thus, it is possible to demonstrate whether certain compounds originate from the surface or from the inner parts of a tissue sample.

For the isolation of the metatacarbolines, a methanolic crude extract from frozen fruiting bodies of *M. metata* was filtered, concentrated, and separated on a preparative C_{18} column. For HPLC separation a water−methanol gradient was used, and the compounds were detected by their UV absorption at *λ* 280 nm. While 10 g of the frozen fruiting bodies yielded 5.8 mg of the major alkaloid 6-hydroxymetatacarboline D (**1d**), most of the other structurally closely related *β*-carboline alkaloids were present only in minor amounts in the fruiting bodies (Figure 1). Therefore, we concentrated first on the structure elucidation of the major alkaloid **1d**.

6-Hydroxymetatacarboline D (**1d**) shows absorption maxima at *λ* 220, 240, 282, 313, and 356 nm in the UV/vis spectrum, which indicate an extended *π*-electron system. When exposed to UV light at *λ* 366 nm **1d** exhibits blue fluorescence. The molecular formula $C_{24}H_{26}N_4O_8$ revealed that **1d** contains 14 degrees of unsaturation. In accordance with the molecular formula the ^{13}C NMR, recorded in DMSO-d_6, exhibits 24 resonances between δ_C 20.9 and 173.8, which were attributed to 11 quaternary carbons, seven CH groups, five CH_2 groups, and one CH_3 group with the help of the HSQC spectrum (Table 1). The ^1H NMR, recorded in DMSO-d_6, reveals the resonances of four aromatic protons between δ_H 7.09 and 8.54. Moreover, the ^1H NMR revealed in the aliphatic region (δ_H 1.83−4.44) the resonances of several CH and CH_2 groups and the resonance of a methyl group at δ_H 1.13. The resonances of two exchangeable protons at δ_H 8.63 and 11.79 were detectable only when DMSO-d_6 rather than CD_3OD was used as a solvent. The correlations in the COSY and HSQC spectra allowed the assignment of five spin systems in compound **1d** (Table 1). The resonances in the aromatic region are attributable to a 1,3,4-trisubstituted benzene moiety and an isolated aromatic CH group. In addition, **1d** contains a $(CH_2)_2$, a $(CH)_2$-CH_3, and a CH-$(CH_2)_3$ moiety. HMBC correlations of the aromatic protons H-4 (δ_H 8.54), H-5 (δ_H 7.57), H-7 (δ_H 7.09), and H-8 (δ_H 7.46) as well as the amide proton NH-2‴

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Table 1. ^{15}N, ^{13}C, and ^{1}H NMR Spectroscopic Data (600 MHz, DMSO-d_6, 292 K) of 6-Hydroxymetatacarboline D (1d)[a]

position	δ_C, mult.	δ_N, mult.	δ_H, mult. (J in Hz)	COSY	NOESY	HMBC$_{H-C}$	HMBC$_{H-N}$
1	143.3, qC						
2		283, N					
3	137.4, qC						
4	112.3, CH		8.54, s		5	1, 4a, 4b, 9a, (3′), 1‴	2
4a	127.1, qC						
4b	122.2, qC						
5	105.9, CH		7.57, d (2.0)	7	4	4a, (4b), 6, 7, 8, 8a	
6	151.5, qC						
7	118.6, CH		7.09 dd (2.0, 8.6)	5, 8		4b, 5, 6, 8a	
8	113.0, CH		7.46, d (8.6)	7	9	(4a), 4b, 5, 6, (7), 8a	9
8a	135.0, qC						
9		111, NH	11.79, s		8, (2′a), (2′b), 3′a, 3′b	(1), (4), 4a, 4b, (5), (8), 8a, 9a	
9a	136.2, qC						
1′	170.0, qC						
2′	30.8, CH_2		3.10, m, H_a	2′b, 3′a, 3′b	5″, (9)	1, 1′, 3′	
			2.91, m, H_b	2′a, 3′a, 3′b	5″, (9)	1, 1′, 3′	
3′	27.5, CH_2		3.44, m, H_a	2′a, 2′b, 3′b	9	1, 9a, 1′, 2′	2
			3.31, m, H_b	2′a, 2′b, 3′a	9	1, 9a, 1′, 2′	2
1″	173.8, qC						
2″	58.4, CH		4.22, dd (3.2, 8.6)	3″a, 3″b		1′, 1″, 3″, 4″	
3″	28.9, CH_2		2.14, m, H_a	2″, 3″b, 4″		1″, 2″, 4″, 5″	
			1.83, m, H_b	(2″), 3″a, 4″		1″, 2″, 4″, 5″	N-5″
4″	24.4, CH_2		1.90, m	3″a, 3″b, 5″	2′a, 2′b	2″, 3″, 5″	N-5″
5″	46.5, CH_2		3.62, m	4″	2′a, 2′b	2″, 3″, 4″	
N-5″		125, N					
1‴	165.1, qC						
1⁗	172.5, qC						
2⁗	57.5, CH		4.44, dd (1.9, 8.9)	3⁗, NH-2⁗	4⁗	1‴, 1⁗, 3⁗, 4⁗	N-2⁗
3⁗	66.6, CH		4.31, dq (2.3, 6.2)	2⁗, 4⁗	NH-2⁗	1⁗, 2⁗, 4⁗	N-2⁗
4⁗	20.9, CH_3		1.13, d (6.2)	3⁗	2⁗, NH-2⁗	1⁗, 2⁗, 3⁗	
N-2⁗		96, NH	8.63, d (9.1)	2⁗	3⁗, 4⁗	(3), 1‴, (1⁗), 2⁗, 3⁗	2

[a]Weak NOESY and HMBC correlations are given in parentheses.

(δ_H 8.63) and the secondary amino proton H-9 (δ_H 11.79) allow the assignment of all the atoms of the β-carboline skeleton. The quaternary carbon C-6 (δ_C 151.5) carries a hydroxy group on account of its chemical shift value. A strong $^3J_{H-C}$ coupling from H-4 to C-1‴ indicates that the quaternary carbon atom C-3 is substituted with C-1‴ (δ_C 165.1), a carboxamide moiety. The $(CH_2)_2$ fragment is directly connected to the β-carboline skeleton via C-1 and C-3′ since there are HMBC correlations from H-2′ to C-1 and from H-3′ to C-1 and C-9a. HMBC correlations from H-2′ and H-3′ to the carbon C-1′ (δ_C 170.0) indicate that the $(CH_2)_2$ moiety is connected with C-1′, a carboxamide unit, via C-2′. On account of its typical chemical shift values the $(CH)_2$-CH_3 fragment could be identified as a part of the amino acid threonine. An HMBC correlation between H-2⁗ and C-1‴ reveals that the threonine residue is attached to the carboxamide carbon C-1‴ of the β-carboline skeleton by a peptide bond between C-1‴ and N-2⁗. The CH-$(CH_2)_3$ fragment is part of the amino acid proline, which is attached to the carboxyethyl side chain by a peptide bond between C-1′ and N-5″ because of the key HMBC correlation between H-2″ and C-1′. The NOE between H-5″ (δ_H 3.62) and H-2′ (δ_H 3.10/2.91) establishes the *trans*-orientation of the proline moiety (Figure 3).

To elucidate the absolute configuration of the three stereogenic centers C-2″, C-2⁗, and C-3⁗, 6-hydroxymetatacarboline D (**1d**) was degraded by hydrolysis,[25] yielding proline (**3**) and threonine (**4**) (Figure 4).

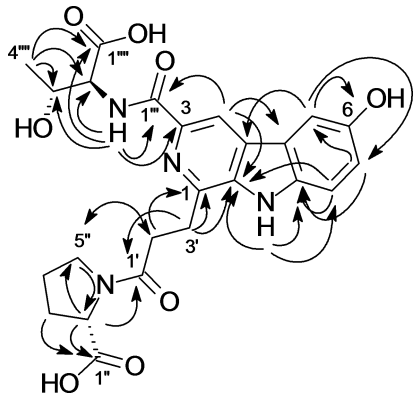

Figure 3. Selected HMBC (→) and NOE (↔) correlations of 6-hydroxymetatacarboline D (**1d**).

The absolute configuration at C-2″ was determined by conversion of **3** to the corresponding methyl ester,[26] which was reacted with *S*-Mosher's acid chloride (*S*-MTPA-Cl)[27,28] to the amide **5** (Figure 4). The GC-MS comparison of the derivatized degradation product **5** with authentic synthetic samples of known configuration revealed the *S*-configuration for C-2″ in **1d**.

To elucidate the absolute configuration at C-2⁗ and C-3⁗, **4** was converted to the corresponding methyl ester **6**,[14] which was then reacted with *N*-methyl-bis-trifluoroacetamide

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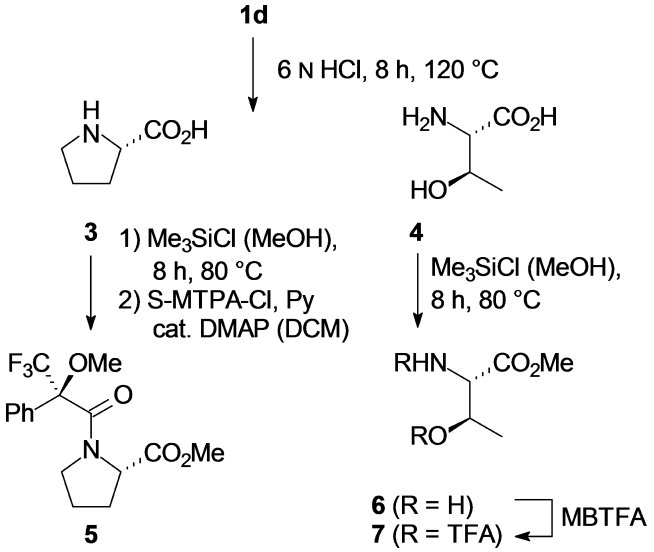

Figure 4. Degradation and derivatization of **1d** for the determination of the absolute configuration of the stereogenic centers (DCM: dichloromethane, DMAP: 4-dimethylaminopyridine; MBTFA: *N*-methyl-bis-trifluoroacetamide; *S*-MTPA-Cl: *S*-Mosher's acid chloride; Py: pyridine).

(MBTFA) to the *O*-methyl-*N*,*O*-bis-trifluoroacetylated derivative **7** (Figure 4).[29,30] The GC-MS comparison of **7** with authentic samples of known configuration revealed the *threo*-configuration for the stereogenic centers in **1d**. The absolute configuration of the stereogenic centers of **6** was determined to be 2*S*,3*R* by subsequent GC-MS comparison of the methyl ester **6** with authentic samples on a chiral GC column (Lipodex E). Consequently, **1d** contains an L-threonine moiety.

Most of the minor alkaloids, the 6-hydroxymetatacarbolines A–C (**1a–1c**), the 6-hydroxymetatacarbolines E–I (**1e–1i**), and the metatacarbolines A–G (**2a–2g**) either occurred only in trace amounts or did not separate well from each other on the preparative HPLC column. Still, they turned out to be closely structurally related to **1d**. Hence, it was possible to determine their structures mainly from key ions present in the corresponding LC-HR-ESIMS, LC-HR-ESIMS/MS, and LC-HR-ESIMS^3 spectra. The molecular formulas of all metatacarbolines derived from the LC-HR-ESIMS spectra indicated that the 6-hydroxymetatacarbolines A–C (**1a–1c**) and E–I (**1e–1i**) differed from **1d** only with respect to the amino acid moiety attached at C-1‴. In the case of the metatacarbolines A–G (**2a–2g**), the molecular ions indicated the presence of different amino acid moieties at C-1‴ and the absence of the OH group at C-6. The LC-HR-ESIMS/MS spectra of all metatacarbolines exhibit a characteristic fragment ion at $[M + H - C_5H_9NO_2]^+$. This ion is generated from the $[M + H]^+$ ion upon loss of the neutral fragment proline (m/z 115, $C_5H_9NO_2$) and subsequent cyclization to a δ-lactam (Figure 5). Consequently, the presence of this fragment indicates that the variable amino acid moiety is located at C-1‴ in all cases. In the LC-HR-ESIMS^3 the daughter ion at $[M + H - C_5H_9NO_2]^+$ loses the variable amino acid residue, yielding a fragment ion either at m/z 265.0608 $[C_{15}H_9N_2O_3]^+$ or at m/z 249.0659 $[C_{15}H_9N_2O_2]^+$ (Figure 5). While the fragment ion at m/z 265.0608 is characteristic of the hydroxylated β-carboline alkaloids **1a–1i**, the fragment ion at m/z 249.0659 is characteristic of the nonhydroxylated β-carboline alkaloids **2a–2g** (Figure 5). By these means the compounds **1e** and **2c** are identified unambiguously despite the fact that both compounds possess the same $[M + H]^+$ ion corresponding to the molecular formula $C_{23}H_{24}N_4O_7$ (Figure 5). Similarly, the structure of **1h** is assigned unambiguously. It contains a phenylalanine residue. What cannot be distinguished by MS methods is **1g** and **1i**, which contain the amino acids isoleucine and leucine, respectively. However, **1g** is likely to contain the more polar isoleucine residue, since **1g** exhibits a shorter retention time than **1i** on an RP-18 column. Furthermore, the exact position of the hydroxyl group in the compounds **1a–1i** cannot be deduced directly from the recorded mass spectra: A systematic shift of 1.6 min in the retention times between the hydroxylated alkaloids **1b–1g** and the corresponding non-hydroxylated alkaloids **2b–2g** indicates that at least **1b–1g** are hydroxylated at C-6. The presence of a weak $[M + H]^+$ ion in the LC-HR-ESIMS representing the same molecular formula as **1d** at another retention time (t_R 10.82 min) may indicate the presence of tiny amounts of a regioisomer of 6-hydroxymetatacarboline D, perhaps 5-hydroxymetatacarboline D.

In order to prove the chemical structure and the absolute configuration of the amino acid moieties at C-1‴ of **1b–1c**, **1e–1i**, and **2b–2g**, these residues were identified by GC-MS. The HPLC fraction containing the β-carboline alkaloids was hydrolyzed and subsequently derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA).[31] Also identified, besides the expected amino acids proline, threonine, alanine, valine, leucine, isoleucine, serine, glutamic acid, pyroglutamic acid, and phenylalanine, were glycine, aspartic acid, and tyrosine as pertrimethylsilylated derivatives, showing the presence of

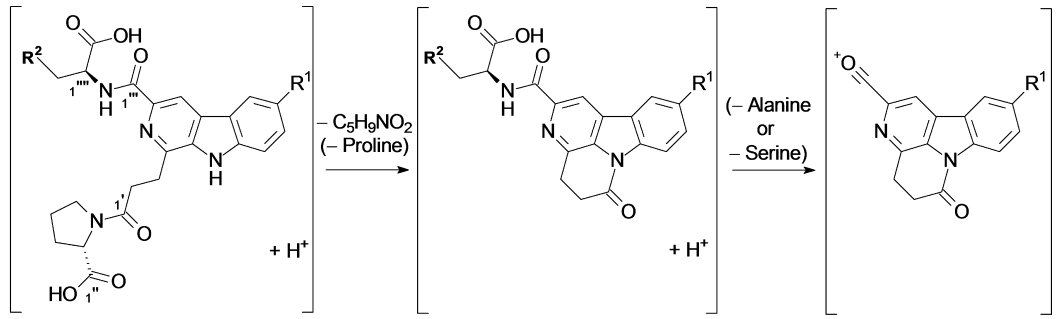

Figure 5. Fragmentation of **1e** and **2c** in the LC-HR-ESIMS/MS and LC-HR-ESIMS^3.

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even more β-carboline alkaloids in the fruiting bodies of *M. metata*. Furthermore, the absolute configuration of alanine, valine, leucine, isoleucine, glutamic acid, phenylalanine, and aspartic acid was determined to be the natural L-configuration by conversion of their methyl esters into the corresponding Mosher amides and subsequent GC-MS comparison with authentic samples of known configuration.[27,28]

The hypothetical biosynthesis of all metatacarbolines obviously starts with condensation of L-tryptophan and 2-oxoglutaric acid, yielding the β-carboline skeleton after decarboxylation at C-1 of the former 2-oxoglutaric acid and subsequent aromatization. Then, L-proline probably forms an amide bond from its nitrogen to the carboxylic group at C-1′, yielding **2a**. 6-Hydroxylation of **2a** would lead to **1a**. The metatacarbolines **2b**−**2g** are likely to originate from **2a** by reaction of the amino acids L-glutamic acid, L-serine, L-threonine, L-alanine, L-valine, L-isoleucine, L-phenylalanine, and L-leucine, respectively, with the carboxylic group at C-1‴. Similarly, the 6-hydroxymetatacarbolines **1b**−**1i** are probably generated from **1a** by reaction with the corresponding amino acids.

β-Carboline alkaloids of various structures are widespread in nature, particularly in plants,[32,33] marine organisms,[34−37] and bacteria,[38−41] but some of these alkaloids have also been isolated from fungi.[32] For instance, the fly agaric *Amanita muscaria* contains tetrahydro-1-methyl-β-carboline-3-carboxylic acid[42] and *Coriolus maximus* contains harman,[43] which is also known from numerous plants.[32] The β-carboline alkaloids 3-(9*H*-β-carboline-1-yl)propionic acid, 3-(7-hydroxy-9*H*-β-carboline-1-yl)propionic acid, and infractopicrin have been isolated from *Cortinarius infractus*.[44,45] *C. brunneus* contains the brunneins A−C and 3-(7-hydroxy-9*H*-β-carboline-1-yl)-propionic acid.[46] So far, the occurrence of β-carboline alkaloids has not been reported from *Mycena* species. Moreover, apart from stellarine B[47] and the dichotomides I and II[48] the known β-carboline alkaloids do not possess an amide bond attached to amino acids at C-1‴ as with **1b**−**1i** and **2b**−**2g**.

Despite the fact that 6-hydroxymetatacarboline D (**1d**) was not effective when tested against some microorganisms, such as *Bacillus brevis*, *Bacillus pumilus*, *Bacillus subtilis*, *Cladosporium cucumerinum*, *Escherichia coli*, and *Saccharomyces cerevisiae*, it is possible that the β-carboline alkaloids of *M. metata* possess other bioactivity and might even play an ecological role for *M. metata*. This hypothesis is supported by the HR-MALDI-MS imaging data, which indicate that **1d** is present only in the upper surface of the fruiting body. In general, β-carboline alkaloids are known for their various biological activities,[33] ranging from monoamine oxidase inhibition,[43] topoisomerase inhibition,[49] and DNA intercalation,[50] to antioxidant,[51] antitumor,[52,53] antiparasitic,[54,55] antimicrobial,[53,56] antiviral,[52,56,57] antiallergic,[48] and hallucinogenic[58] properties.

As far as we know, HR-MALDI-MS imaging has not been applied before either to the screening for new natural products or to fruiting bodies of mushrooms. After optimization of the matrix preparation technique the major β-carboline alkaloid 6-hydroxymetatacarboline D (**1d**) was readily detectable by HR-MALDI-MS imaging. The signal originating from the $[M + K]^+$ ion of **1d** represented the basis ion in the mass range between *m*/*z* 150 and 1000 (Figure 2) in many regions of the upper cap surface. The imaging measurement in the high-resolution mode allowed us to deduce the molecular formula for every prominent ion and to evaluate its potential structural novelty (Figure 2). Due to lack of mushroom material of *M. metata*, we have not been able to perform further HR-MALDI-MS imaging experiments. Due to the encouraging first results, we are planning to apply this method to other mushroom species and to perform further investigations on *M. metata*. In general, the same results can be obtained with HR-ESIMS. A drawback of HR-MALDI-MS imaging is the high costs in comparison with HR-ESIMS and particularly in comparison with HPLC-UV; however, HR-MALDI-MS imaging provides additional data on the spatial distribution of a compound. The spatial distribution of a certain metabolite gives some hints at its metabolic origin, since secondary metabolites are more likely to be restricted to certain parts of an organism than primary metabolites. Moreover, the localization of a certain metabolite is valuable for the optimization of the isolation process, since the extraction can be focused on compound-containing tissue parts.

EXPERIMENTAL SECTION

General Experimental Procedures. Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Preparative HPLC separations were performed using two Waters 590EF pumps equipped with an automated gradient controller 680 and a Kratos Spectroflow 783 UV/vis detector. The samples were fractionated on a Nucleodur C_{18} EC column (5 μm, 21 × 250 mm; Macherey-Nagel) using the following gradient program: 10 min 99.9% H_2O/0.1% AcOH, then within 30 min linear to 100% MeOH, then 20 min at 100% MeOH; flow rate: 12 mL/min; detection: UV at 280 nm. UV spectra were recorded on a Varian Cary 100 Bio UV/vis spectrometer. NMR spectra were recorded with a 600 MHz Bruker Avance III spectrometer equipped with a TXI cryo probe (^{1}H at 600.13, ^{13}C at 150.9, ^{15}N at 61 MHz). Chemical shifts were determined in δ relative to the solvent DMSO-d_6 (δ_H 2.49, δ_C 39.5 ppm) as internal standard. GC-MS spectra were recorded with a Thermo Electron Trace DSQ mass spectrometer coupled with a Thermo Electron Trace GC Ultra equipped with a PTV injector. For sample separation, either a fused silica OPTIMA-5-Accent capillary column (15 m × 0.25 mm, coated with a 0.25 μm layer of liquid phase, Macherey-Nagel) or a Lipodex E capillary column (25 m × 0.25 mm, Macherey-Nagel) was used. Helium served as carrier gas. Injection volumes were 0.2−0.5 μL of a 1−2% (w/v) solution. MSTFA was used for pertrimethylsilylation and MBTFA for pertrifluoroacetylation. Temperature programs: 1 min isothermal at 50 °C, then 5 °C/min up to 300 °C, finally 10 min isothermal at 300 °C (OPTIMA-5-Accent); 1 min isothermal at 50 °C, then 5 °C/min up to 200 °C, finally 15 min isothermal at 200 °C (Lipodex E). Retention indices R_i according to Kováts were determined by injection of a 0.2 μL sample of a standard mixture of saturated straight-chain alkanes (C_{10}−C_{36}).[59] LC-HR-ESIMS spectra were obtained with a Thermo Scientific LTQ Orbitrap mass spectrometer equipped with an H-ESI-II source. The spectrometer was operated in positive mode (1 spectrum/s; mass range: 250−1000) with a nominal mass resolving power of 60 000 at *m*/*z* 400. For sample separation an Agilent 1200 HPLC system consisting of an LC pump, PDA detector, autosampler (injection volume 10 μL), and column oven (30 °C) was coupled with the mass spectrometer. The separations were performed using a Nucleodur Gravity C_{18} column (1.8 μm, 3 × 50 mm, Macherey-Nagel) using an H_2O (+ 0.1% HCO_2H, + 10 mM NH_4OAc) (A)−MeCN (+ 0.1% HCO_2H) (B) gradient at a flow rate of 250 μL/min as follows: 95% A isocratic for 3 min, linear gradient to 100% B over 20 min, followed by 100% B isocratic for 10 min; the system returned to its initial condition (95% A) within 1 min, and was equilibrated for 5 min. HR-MALDI-MS imaging of secondary metabolites of *M. metata* was performed with an LTQ Orbitrap XL mass spectrometer (Thermo Scientific) coupled to a MALDI source to provide high-resolution mass spectra and images. The spectrometer was operated in positive mode (mass range *m*/*z* 150−1000) with nominal mass resolving power of 60 000 at *m*/*z* 400 and a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation, using the internal calibration (lock mass) at *m*/*z*

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273.03936 (2 × DHB − 2 × H_2O + H^+) . The laser power was set at 30 μJ, with raster plate motion and raster step size of 50 or 100 μm; one microscan per step was used for the analysis. All data were processed by the Thermo ImageQuest 1.0.1 software to select masses for visualization. Masses were normalized to total ion current and visualized in a mass window ±1 mmu. Microscopic pictures (magnification = 1−1.25×) were taken using a Leica S8 APO Greenough stereomicroscope equipped with a Schott KL 1500 compact halogen cold light source. The images were captured by a digital camera and processed using the Leica Application Suite LAS EZ version 1.6.0.

Mushroom Material. Fruiting bodies of *M. metata* (leg. Margot Spiteller and G. Spiteller; det. C. Gubitz and P. Spiteller) were collected between September and December, 2005 to 2011, in coniferous and deciduous forests near Kamerun (49.8969° N, 11.6205° E), a restaurant located 10 km southeast of Bayreuth, in the Studentenwald (49.9231° N, 11.5737° E) in Bayreuth, and near the peak of the Schneeberg (50.0498° N, 11.8566° E) in the Fichtelgebirge (Germany). Voucher samples of *M. metata* are deposited at the Institut für Organische Chemie, Universität Bremen, Germany. The mushrooms were stored at −32 °C after collection.

Test Organisms. *Bacillus brevis* (NCCB 89173, Centraalbureau voor Schimmelcultures, Amsterdam), *Bacillus pumilus* (Max-Planck-Institut für Chemische Ökologie, Jena), *Bacillus subtilis* (NCCB 1089, Centraalbureau voor Schimmelcultures, Amsterdam), *Cladosporium cucumerinum* (CBS 177.54, Centraalbureau voor Schimmelcultures, Amsterdam), *Escherichia coli* (Technische Universität München), and *Saccharomyces cerevisiae* (Technische Universität München) were used.

Sample Preparation for HR-MALDI-MS Imaging. Fruiting bodies of *Mycena metata* were freeze-dried, and a fragment of the cap was excised with a scalpel. To examine the compound composition inside the cap, the surface at the top right edge of the cap was scraped off. The cap fragment was attached to a MALDI glass slide (Thermo Scientific) with sticky tape. Samples were spray-coated with 2,5-dihydroxybenzoic acid (DHB), initially from a saturated solution in 80% methanol and 1% TFA, and then automatically using an ImagePrep device (Bruker Daltonics) with a solution of 30 mg/mL DHB in 50% methanol and 0.2% TFA. The ImagePrep instrument deposits matrix solution onto the tissue in a controlled manner. The initial spray-coating was performed using the ImagePrep device in a user-controlled fashion, giving six spray cycles of 0.5 s interrupted by 30 s of drying. The reproducibility of the sample preparation was monitored by a scattering light sensor controlling the matrix thickness.

Extraction and Isolation of 1a−1i and 2a−2g. Frozen fruiting bodies (10 g) were crushed after addition of MeOH (10 mL) and extracted twice with MeOH (2 × 100 mL) at room temperature. The combined extracts were concentrated in vacuo at 35 °C. The resulting residue was dissolved in H_2O−MeOH (1:1, 5 mL), prepurified with a C_{18} cartridge, and separated on a C_{18} column by preparative HPLC (UV detection at 280 nm), yielding **1d** (5.8 mg, t_R 37.0 min) or a mixture of **1a−1i** and **2a−2g** (15 mg including **1d**, t_R 16−46 min). The relative amounts of the β-carboline alkaloids of *M. metata* to each other can be roughly estimated from the relative intensities of the total ion current in the LC-HR-ESIMS: **1a** (2.7%), **1b** (1.4%), **1c** (2.6%), **1d** (100%), regioisomer of **1d** (3.6%), **1e** (1.3%), **1f** (7.9%), **1g** (11.8%), **1h** (0.06%), **1i** (0.1%), **2a** (5.4%), **2b** (2.0%), **2c** (1.1%), **2d** (48.0%), **2e** (0.2%), **2f** (0.8%), **2g** (0.8%).

*6-Hydroxymetatacarboline A (**1a**):* LC-HR-ESIMS (t_R 8.8 min) m/z 398.1348 (calcd for $C_{20}H_{20}N_3O_6$ $[M + H]^+$, 398.1352); LC-HR-ESIMS/MS (parent ion m/z 398, 35 eV) m/z (%) 283.0714 (100) (calcd for $C_{15}H_{11}N_2O_4$ $[M + H - C_5H_9NO_2]^+$, 283.0713).

*6-Hydroxymetatacarboline B (**1b**):* LC-HR-ESIMS (t_R 9.5 min) m/z 526.1934 (calcd for $C_{25}H_{28}N_5O_8$ $[M + H]^+$, 526.1938); LC-HR-ESIMS/MS (parent ion m/z 526, 35 eV) m/z (%) 411.1299 (100) (calcd for $C_{20}H_{19}N_4O_6$ $[M + H - C_5H_9NO_2]^+$, 411.1299).

*6-Hydroxymetatacarboline C (**1c**):* LC-HR-ESIMS (t_R 9.7 min) m/z 485.1668 (calcd for $C_{23}H_{25}N_4O_8$ $[M + H]^+$, 485.1672); LC-HR-ESIMS/MS (parent ion m/z 485, 35 eV) m/z (%) 467.1567 (1) (calcd for $C_{23}H_{23}N_4O_7$ $[M + H - H_2O]^+$, 467.1561), 370.1034 (100) (calcd for $C_{18}H_{16}N_3O_6$ $[M + H - C_5H_9NO_2]^+$, 370.1034).

*6-Hydroxymetatacarboline D (**1d**):* slightly yellowish solid; UV/vis (MeOH) λ_{max} (lg ε) 220 (4.09), 240 (4.07), 282 (4.20), 313 (3.47), 356 (3.32) nm; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 1); ^{15}N NMR (see Table 1); LC-HR-ESIMS (t_R 10.1 min) m/z 499.1828 (calcd for $C_{24}H_{27}N_4O_8$ $[M + H]^+$, 499.1829); LC-HR-ESIMS/MS (parent ion m/z 499, 35 eV) m/z (%) 481.1716 (1) (calcd for $C_{24}H_{25}N_4O_7$ $[M + H - H_2O]^+$, 481.1718), 384.1190 (100) (calcd for $C_{19}H_{18}N_3O_6$ $[M + H - C_5H_9NO_2]^+$, 384.1190); LC-HR-ESIMS^3 (parent ion m/z 499, 35 eV; daughter ion m/z 384, 35 eV) m/z (%) 265.0608 (100) (calcd for $C_{15}H_9N_2O_3$ $[M + H - C_5H_9NO_2 - C_4H_9NO_3]^+$, 265.0608).

*6-Hydroxymetatacarboline E (**1e**):* LC-HR-ESIMS (t_R 10.6 min) m/z 469.1721 (calcd for $C_{23}H_{25}N_4O_7$ $[M + H]^+$, 469.1723); LC-HR-ESIMS/MS (parent ion m/z 469, 35 eV) m/z (%) 354.1085 (100) (calcd for $C_{18}H_{16}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 354.1084); LC-HR-ESIMS^3 (parent ion m/z 469, 35 eV; daughter ion m/z 354, 35 eV) m/z (%) 265.0609 (100) (calcd for $C_{15}H_9N_2O_3$ $[M + H - C_5H_9NO_2 - C_3H_7NO_2]^+$, 265.0608).

*6-Hydroxymetatacarboline F (**1f**):* LC-HR-ESIMS (t_R 11.9 min) m/z 497.2032 (calcd for $C_{25}H_{29}N_4O_7$ $[M + H]^+$, 497.2036); LC-HR-ESIMS/MS (parent ion m/z 497, 35 eV) m/z (%) 382.1399 (100) (calcd for $C_{20}H_{20}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 382.1397).

*6-Hydroxymetatacarboline G (**1g**):* LC-HR-ESIMS (t_R 12.5 min) m/z 511.2192 (calcd for $C_{26}H_{31}N_4O_7$ $[M + H]^+$, 511.2193); LC-HR-ESIMS/MS (parent ion m/z 511, 35 eV) m/z (%) 396.1558 (100) (calcd for $C_{21}H_{22}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 396.1554).

*6-Hydroxymetatacarboline H (**1h**):* LC-HR-ESIMS (t_R 12.7 min) m/z 545.2031 (calcd for $C_{29}H_{29}N_4O_7$ $[M + H]^+$, 545.2036); LC-HR-ESIMS/MS (parent ion m/z 545, 35 eV) m/z (%) 430.1402 (100) (calcd for $C_{24}H_{20}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 430.1397); LC-HR-ESIMS^3 (parent ion m/z 545, 35 eV; daughter ion m/z 430, 35 eV) m/z (%) 265.0608 (100) (calcd for $C_{15}H_9N_2O_3$ $[M + H - C_5H_9NO_2 - C_9H_{11}NO_2]^+$, 265.0608).

*6-Hydroxymetatacarboline I (**1i**):* LC-HR-ESIMS (t_R 13.2 min) m/z 511.2192 (calcd for $C_{26}H_{31}N_4O_7$ $[M + H]^+$, 511.2193); LC-HR-ESIMS/MS (parent ion m/z 511, 35 eV) m/z (%) 396.1556 (100) (calcd for $C_{21}H_{22}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 396.1554).

*Metatacarboline A (**2a**):* LC-HR-ESIMS (t_R 9.9 min) m/z 382.1398 (calcd for $C_{20}H_{20}N_3O_5$ $[M + H]^+$, 382.1403); LC-HR-ESIMS/MS (parent ion m/z 382, 35 eV) m/z (%) 267.0764 (100) (calcd for $C_{15}H_{11}N_2O_3$ $[M + H - C_5H_9NO_2]^+$, 267.0764).

*Metatacarboline B (**2b**):* LC-HR-ESIMS (t_R 11.0 min) m/z 510.1987 (calcd for $C_{25}H_{28}N_5O_7$ $[M + H]^+$, 510.1989); LC-HR-ESIMS/MS (parent ion m/z 510, 35 eV) m/z (%) 395.1350 (100) (calcd for $C_{20}H_{19}N_4O_5$ $[M + H - C_5H_9NO_2]^+$, 395.1350).

*Metatacarboline C (**2c**):* LC-HR-ESIMS (t_R 11.3 min) m/z 469.1721 (calcd for $C_{23}H_{25}N_4O_7$ $[M + H]^+$, 469.1723); LC-HR-ESIMS/MS (parent ion m/z 469, 35 eV) m/z (%) 354.1085 (100) (calcd for $C_{18}H_{16}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 354.1084); LC-HR-ESIMS^3 (parent ion m/z 469, 35 eV; daughter ion m/z 354, 35 eV) m/z (%) 267.0765 (100) (calcd for $C_{15}H_{11}N_2O_3$ $[M + H - C_5H_9NO_2 - C_3H_5NO_2]^+$, 267.0764), 249.0659 (85) (calcd for $C_{15}H_9N_2O_2$ $[M + H - C_5H_9NO_2 - C_3H_7NO_3]^+$, 249.0659).

*Metatacarboline D (**2d**):* LC-HR-ESIMS (t_R 11.8 min) m/z 483.1879 (calcd for $C_{24}H_{27}N_4O_7$ $[M + H]^+$, 483.1880); LC-HR-ESIMS/MS (parent ion m/z 483, 35 eV) m/z (%) 465.1768 (6) (calcd for $C_{24}H_{25}N_4O_6$ $[M + H - H_2O]^+$, 465.1769), 368.1244 (100) (calcd for $C_{19}H_{18}N_3O_5$ $[M + H - C_5H_9NO_2]^+$, 368.1241).

*Metatacarboline E (**2e**):* LC-HR-ESIMS (t_R 12.2 min) m/z 453.1770 (calcd for $C_{23}H_{25}N_4O_6$ $[M + H]^+$, 453.1774); LC-HR-ESIMS/MS (parent ion m/z 453, 35 eV) m/z (%) 338.1139 (100) (calcd for $C_{18}H_{16}N_3O_4$ $[M + H - C_5H_9NO_2]^+$, 338.1135).

*Metatacarboline F (**2f**):* LC-HR-ESIMS (t_R 13.5 min) m/z 481.2084 (calcd for $C_{25}H_{29}N_4O_6$ $[M + H]^+$, 481.2087); LC-HR-ESIMS/MS (parent ion m/z 481, 35 eV) m/z (%) 366.1448 (100) (calcd for $C_{20}H_{20}N_3O_4$ $[M + H - C_5H_9NO_2]^+$, 366.1448).

*Metatacarboline G (**2g**):* LC-HR-ESIMS (t_R 14.1 min) m/z 495.2241 (calcd for $C_{26}H_{31}N_4O_6$ $[M + H]^+$, 495.2244); LC-HR-ESIMS/MS (parent ion m/z 495, 35 eV) m/z (%) 380.1604 (100) (calcd for $C_{21}H_{22}N_3O_4$ $[M + H - C_5H_9NO_2]^+$, 380.1605).

Determination of the Absolute Configuration at C-2″ of ***1d****.* Degradation and derivatization of **1d** to (*S*)-methyl 1-((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)pyrrolidine-2-carboxylate (2*S*,2′*R*-**5**) was conducted as follows. A 5 mg amount of 6-hydroxymetatacarboline D (**1d**) was heated under reflux (120 °C) in 1 mL of 6 N hydrochloric acid for 8 h. After neutralization with 2 N sodium hydroxide the solvent was removed completely. The residue was heated under reflux (80 °C) in 2 mL of methanol and 0.2 mL of $(CH_3)_3SiCl$ for 8 h. After complete removal of the solvent the residue was dissolved in 0.5 mL of CH_2Cl_2. Then, 5 μL (62 μmol) of pyridine, 3 μL (16 μmol) of (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid chloride (*S*-MTPA-Cl), and catalytic amounts of 4-dimethylaminopyridine (DMAP) were added, and the solution was stirred under argon at 37 °C overnight. The solvent was removed completely, and the residue was dissolved in 1 mL of H_2O and extracted three times with 1 mL of $CHCl_3$. The organic phase was dried over Na_2SO_4, filtered, concentrated to dryness, diluted in ethyl acetate, and transferred into a GC vial. GC-MS (OPTIMA-5-Accent) R_i 1952; *m*/*z* (%) 286 (12) $[M - CO_2CH_3]^+$, 190 (9), 189 (68) $[C(CF_3)(OCH_3)(C_6H_5)]^+$, 186 (6), 158 (6), 156 (44) $[M - C(CF_3)(OCH_3)(C_6H_5)]^+$, 139 (5), 129 (9), 128 (100) $[M - (C{=}O)C(CF_3)(OCH_3)(C_6H_5)]^+$, 127 (9), 119 (5), 105 (12) $[C_7H_5O]^+$, 91 (7), 84 (5), 77 (7) $[C_6H_5]^+$, 44 (9), 42 (5).

*Synthetic 2S,2′R-****5****.* Compound 2*S*,2′*R*-**5** was synthesized from L-proline by methylation and reaction with *S*-MTPA-Cl as described above. GC-MS (OPTIMA-5-Accent) R_i 1952; *m*/*z* (%) 286 (12) $[M - CO_2CH_3]^+$, 190 (6), 189 (69) $[C(CF_3)(OCH_3)(C_6H_5)]^+$, 186 (7), 158 (6), 157 (5), 156 (45) $[M - C(CF_3)(OCH_3)(C_6H_5)]^+$, 139 (5), 129 (7), 128 (100) $[M - (C{=}O)C(CF_3)(OCH_3)(C_6H_5)]^+$, 127 (9), 119 (7), 105 (11) $[C_7H_5O]^+$, 84 (5), 77 (7) $[C_6H_5]^+$, 69 (7) $[CF_3]^+$, 42 (5), 40 (19).

*Synthetic 2R,2′R-****5****.* Compound 2*R*,2′*R*-**5** was synthesized from D-proline by methylation and reaction with *S*-MTPA-Cl as described above. GC-MS (OPTIMA-5-Accent) R_i 1958; *m*/*z* (see mass spectrum of 2*S*,2′*R*-**5**).

Determination of the Relative Configuration at C-2‴ and C-3‴ of ***1d****.* Degradation and derivatization of **1d** to (2*S*,3*R*)-methyl 2-(2,2,2-trifluoroacetamido)-3-(2,2,2-trifluoroacetoyloxy)butanoate (2*S*,3*R*-**7**) was conducted as follows. A 5 mg amount of 6-hydroxymetatacarboline D (**1d**) was hydrolyzed and methylated as described above. The reaction product was derivatized for 1 h in a GC vial with 20 μL of MBTFA. GC-MS (OPTIMA-5-Accent) R_i 1096; *m*/*z* (%) 281 (12), 266 (11) $[M - CO_2CH_3]^+$, 185 (38) $[M - CF_3CO_2CH{=}CH_2]^+$, 184 (15) $[M - CF_3CO_2CHCH_3]^+$, 180 (9), 167 (6), 153 (31), 152 (100) $[M - CO_2CH_3 - CF_3CO_2H]$, 141 (27) $[CF_3CO_2CHCH_3]^+$, 132 (5), 113 (6), 96 (6), 69 (36) $[CF_3]^+$, 59 (10) $[CO_2CH_3]^+$, 57 (18), 44 (6), 43 (7), 40 (6).

*Synthetic 2S,3R-****7****.* Compound 2*S*,3*R*-7 was synthesized from L-threonine by methylation and derivatization with MBTFA as described above. GC-MS (OPTIMA-5-Accent) R_i 1096; *m*/*z* (%) 281 (10), 266 (10) $[M - CO_2CH_3]^+$, 185 (31) $[M - CF_3CO_2CH{=}CH_2]^+$, 184 (14) $[M - CF_3CO_2CHCH_3]^+$, 180 (8), 167 (7), 153 (34), 152 (100) $[M - CO_2CH_3 - CF_3CO_2H]$, 141 (22) $[CF_3CO_2CHCH_3]^+$, 132 (5), 96 (6), 69 (32) $[CF_3]^+$, 59 (9) $[CO_2CH_3]^+$, 57 (18), 45 (7), 44 (13), 41 (5), 40 (13).

*Synthetic 2S,3S-****7****.* Compound 2*S*,3*S*-7 was synthesized from L-*allo*-threonine by methylation and derivatization with MBTFA as described above. GC-MS (OPTIMA-5-Accent) R_i 1130; *m*/*z* (see mass spectrum of 2*S*,3*R*-7).

Determination of the Absolute Configuration at C-2‴ and C-3‴ of ***1d****.* Degradation and derivatization of **1d** to (2*S*,3*R*)-methyl 2-amino-3-hydroxybutanoate (2*S*,3*R*-**6**) was conducted as follows. A 5 mg portion of 6-hydroxymetatacarboline D (**1d**) was hydrolyzed and methylated as described above. The reaction product was diluted in ethyl acetate, transferred into a GC vial, and separated on a chiral GC column. GC-MS (Lipodex E) R_i 1450; *m*/*z* (%) 118 (5) $[M - CH_3]^+$, 89 (53) $[M - CH_3CHO]^+$, 88 (13) $[M - CH_3CHOH]^+$, 75 (7), 74 (100) $[M - CO_2CH_3]^+$, 71 (5), 58 (7), 57 (42), 56 (18), 46 (5), 45 (9) $[CH_3CHOH]^+$, 44 (7) $[CH_3CHO]^+$, 43 (8) $[CH_3CO]^+$, 40 (18).

*Synthetic 2S,3R-****6****.* Compound 2*S*,3*R*-**6** was synthesized from L-threonine by methylation as described above. GC-MS (Lipodex E) R_i 1446; *m*/*z* (%) 118 (4) $[M - CH_3]^+$, 89 (61) $[M - CH_3CHO]^+$, 88 (15) $[M - CH_3CHOH]^+$, 74 (100) $[M - CO_2CH_3]^+$, 58 (7), 57 (41), 56 (17), 45 (8) $[CH_3CHOH]^+$.

*Synthetic 2R,3S-****6****.* Compound 2*R*,3*S*-**6** was synthesized from D-threonine by methylation as described above. GC-MS (Lipodex E) R_i 1433; *m*/*z* (see mass spectrum of 2*S*,3*R*-**6**).

Identification of Pertrimethylsilylated Amino Acids after Hydrolysis of the Metatacarbolines. The β-carboline alkaloids **1a**−**1i** and **2a**−**2g** (15 mg including **1d**) were hydrolyzed in 1 mL of 6 N HCl at 120 °C for 1 h. After removal of the solvent an aliquot was pertrimethylsilylated with MSTFA (10−20 μL) for GC-MS analysis. Samples derived from authentic amino acids (0.5 mg) were used for GC comparison.

Determination of the Absolute Configuration at C-2⁗ of the Metatacarbolines. The hydrolysis products of the β-carboline alkaloids **1a**−**1i** and **2a**−**2g** were dissolved in MeOH (2 mL) and methylated for 10 min with an ethereal solution of CH_2N_2 (2−5 mL). The solvent was removed completely, and the residue was dissolved in 0.5 mL of pyridine. After addition of 3 μL (16 μmol) of *R*-MTPA-Cl and catalytic amounts of DMAP the solution was stirred under argon at 37 °C overnight. The solvent was removed completely, and the residue was dissolved in 2 mL of H_2O and extracted three times with 2 mL of CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and transferred into a GC vial for GC-MS analysis. Diastereomeric samples derived from authentic amino acids (0.5 mg) by reaction with *R*- and *S*-MTPA-Cl were used for GC comparison.

Biological Tests. For agar diffusion assays, **1d** (0.5 μmol) was dissolved in MeOH (5 μL) and dropped onto paper discs (diameter 5 mm, thickness 0.5 mm). These discs were dried under sterile conditions, placed on agar plates inoculated with the test organisms, and incubated at appropriate conditions (*Bacillus brevis* at 37 °C for 24 h on peptone agar, *Bacillus pumilus* at 26 °C for 24 h on peptone agar, *Bacillus subtilis* at 37 °C for 24 h on LB agar, *Cladosporium cucumerinum* at 18 °C for 3−5 d on malt extract agar, *Escherichia coli* at 37 °C for 24 h on LB agar, *Saccharomyces cerevisiae* at 26 °C for 24 h on YMG agar). Nourseothricin (0.5 μmol) was used as a positive control (*B. brevis* and *B. pumilus*: 25 mm inhibition zone, *B. subtilis*: 14 mm inhibition zone) and mannitol (0.5 μmol) as a negative control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Selected UV/vis and NMR spectra of **1d** and mass spectra of **1a**−**1i** and **2a**−**2g** and the degradation products **5**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ 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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