Stachylines A−D from the Sponge-Derived Fungus *Stachylidium* sp.

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The marine-derived fungus *Stachylidium* sp. was isolated from the sponge *Callyspongia* cf. *C. flammea.* Four new, putatively tyrosine-derived and O-prenylated natural products, stachylines A−D (**1**−**4**), were obtained from the fungal extract. The structures of **1**−**4** were elucidated on the basis of extensive spectroscopic analyses. The absolute configuration of compound **2** was established by Mosher's method. Stachyline A (**1**) possesses a rare terminal oxime group and occurs as an interchangeable mixture of *E*/*Z*-isomers.

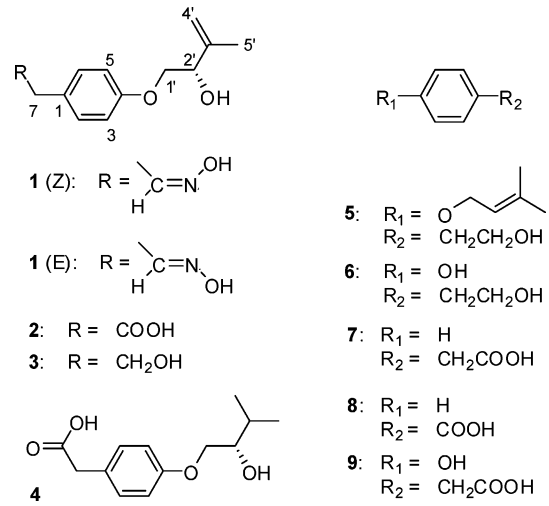
The marine environment harbors approximately half of the global biodiversity and is estimated to contain between 3 and 500 million different species, offering an almost infinite resource for novel compounds.[1] Among these organisms marine-derived fungi became known as prolific producers of structurally most intriguing compounds.[2] In general, tyrosine derivatives have only rarely been reported from fungi, and in most cases such compounds were obtained from strains originating from environmentally extreme habitats, e.g., tyrosol carbamate, which was isolated from the deep-water fungus *Arthrinium* sp.[3] *Phytomyces* sp., producing O-prenylated tyrosine derivatives, is an extremophile collected from an acid mine waste rich in toxic metals.[4] Another unusual case is aspergillusol A, an α-glucosidase inhibitor obtained from the sponge-derived fungus *Aspergillus aculeatus*, which is reported to be the only known fungal tyrosine derivative to possess an oxime group.[5]

Secondary metabolites with an oxime substituent are rare, and most of the reported examples have potent bioactivity; for example, the actinomycete-derived nocardicins displayed strong antibiotic activity,[6] and brevioxime from *Penicillium brevicompactum* inhibited the biosynthesis of insect juvenile hormones.[7] *Penicillium olsonii* produced 2-(4-hydroxyphenyl)-2-oxoacetaldehyde oxime (PHBA), which regenerated phosphorylated cholinesterase.[8] The oxime geometrical isomers collismycins A and B were isolated from *Streptomyces* sp. MQ22, which inhibited dexamethasone glucocorticoid receptor binding.[9]

During our search for new cytotoxic natural products an extract of the marine-derived fungus *Stachylidium* sp. was found to be active. During chromatographic separations it became clear that this fungus produces a vast array of secondary metabolites with intriguing structural features, among them the four novel, putatively tyrosine-derived and O-prenylated natural products stachylines A−D (**1**−**4**). Stachyline A (**1**) is distinguished by an oxime terminal group, probably derived through biosynthetic reactions similar to those known for cyanogenic glycosides and nocardicin A formation.[10−14] The molecules were evaluated in a number of biological assays; to date, however, no considerable activity was detected.

Results and Discussion

The RP-18 HPLC chromatogram of **1** contained two peaks (ratio 1:1), which when reinjected after their individual isolation, again resulted in the same chromatogram. This result suggested that compound **1** exists as a mixture of interchangeable isomers. NMR spectra also presented two sets of data, one for each isomer (see Tables 1 and 2). For clarity in the description of the structure elucidation, only one set of data will be considered initially.



The molecular formula of **1** was deduced by accurate mass measurement (HREIMS/HRESIMS) to be $C_{13}H_{17}NO_3$, requiring six sites of unsaturation. The ^{13}C NMR and DEPT135 spectra showed the presence of 13 resonances for one methyl, five sp^2 methine, one sp^3 methine, one sp^2 methylene, and two sp^3 methylene groups and three quaternary carbons (see Tables 1 and 2). The UV maximum at 276 nm indicated the presence of an aromatic moiety, whereas an IR absorption at around 3300 cm^{-1} arose from a hydroxy group in the molecule.

^1H NMR and $^1H-^{13}C$ HSQC data (see Tables 1 and 2) included two resonances for magnetically equivalent protons, i.e., H-2/H-6 and H-3/H-5 resonating at δ_H 7.17 and 6.88, respectively. This spectroscopic feature was interpreted as characteristic for a *para*-substituted aromatic ring. From the same spectra the presence of an exomethylene moiety, CH_2-4′, was evident (δ_H 4.89, 5.08; δ_C 112.1). The ^{13}C NMR spectrum showed two resonances for carbons connected to oxygen atoms, namely, at δ_C 72.1 (C-1′) and 73.9 (C-2′) (see Table 1), and the protons attached to these carbon atoms, H_2-1′ and H-2′, were coupled, as evidenced by $^1H-^1H$ COSY correlations. $^1H-^1H$ COSY correlations indicated the presence of another spin system between H_2-4′ and H_3-5′. The partial structures deduced from these two spin systems were connected making use of $^1H-^{13}C$ HMBC correlations. Thus, correlations from H_3-5′ to C-2′, C-3′, and C-4′ delineated an unsaturated and hydroxylated isoprene unit (see Table S1 in the Supporting Information). This was confirmed by further heteronuclear long-range correlations from H_2-4′ to C-3′ and C-2′. $^1H-^{13}C$ HMBC correlations between H-1′ and qC-4 showed that the isoprene unit was connected via C-4 to the *para*-substituted aromatic moiety. Due to the downfield shift of C-1′ and C-4 (δ_C 72.1, 158.5), connection of both parts of the molecule occurred through an oxygen atom.

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Table 1. ^{13}C NMR Spectroscopic Data for Compounds **1**−**4**

	1[d]		**2**	**3**	**4**
position	δ_C, mult.[a,b,c]		δ_C, mult.[a,b,c]	δ_C, mult.[a,b,c]	δ_C, mult.[a,b,c]
1	130.1, qC		128.0, qC	132.5, qC	127.9, qC
2	130.5, CH		131.2, CH	130.7, CH	131.2, CH
3	115.5, CH		115.3, CH	115.2, CH	115.2, CH
4	(*Z*-), 158.5, qC	(*E*-), 158.7, qC	158.8, qC	158.3, qC	159.0, qC
5	115.5, CH		115.3, CH	115.2, CH	115.2, CH
6	130.5, CH		131.2, CH	130.7, CH	131.2, CH
7	(*Z*-), 31.0, CH_2	(*E*-), 35.4, CH_2	40.5, CH_2	39.4, CH_2	40.4, CH_2
8	(*Z*-), 150.0, CH	(*E*-), 149.7, CH	173.0, qC	64.1, CH_2	173.2, qC
1′	72.1, CH_2		72.1, CH_2	72.1, CH_2	71.6, CH_2
2′	73.9, CH		73.9, CH	74.0, CH	74.6, CH
3′	146.1, qC		146.1, qC	146.2, qC	31.6, CH
4′	112.1, CH_2		112.1, CH_2	112.0, CH_2	17.7, CH_3
5′	18.7, CH_3		18.8, CH_3	18.8, CH_3	19.4, CH_3

[a] Acetone-d_6, 75.5 MHz. [b] Assignments are based on extensive 1D and 2D NMR experiments (HMBC, HSQC, COSY; see Supporting Information). [c] Implied multiplicities determined by DEPT. [d] Carbon resonances were attributed to the *Z*- or *E*-configuration, according to ACD/NMR Predictor software.

Table 2. ^1H NMR Spectroscopic Data for Compounds **1**−**4**

	1[c]		**2**	**3**	**4**
position	δ_H[a,b] (*J* in Hz)		δ_H[a,b] (*J* in Hz)	δ_H[a,b] (*J* in Hz)	δ_H[a,b] (*J* in Hz)
1					
2	(*Z*-), 7.17, d (8.4)	(*E*-), 7.13, d (8.4)	7.20, d (8.4)	7.13, d (8.4)	7.19, d (8.4)
3	6.88, d (8.4)		6.87, d (8.4)	6.84, d (8.4)	6.87, d (8.4)
4					
5	6.88, d (8.4)		6.87, d (8.4)	6.84, d (8.4)	6.87, d (8.4)
6	(*Z*-), 7.17, d (8.4)	(*E*-), 7.13, d (8.4)	7.20, d (8.4)	7.13, d (8.4)	7.19, d (8.4)
7	(*Z*-), 3.60, d (5.5)	(*E*-), 3.39, d (6.2)	3.51, br s	2.72, t (7.1)	3.52, br s
8	(*Z*-), 6.73, t (5.5)	(*E*-), 7.40, t (6.2)		3.68, t (7.1)	
1′	a: 4.01, dd (3.7, 9.5)		a: 4.01, dd (4.0, 9.8)	a: 4.00, dd (4.3, 9.8)	a: 4.00, dd (4.0, 9.8)
	b: 3.90, dd (7.3, 9.5)		b: 3.90, dd (7.2, 9.8)	b: 3.89, dd (7.2, 9.8)	b: 3.89, dd (6.6, 9.8)
2′	4.39, m		4.40, dd (4.0, 7.2)	4.39, dd (4.3, 7.2)	3.68, m
3′					1.86, m
4′	a: 4.89, br s		a: 4.88, br s	a: 4.89, br s	0.97, d (6.8)
	b: 5.08, br s		b: 5.08, br s	b: 5.09, br s	
5′	1.78, s		1.79, s	1.79, s	0.97, d (6.8)

[a] Acetone-d_6, 300 MHz. [b] Assignments are based on extensive 1D and 2D NMR experiments (HMBC, HSQC, COSY; see Supporting Information). [c] Proton resonances were attributed to the *Z*- or *E*-configuration, according to ACD/NMR Predictor software.

According to the molecular formula, the second substituent on the aromatic ring of **1** must have the composition C_2H_4NO. $^1H-^{13}C$ HMBC correlations could be detected from the aromatic protons H-2/H-6 to the methylene carbon C-7, making the connection of the second side chain via C-1 most likely. Through an $^1H-^1H$ COSY experiment, the methylene protons CH_2-7 were shown to be part of a spin system including H-8 (δ_H 6.73), whereby the latter was bound to an sp^2 hybridized carbon. The remaining presence of a nitrogen, oxygen, and proton atom was assigned to an unusual oxime moiety (see Tables 1 and 2). The latter is known to be able to adopt interchangeable configurations.[24] *E*- and *Z*-isomers in the case of **1** presented clearly distinguishable NMR shifts for the C-8 to C-2/C-6 part of the molecules (see Tables 1 and 2). The assignment of NMR shifts for the *E*- and *Z*-isomers was supported using the ACD NMR Predictor software (ACD/Laboratories). The configuration at C-2′ of compound **2** was found to be *S*, as deduced by Mosher's method. Based on the negative optical rotation of compound **1**, which was also observed for **2**, and the close biosynthetic relationship between both compounds, the *S* configuration is also proposed for C-2′ of **1**. For compound **1** the trivial name *E*- and *Z*-stachyline A is proposed.

The molecular formula of **2** was deduced by accurate mass measurement (HREIMS) to be $C_{13}H_{16}O_4$, requiring six sites of unsaturation. The ^{13}C and ^1H NMR spectra were closely related to those of compound **1** (see Table 2). One of the substituents on the aromatic ring was again an O-prenyl moiety, as in **1**, also connected via qC-4 and C-1′ (see Tables 1, 2 and Supporting Information). The second substituent on the aromatic ring of **2** was, however, different and established by interpretation of $^1H-^{13}C$ HMBC long-range correlations. Thus, the methylene protons H_2-7 had a heteronuclear coupling with C-2 and C-6 of the aromatic ring. Additionally, H_2-7 had HMBC long-range correlations to the quaternary carbon C-8, which according to its characteristic ^{13}C NMR resonance was part of a carboxylic acid group (δ_C 173.0). Compound **2** was thus an O-prenylated phenyl acetic acid.

The absolute configuration at C-2′ was determined by modified Mosher's method. The evaluation of the corresponding $^1H-^{13}C$ HSQC NMR spectra revealed differences of the proton shifts between the (*S*)- and (*R*)-MTPA esters (see Figure 1 and Supporting Information). The calculated differences in chemical shifts [δ of protons in the (*S*)-MTPA-ester minus δ of the corresponding protons in the (*R*)-MTPA-ester] led to the assignment of the *S*-absolute configuration for C-2′. Since compounds **1**−**4** all showed a negative specific optical rotation, it was assumed that they all share the *S*-configuration at C-2′. Compound **2** is an O-prenylated phenyl acetic acid, for which we propose the trivial name stachyline B.

The molecular formula of **3** was deduced by accurate mass measurement (HREIMS) to be $C_{13}H_{18}O_3$, requiring five sites of unsaturation. 1D and 2D NMR data of **3** were closely similar to those of **2** (see Tables 1, 2 and Supporting Information), except for the absence of resonances for the carboxylic acid group in the ^{13}C NMR spectrum and the presence of an additional resonance for a sp^3 methylene group. The latter had a downfield shifted ^{13}C NMR resonance (δ_C 64.1), which evidenced connection to an oxygen atom. Thus, **3** can be regarded as the C-8 reduction product of **2**, for which the trivial name stachyline C is suggested.

The molecular formula of **4** was deduced by accurate mass measurement (HRESIMS) to be $C_{13}H_{18}O_4$, requiring five sites of

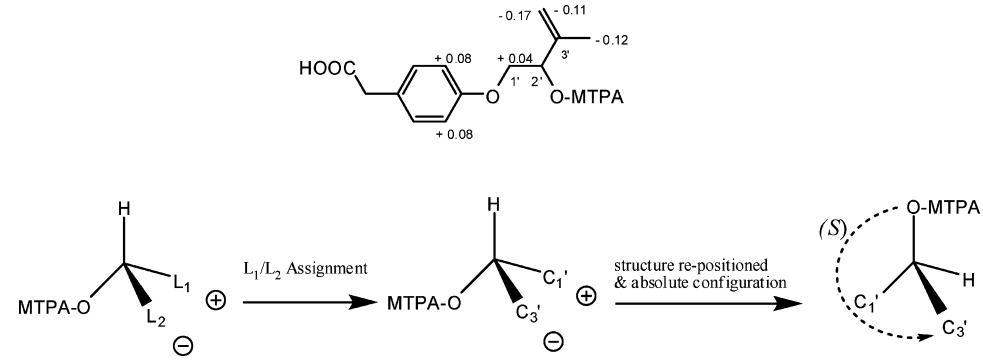

Figure 1. Deduction of the absolute configuration at C-2′ using modified Mosher's method with Δ^{S-R} values of MTPA esters of compound **2**.

unsaturation. 1D and 2D NMR spectroscopic data of compound **4** exhibited many identical features when compared with those of **2** (see Tables 1, 2 and Supporting Information). A difference, however, concerned the exomethylene proton signals, which were absent in the ^{1}H NMR spectrum of **4**, while signals for a methyl (H_3-4′, δ_H 0.97) and a methine group were present (H-3′, δ_H 1.86). ^{1}H−^{1}H COSY correlations confirmed the structure, since a spin system connected all atoms within the isoprene unit from H_2-1′ to H_3-5′ and H_3-4′ (see Table 2 and Supporting Information). For compound **4** the trivial name stachyline D is proposed.

Further compounds isolated were established to be known molecules, including etrogol (**5**),[4] tyrosol (**6**),[15] phenyl acetic acid (**7**),[16] benzoic acid (**8**)[17] and *para*-hydroxyphenyl acetic acid (**9**).[18,19]

Stachylines A, B, and C (**1**−**3**) were evaluated for a large number of pharmacological activities, including tumor cell cytotoxic activity, affinity to receptors from the central nervous system, antidiabetic activity, protein kinase inhibition, NF-κB protein complex inhibition, antibacterial and antifungal activity, antiplasmodial activity, and antiviral activity (HIV and influenza viruses). No activity in any of the test systems was found (see Supporting Information).

Stachylines A−D are presumably tyrosine-derived metabolites, a group of compounds rarely encountered in fungal metabolism. The oxime group in compound **1** is even more intriguing. Phenylpyruvic acid oxime derivatives,[11] brominated tyrosine-derived compounds like the bastadins and psammaplins,[20] and possibly biosynthetically related nitrile-containing metabolites[21] occur in marine sponges. The *Stachylidium* sp. used for this investigation was isolated from a marine sponge.

It was observed that the configuration of the oxime moiety in compound **1** was labile, and upon chromatographic isolation of the *E*- and *Z*-isomers, respective isomerization took place instantaneously. None of the isomers seemed to be thermodynamically more stable under the prevailing conditions, since a 1:1 ratio of isomers was obtained (see Supporting Information). In contrast to that, the geometrical isomers collismycins A and B from *Streptomyces* sp. MQ22 were reported to be non-interchangeable in many organic solvents and at room temperature. Only by heating in *ortho*-dichlorobenzene at 120 °C were they gradually interchangeable.[9] For 2-(4-hydroxyphenyl)-2-oxoacetaldehyde oxime (PHBA) isolated from *Penicillium olsonii*, the *E*-configuration was described without mentioning any isomerization reactions.[7]

Although the oxime functional groups are rare in natural products, they occur in a variety of phyla, e.g., sponges, bacteria, fungi, and plants. Tyrosine derivatives with an oxime moiety were found in actinomycetes, such as the β-lactam antibiotic nocardicin A from *Nocardia uniformis*.[6] Higher plants are also capable of producing oxime groups, since the biosynthesis of glucosinolates and cyanogenic glycosides, e.g., dhurrin from *Sorghum bicolor*, proceeds via aldoximes.[12,21−23] From these studies, oximes can be regarded as biosynthetic precursors of nitriles. Aspergillusol A was reported to be the first fungal tyrosine derivative with an oxime moiety;[5] however a previous publication had described the isolation of 2-(4-hydroxyphenyl)-2-oxoacetaldehyde oxime (PHBA) from *Penicillium olsonii*.[7] Hence, the current report is the third one of a fungal tyrosine-derived oxime derivative.

Concerning the biosynthesis of naturally occurring oximes, it is not clear which isomer is produced initially. The aldoxime intermediate in the biosynthesis of the cyanogenic glycoside dhurrin has been reported to initially have the *E*-configuration, subsequently undergoing nonenzymatic isomerization.[12,23] Nocardicin A, with *Z*-oxime configuration, is thought to be preferentially produced over the *E*-configurated nocardicin B, due to favorable intramolecular hydrogen bonding in biosynthetic intermediates.[13,14] In the case of bastadins and psammaplins, it was assumed that the *Z*-oxime configuration is initially produced during biosynthesis, followed by isomerization to the thermodynamically more favored *E*-isomers during extraction and isolation of the compounds.[20]

Stachylines A−D are proposed to be biosynthetically derived from tyrosine (Figure 2). For stachyline A the biosynthetic process may be similar to that of cyanogenic glycosides, e.g., dhurrin, in that the precursor tyrosine is N-hydroxylated by CYP 450 enzymes to *N*,*N*-dihydroxytyrosine and subsequently decarboxylated and dehydrated to form an aldoxime.[12] A similar pathway was described for the norcardicin biosynthesis.[13,14]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 140 polarimeter. UV and IR spectra were obtained employing a Perkin-Elmer Spectrum BX instrument. CD spectra were recorded in MeOH at room temperature using a JASCO J-810-150S spectropolarimeter. All NMR spectra were recorded in CD_3OD or $(CD_3)_2CO$ employing a Bruker Avance 300 DPX spectrometer. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 for CD_3OD and $\delta_{H/C}$ 2.04/29.8 for $(CD_3)_2CO$. HRESIMS were recorded on a Bruker Daltonik micrOTOF-Q time-of-flight mass spectrometer with ESI source. HPLC was carried out using a system composed of a Waters 515 pump together with a Knauer K-2300 differential refractometer. HPLC columns were from Knauer (250 × 8 mm, 5 μm, Eurospher-100 Si and 250 × 8 mm Eurospher-100, 5 μm, C18), flow rate 2 mL/min. Merck silica gel 60 (0.040−0.063 mm, 70−230 mesh) was used for vacuum liquid chromatography (VLC). Columns were wet-packed under vacuum using petroleum ether (PE). Before applying the sample solution, the columns were equilibrated with the first designated eluent. Standard columns for crude extract fractionation had dimensions of 13 × 4 cm.

Fungal Material. The marine-derived fungus *Stachylidium* sp. was isolated from the sponge *Callyspongia* sp. cf. *C. flammea* (collected at Bear Island, Sydney, Australia) and identified by P. Massart and C. Decock, BCCM/MUCL, Catholic University of Louvain, Belgium. A specimen is deposited at the Institute for Pharmaceutical Biology, University of Bonn, isolation number "293K04", strain number 220.

Culture, Extraction, and Isolation. Compounds **1** to **4** were isolated from three different cultures of *Stachylidium* sp. The first and second

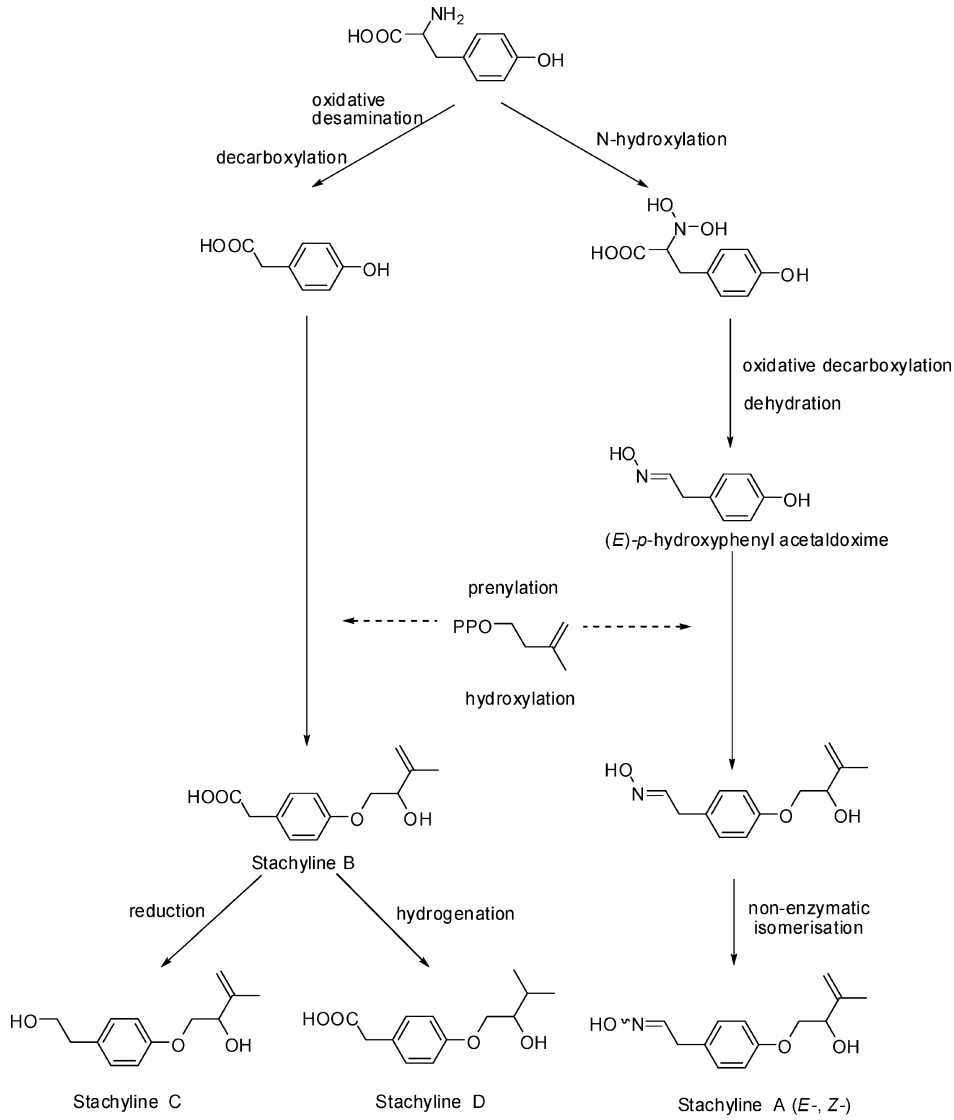

Figure 2. Proposed biosynthesis of stachylines A−D.

culture comprised 12 and 10 L of agar-biomalt medium (biomalt 20 g/L, 15 g/L agar) supplemented with sea salt incubated for 2 months (12 L) and 40 days (10 L) in Fernbach flasks at room temperature. The third culture was performed with 9.6 L of liquid YPM medium (yeast extract 5 g/L, peptone from YPM 3 g/L, mannitol 25 g/L) incubated for 10 days. An extraction with 5 L of EtOAc yielded 5.9, 2.1, and 1.0 g of extract, respectively, which were subjected to a VLC fractionation on silica gel, using a step gradient solvent system with petroleum ether−acetone from 10:1, 5:1, 2:1, 1:1, to 100% acetone and, subsequently, 100% MeOH, resulting in six VLC fractions for each culture. Compounds **2** and **3** were isolated from the first culture (12 L), while compound **4** was isolated from the second culture (10 L), and compound **1** was isolated from the third culture (YPM medium). The known compounds **6** and **9** were isolated from the first culture, and the known compounds **5**, **7**, and **8** from the first and third culture (isolation described for the YPM culture).

Compound **1** was isolated from VLC fraction 3 by NP-HPLC separation using petroleum ether−acetone (7:1) to yield six fractions. Subfractions 3 and 4 corresponded to a mixture of isomers of compound **1** (*E*- and *Z*-, 4.4 mg), which was again separated by RP-HPLC using 50% MeOH (t_R 11 and 15 min), but immediately formed the initial mixture of isomers in a ratio of 1:1. Compounds **2** and **3** were isolated from VLC fraction 3 by NP-HPLC using petroleum ether−acetone (11:2) to yield 10 fractions. Further RP-HPLC fractionation with subfraction 5 using 50% MeOH yielded five more subfractions, in which subfraction 4 was a mixture of compounds **2** and **3**. Finally, we performed NP-HPLC fractionation using petroleum ether−acetone (7:1) to afford compound **2** (fraction 1 of 2, 4.1 mg, t_R 19 min) and compound **3** (fraction 2 of 2, 2.2 mg, t_R 26 min). Compound **4** was isolated from VLC fraction 4, followed by HPLC fractionation using petroleum ether−acetone (9:2; subfraction 2 of 7), which was further purified using RP-HPLC with 60% MeOH (fraction 2 of 4, 1.8 mg, t_R 10 min).

Etrogol (**5**) was isolated from VLC fraction 3, followed by HPLC fractionation using petroleum ether−acetone (7:1) to yield five subfractions. Subfraction 1 was further purified using petroleum ether−acetone (7:1) to yield pure etrogol (fraction 3 of 3, 3.1 mg, t_R 26 min). Tyrosol (**6**) was isolated from VLC fraction 3 followed by HPLC fractionation using petroleum ether−acetone (5:1) to yield 10 fractions. Further RP-HPLC fractionation with subfraction 6 using 30% MeOH yielded the pure compound (fraction 3 of 7, 2.5 mg, t_R 19 min). Phenylacetic acid (**7**) and benzoic acid (**8**) were isolated from VLC fraction 3, followed by HPLC fractionation using petroleum ether−acetone (11:1) to yield five fractions. Benzoic acid was present in fraction 2 (7.9 mg, t_R 16 min), and phenylacetic acid in fraction 3 (11.2 mg, t_R 25 min). *para*-Hydroxyphenylacetic acid (**9**) was isolated from VLC fraction 3 followed by HPLC fractionation using petroleum ether−acetone (5:1) to yield 10 fractions. Further RP-HPLC fractionation with subfraction 6 using 30% MeOH yielded the pure compound (fraction 4 of 7, 1.8 mg, t_R 27 min).

Stachyline A, mixture of *E*-/*Z*-isomers, 1:1 (1): white, amorphous solid (458 μg/L, 0.43%); $[\alpha]^{23}_D$ −15 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.60), 222 (2.84), 276 nm (2.76); IR (ATR) ν_{max} 3300 (br), 2922, 1610, 1510 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2 and Supporting Information); ESIMS *m*/*z* 236.1 $[M + H]^+$; HREIMS *m*/*z* 235.1208 $[M]^+$ (calcd for $C_{13}H_{17}NO_3$, *m*/*z* 235.1208); HRESIMS *m*/*z* 258.1125 $[M + Na]^+$ (calcd for $C_{13}H_{17}NNaO_3$, *m*/*z* 258.1101).

Stachyline B (2): white, amorphous solid (340 μg/L, 0.07%); $[\alpha]^{23}_D$ −12 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.35), 227 (3.75), 276 (3.05), 283 nm (2.75); CD (*c* 1.06 × 10^{-6} mol/L, MeOH), λ ($\Delta\varepsilon$) = 197 (+1.78); IR (ATR) ν_{max} 3364 (br), 2929, 1610, 1511 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2 and Supporting Information); EIMS *m*/*z* 236.1 $[M]^+$; HREIMS *m*/*z* 236.1051 $[M]^+$ (calcd for $C_{13}H_{16}O_4$, *m*/*z* 236.1049).

Stachyline C (3): white, amorphous solid (183 μg/L, 0.04%); $[\alpha]^{23}_D$ −16 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.43), 225 (3.85), 277 (2.95), 283 nm (2.95); CD (*c* 1.25 × 10^{-6} mol/L, MeOH) λ ($\Delta\varepsilon$) = 199 (+1.94); IR (ATR) ν_{max} 3235 (br), 2937, 1685, 1509 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2 and Supporting Information); EIMS *m*/*z* 222.1 $[M]^+$; HRESIMS *m*/*z* 245.1162 $[M + Na]^+$ (calcd for $C_{13}H_{18}NaO_3$, *m*/*z* 245.1148).

Stachyline D (4): white, amorphous solid (180 μg/L 0.09%); $[\alpha]^{23}_D$ −6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 205 nm (3.86), 226 (3.86), 276 (3.08), 283 nm (2.90); IR (ATR) ν_{max} 3265 (br), 1687, 1512 cm^{-1}; ^{1}H NMR and ^{13}C NMR (Tables 1 and 2 and Supporting Information); ESIMS *m*/*z* 237.1 $[M - H]^-$; HRESIMS *m*/*z* 261.1096 $[M + Na]^+$ (calcd for $C_{13}H_{18}NaO_4$, *m*/*z* 261.1097).

Preparation of the (*R*)- and (*S*)-MTPA Esters of Compound 2. The secondary alcohol (1 equiv, 1 mg, 4.23 × 10^{-6} μmol) was dissolved with the corresponding MTPA-Cl (10 equiv) in an adequate quantity of deuterated pyridine and DMAP (1 equiv) in an NMR tube. The reaction was followed by ^{1}H NMR to observe the downfield proton shift of H-2′. After 1 h reaction time, the final ^{1}H NMR was recorded, and the sample dried and redissolved in D-acetone for ^{1}H−^{13}C HSQC measurements. The $\Delta^{(S-R)}$ values between (*S*)- and (*R*)-MTPA esters were recorded with the help of both ^{1}H NMR and ^{1}H−^{13}C HSQC (see Figure 1 and Supporting Information).

Bioassays. The referenced compounds were tested in antibacterial (*Escherichia coli*, *Bacillus megaterium*), antifungal (*Mycotypha microspora*, *Eurotium rubrum*, and *Microbotryum violaceum*), and antialgal (*Chlorella fusca*) assays,[25,26] protein kinase DYRK1A and CDK5 inhibition assays,[27] HIV-1 and HIV-2 virus assays,[28,29] and the cytotoxic activity assay against a panel of five tumor cell lines, NCI-H460/lung, A549/lung, MCF7/breast, SF268/CNS, and CAKI/renal.[30,31] Compounds were further tested for antiplasmodial activity against *Plasmodium berghei*,[32] in a influenza B virus (Flu B) assay,[33] in binding assays against a panel of 44 psychoactive receptors,[34] for the inhibition of the NF-κB protein complex assay,[35] and in a panel of assays toward antidiabetic activity.[36−38]

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Supporting Information Available: Spectroscopic data and other relevant information are included for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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