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Cohaerins C–F, four azaphilones from the xylariaceous fungus *Annulohypoxylon cohaerens*

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Dedicated to the celebration of the 65th birthday of Professor Yoshinori Asakawa

Abstract—Four azaphilones named cohaerins C–F, along with 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl were isolated from the methanolic extract of the stromata of *Annulohypoxylon cohaerens* (Ascomycetes, Xylariaceae). Cohaerins C–E constitute typical azaphilones, bearing a γ-lactone ring, while cohaerin F has an unprecedented carbon skeleton, lacking the lactone ring of the azaphilones and with an aliphatic side chain being attached directly to the azaphilone backbone by a C–C bond. Their structures were determined by 2D NMR, IR, UV, and CD spectroscopy. They showed moderate inhibitory activity of nitric oxide production in RAW cells, and strong and nonselective antimicrobial effects.

1. Introduction

The new genus *Annulohypoxylon* (Ascomycota, Xylariaceae) has recently been recognized from a comparison of morphological features and molecular taxonomy and segregated from the related genus *Hypoxylon*.[1] *Annulohypoxylon* had until recently been treated as *Hypoxylon* sect. *Annulata*[2] and is believed to have evolved from the same evolutionary lineage as *Hypoxylon* and *Daldinia*. The fruit bodies (stromata) of all these genera are usually associated with—and commonly encountered on—woody angiosperms.

This study relates to our ongoing projects on the pigments and other secondary metabolites produced in abundance by *Hypoxylon* and its relatives, which have been known to be chemotaxonomically significant for a long time.[3,4] In fact, the characteristic pigments of certain *Annulohypoxylon* spp. [multiformins from *Hypoxylon* (=*Annulohypoxylon*) *multiforme*[5] and cohaerins from *Hypoxylon* (=*Annulohypoxylon*) *cohaerens*][6] were only recently isolated and identified. Using HPLC profiling based on diode array and ESIMS data, a broad range of species, including type and other authentic material, were examined in the latter study.[6] These chemotaxonomic results also confirmed the new generic concept:[1] *Annulohypoxylon* and *Hypoxylon* as understood today (i.e., sect. *Annulata* and *Hypoxylon* in Refs. 2 and 5) significantly differ in the distribution of stromatal secondary metabolites. Several hundreds of their specimens, including representatives of most currently accepted species, were already studied by HPLC profiling. According to these results, no azaphilone type contained in *Hypoxylon* was ever found in *Annulohypoxylon* and vice versa. Further, the metabolites that are frequently encountered in *Annulohypoxylon* are BNT (**5**, ubiquitous in all of its species and many other Xylariaceae), daldinone A (also present in a few other genera), truncatone[6,7] (apparently restricted to *Annulohypoxylon*[2,6]), and spider sex pheromones that were hitherto only found in *Annulohypoxylon annulatum*.[8] The genus *Hypoxylon* is instead characterized by the occurrence of, e.g., mitorubrins, entonaemins, rubiginosins, and daldinin type azaphilones, and by the lack of truncatone, cohaerins, and multiformins.[4]

The above HPLC-based study[6] was rather conclusive with respect to the general occurrence of chemical types of

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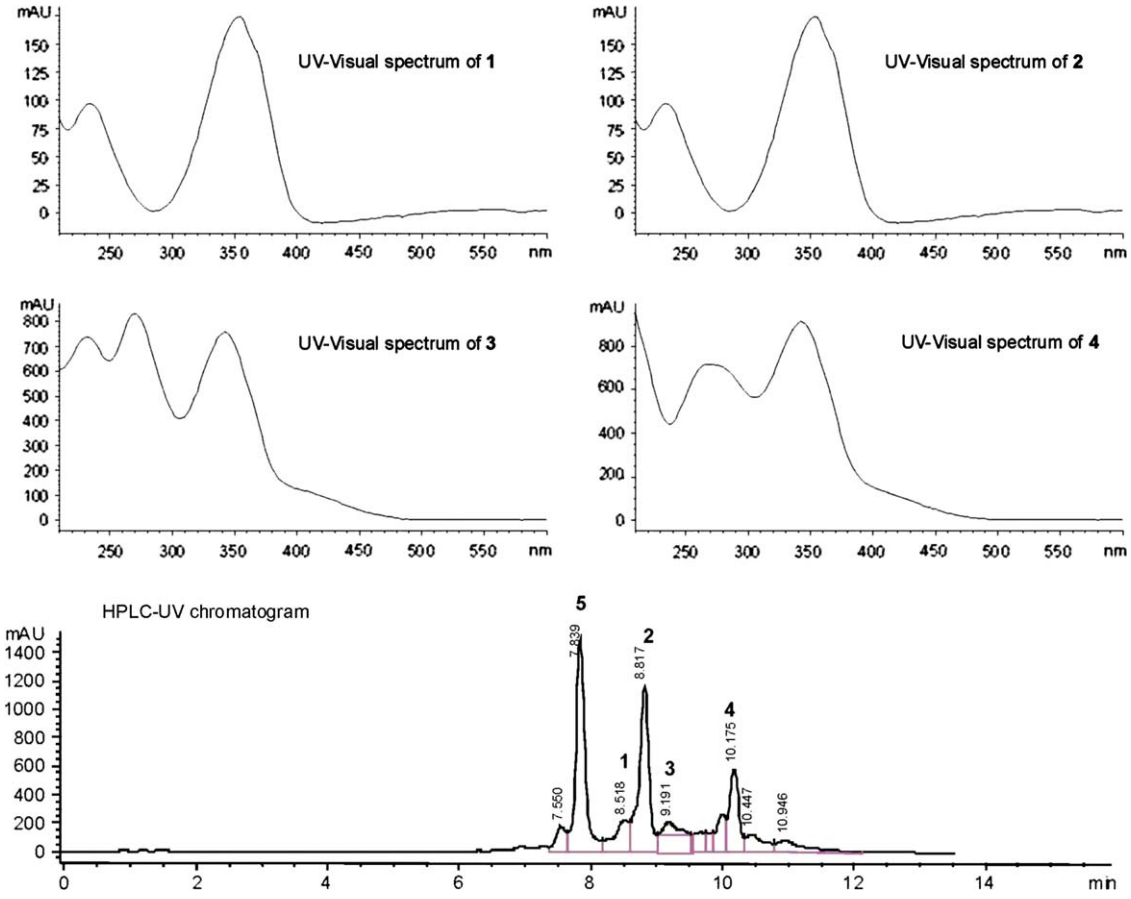

Figure 1. HPLC–UV chromatogram (210 nm) of the crude stromatal MeOH extract of *A. cohaerens* STMA 05296 and UV–vis spectra of cohaerins C–F (**1**–**4**). Besides the new compounds, BNT (**5**), another major component, is also indicated in the chromatogram trace; other peaks relate to unknown minor components. Column: Merck Lichrospher C18 (5 mm, 125×4 mm); mobile phase: 0.1% H_3PO_4 (A):acetonitrile (B); linear gradient from 0 to 100% B in 10 min; thereafter continuing at 100% B (for details on method see Refs. 6,9, and 10).

pigments in these fungi, and their characteristic metabolites were still detectable in type specimens over 200 years old. Nonetheless, little is known about the aspects such as the host-specific metabolite production in the species of *Annulohypoxylon*, or on the occurrence of chemical races in certain geographic regions. HPLC profiling is being used as a routine procedure in our floristic and chemotaxonomic studies of *Hypoxylon* and allied genera,[9–12] and so far all materials referable to *A. cohaerens* showed the same HPLC profile, with BNT (**5**) and cohaerins A (**6**) and B[6] as prevailing components. However, we recently noted that some specimens of *A. cohaerens* from Central and Southeastern Europe showed a deviating HPLC profile (see Fig. 1). Despite their typical morphology, cohaerins A (**6**) and B[6] lacked in their HPLC chromatograms, and other components with similar UV–vis spectra were observed. One of these specimens was therefore obtained in sufficient quantities, which allowed us to study its chemical constituents, resulting in the identification of four new azaphilones named cohaerins C–F (**1**–**4**). We would like to report here their isolation and biological activities.

2. Results and discussion

The MeOH extract of *A. cohaerens* STMA 04158 was subjected to conventional purification procedures, resulting in the isolation of four new azaphilone derivatives (**1**–**4**), along with the known compound, 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl (BNT, **5**).[11]

Compound **1** gave a molecular ion peak at *m*/*z* 505 $(M+Na)^+$ in the FABMS, which, is in accordance with the data obtained from the NMR spectra, corresponded to the molecular formula $C_{28}H_{34}O_7$, which was determined by HRFABMS. Its IR and UV–vis spectra showed the presence of a γ-lactone (1784 cm^{-1}), a conjugated ketone (1717 cm^{-1}; 269 and 341 nm), and an olefinic functional group (1637 cm^{-1}). The ^1H NMR spectrum of **1** displayed the typical pattern of an azaphilone skeleton with three olefinic protons attributed to H-1, H-4, and H-5. In addition, a singlet methyl was located at C-9. Interpretation of 2D NMR spectrum indicated the presence of two subunits linked to the main azaphilone backbone at C-3 and C-18. The spectral data of the first subunit were identical to those of 4-hydroxy-2-methyl-6-oxocyclohex-1-enyl, i.e., a partial structure of cohaerin B.[6] This unit was also connected with the main azaphilone structure at C-3 by HMBC correlations between H-4 and C-10, and H-16 and C-3. Interpretation of ^1H–^1H COSY spectrum of **1** suggested that the second unit was an aliphatic 2-methyl-octanoyl side chain. The connection of this unit and the main azaphilone backbone was determined at C-18, based on HMBC correlations between H-18 and C-19, and H-20 and C-18 and C-19. Furthermore, H-8 coupled to C-17 and

C-18, and H-9 coupled to C-7, C-8, and C-17 in the HMBC spectrum, which confirmed the presence of a γ-lactone in the molecule. The relative stereochemistries of H-8, H-9, and H-18 were established to be α-oriented, due to their NOESY correlations between H-8 and H-18, and H-9 and H-18, respectively. However, the relative stereochemistry of the hydroxyl group at C-13 and the secondary methyl at C-20 remained unclear. In addition, the absolute configuration at C-7 was found to be *R* based on its CD spectrum, which showed positive (354 and 230 nm) and negative (257 nm) Cotton effects.[13] Based on the above spectral evidence, cohaerin C (**1**) was determined to be 3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-6*a*(*R*)-methyl-9-(2-methyloctanoyl)-9(*S*),9*a*(*S*)-dihydro-6*aH*-furo[2,3-*h*]isochromene-6,8-dione as shown in Fig. 2.

Cohaerin D (**2**) has the molecular formula $C_{28}H_{32}O_7$ based on HRFABMS with two hydrogen atoms less than cohaerin C (**1**). Its spectral data are similar to those of **1**, except for the presence of a double bond at C-8 and C-18 to form an unsaturated lactone, which was revealed by IR absorption band at 1764 cm^{-1} and the downfield shifts of C-8 and C-18 compared with those of **1** in ^{13}C NMR. The absolute configuration at C-7 was also determined to be *R* based on the CD spectrum with positive (353 nm) and negative (280 nm) Cotton effects.[13] Thus, cohaerin D (**2**) was elucidated to be 3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-6*a*(*R*)-methyl-9-(2-methyloctanoyl)-6*aH*-furo[2,3-*h*]isochromene-6,8-dione.

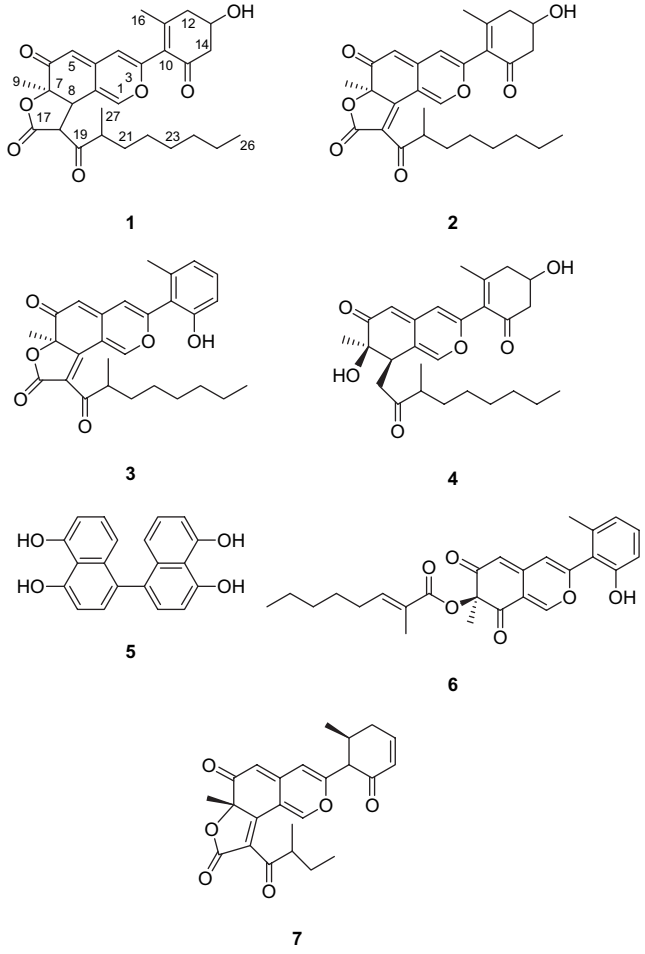

Figure 2. Structures of **1**–**7**.

A molecular formula of $C_{28}H_{30}O_6$ was determined for cohaerin E (**3**) on the basis of the observed molecular ion peak at *m*/*z* 463.2148 [Calcd for $C_{28}H_{31}O_6$ $(M+H)^+$, 463.2121] in its HRFABMS. The NMR spectra of **3** resembled those of **2**. The only notable difference was observed in the signals of the partial structure at C-3, which was found to be 2-hydroxy-6-methylphenyl as deduced from its 2D NMR spectra and comparison with the data for cohaerin A (**6**).[6] The absolute configuration at C-7 was also found to be *R* by comparing its CD spectrum (see Section 3) with those of cohaerins C and D (**1**–**2**). From the above spectral evidence, cohaerin E (**3**) was determined to be 3-(2-hydroxy-6-methylphenyl)-6*aR*-methyl-9-(2-methyloctanoyl)-6*aH*-furo[2,3-*h*]isochromene-6,8-dione.

Cohaerin F (**4**) exhibited a *quasi*-molecular ion peak at *m*/*z* 479.2442 $(M+Na)^+$ corresponding to a molecular formula of $C_{27}H_{36}O_6Na$ as determined by HRFABMS [Calcd for $C_{27}H_{36}O_6Na$ $(M+Na)^+$, 479.2410]. Its IR spectrum showed only the absorption bands of ketones (1711 and 1678 cm^{-1}). Further interpretation of its NMR spectral data revealed that **4** contained the same cyclohexadienone group at C-3 as found in **1**. In comparison with the structure of cohaerin C (**1**), the lactone group had disappeared, but signals for a methylene group at C-18 were noted and correlated with C-8 and C-19 in its HMBC spectrum (Fig. 3). Taking into account the 2D NMR spectra and comparing with those of **1**–**3**, we could deduce the structure of **4** as shown in Figure 2. Furthermore, the absolute configuration of C-7 was established to be *R* by comparing its CD spectrum (see Section 3) with those of **1**–**3**. In addition, both H-8 and H-9 were in α-face, since they correlated in NOESY spectrum. Consequently, cohaerin F (**4**) is 7-hydroxy-3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-7(*R*)-methyl-8-(3-methyl-2-oxononyl)-7,8-dihydro-isochromen-6-one.

Recent studies have revealed that the Xylariaceae azaphilones possess broad-spectral activities in biological systems.[5,13,14] Especially, they exhibited nonselective antimicrobial activities, which suggested their role for protection of the fungal stromata from feeding enemies in the environment.[2,6,15] Some of their pigments are also potent nitric oxide inhibitors in macrophages.[16] In this paper, we continued to screen the inhibitory activity of nitric oxide production in RAW 264.7 cells by the cohaerins C–F, which showed moderate activity with their IC_{50} values of 30.2 (**1**), 19.6 (**2**), 26.1 (**3**), and 41.2 μM (**4**), respectively. These

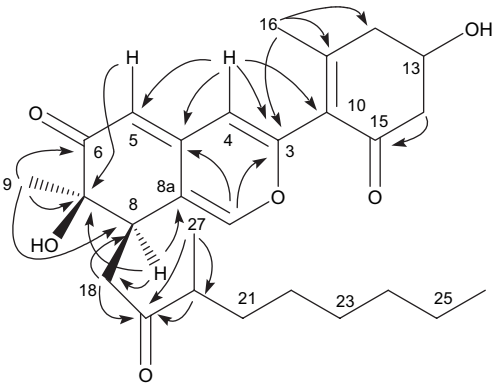

Figure 3. Important HMBC correlations of **4**.

Table 1. Antimicrobial activities of metabolites from *Annulohypoxylon* and two standard antibiotics in the agar diffusion assay against *Bacillus subtilis* (NB medium, after 18 h of incubation) and *Yarrowia lipolytica* (YMG medium, after 24 h of incubation)

Compound	*B. subtilis*	*Y. lipolytica*
Cohaerin C (**1**)	**13**	**11**
Cohaerin D (**2**)	**12**	**12**
Cohaerin E (**3**)	**14**	**12**
Cohaerin F (**4**)	**14**	**12**
From previous work		
Cohaerin A (**6**)	**12**	**10**
Multiformin B (**7**)	**11**	**9**
Standards		
Penicillin G	**23**	—
Actinomycin D	—	**17**

Inhibitory concentrations are given in millimeter for diameter of inhibition zone for concentrations of 50 mg/paper disk (diameter of paper disk; 6 mm). (—) Indicates lack of activity.

results again suggested that the presence of a lactone ring in the molecule increased the activity, but the activities were substantially weaker than those of some azaphilones from *Hypoxylon*, such as rutilins and rubiginosin A.[16] By contrast, the antimicrobial activities (Table 1) did not deviate much from other compounds previously evaluated, including cohaerin A (**6**) and multiformin B (**7**), which were tested for comparison. No particular selectivity against the yeast *Yarrowia lipolytica* or the bacterium *Bacillus subtilis* was observed.

Previous work on the related genera *Daldinia*[10] and *Hypoxylon*[11,12] had revealed rather constant secondary metabolite profiles in a given species, and this was confirmed by examination of hundreds of specimens. Nonetheless, so far there is no evidence that the specimens used for isolation of cohaerins C–F belong to a new morphological species or variety, despite it yielded four unprecedented azaphilones and lacked the cohaerins A and B that were previously attributed to this species.[6] Neither the morphological features (asci, ascospores, perithecia, ostioles, etc.) of the teleomorph nor the conidiogenous structures of the anamorph deviated from typical *A. cohaerens.* All of them were even collected from *Fagus*, as typical for this highly host-specific species. Since the stromata of the specimens STMA 04158, STMA 05161, and STMA 05296 also gave the typical olivaceous color in KOH that is useful to distinguish *A. cohaerens* from morphologically similar, related species, including *A. multiforme*[2] (as *Hypoxylon* spp.), it is not possible to distinguish the 'chemical races' of *A. cohaerens* without the aid of HPLC or, possibly, TLC.

A comparison of the structural features of **1**–**4** with those of other known azaphilones of *Annulohypoxylon* suggests that their biogenesis maybe quite different from those of the cohaerins A and B, and multiformins. Compounds **1**–**3** and the multiformins[5] possess a tricyclic ring attached by an ester bond to a branched unsaturated C-8 fatty acid and an additional lactone ring is present. In contrast, **4** remains tricyclic but lacks the ester bond of the cohaerins. The multiformins differ from all cohaerins in having a shorter side chain attached to their tetracyclic ring. All of them indeed have carbon skeletons quite different from those of the daldinins or the orsellinic acid containing azaphilones such as mitorubrin that are found in the related genera *Daldinia* and *Hypoxylon*, respectively.[7,10–12] Molecular taxonomy, including studies on polyketide synthases and other enzymes of secondary metabolite biogenesis, may eventually show whether the current fungus constitutes a cryptic species within *A. cohaerens.* For such purposes, a culture of the fungus was deposited in a public collection.

Trace amounts of mitorubrin derivatives[10] were also isolated from the stromatal extract, which was later shown to be due to the fact that the substrate contained some small stromata of *Hypoxylon fragiforme*, another fungus commonly encountered on *Fagus* in Europe. Stromata of *A. cohaerens* from the voucher specimens were found devoid of mitorubrins by HPLC–MS. This observation relates to the problem that different Xylariaceae species may frequently colonize the same substrate, with their fruit bodies becoming intermingled.[11] Special care must therefore be taken in the examination of such material to be extracted.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-1000 polarimeter in $CHCl_3$. IR spectra were measured on a Perkin–Elmer Spectrum One FTIR spectrometer. UV–vis spectra were obtained on a Shimadzu UV-1650PC in MeOH. CD spectra were measured on a JASCO J-725 spectrometer in MeOH. Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR). Column chromatography was carried out on silica gel 60 (0.2–0.5 and 0.04–0.063 mm, Merck), reverse-phase C_{18} silica gel (Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, $CHCl_3$–MeOH, 1:1). HPLC analyses were carried out as described earlier.[6]

3.2. Fungal material

Stromata of *A. cohaerens* was collected by N. Radulovíc at Mt. Suva Planina, near Niš city, Serbia and Montenegro, from decaying tree trunks of *Fagus sylvatica* in September 2004 (STMA 04158, used for preparative work) and again on the same site in September 2005 (STMA 05296, see Fig. 4). Another specimen (STMA 05161) that showed the same chemotype was collected by M.S. on 17 July 2005 from *F. sylvatica* in Austria, Lower Austria Prov., Mauerbach, Nature reserve Kartause, in the course of the mycological excursion of the 16th International Botanical Congress (Vienna). The material was identified by the authors. Voucher specimens bearing the above STMA numbers are deposited at the mycological herbarium, Staatliches Museum für Naturkunde, Karlsruhe, Germany. A culture obtained from specimen STMA 05296 has been deposited with CBS, Utrecht, The Netherlands as Strain nr CBS 119311.

3.3. Extraction and isolation

The crude extract (4.9 g from 47 g of dry stromata) was chromatographed by Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) to give six fractions.

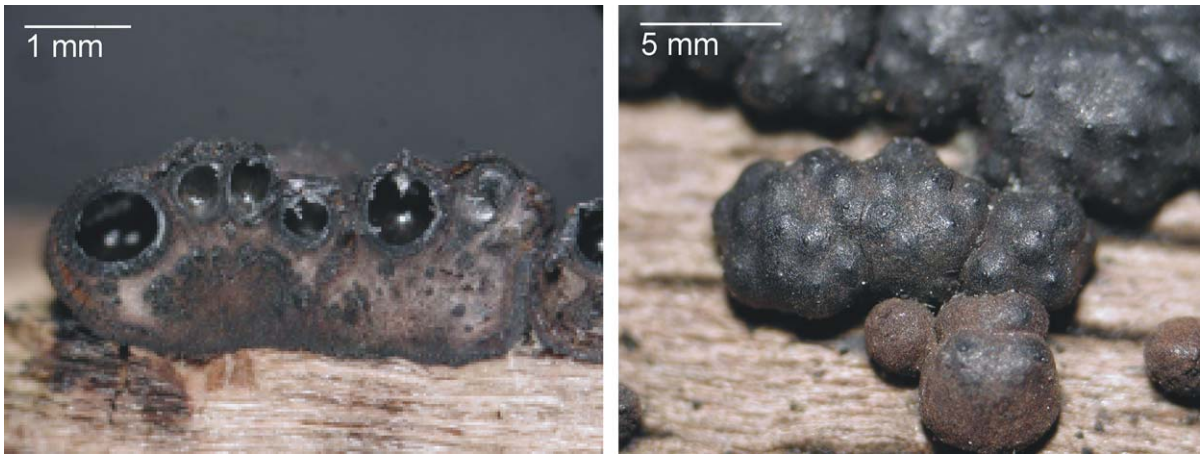

Figure 4. Stromata of *A. cohaerens* STMA 05296 on decorticated wood of *F. sylvatica*. Left: section through stromata. Right: stromatal habit. Scale is indicated by bars.

The first fraction (1330.5 mg) was purified by SiO_2 column chromatography ($CHCl_3$–MeOH, 30:1) to afford **1** (95.3 mg), **2** (176.2 mg), and two sub-fractions 1–1 (154.1 mg) and 1–2 (114.2 mg), which were further separated by reversed-phase column chromatography, using MeOH–H_2O (4:1) as mobile phase to yield **3** (27.9 mg) and **4** (12.0 mg), respectively. Second fraction (1079.7 mg) was subjected to SiO_2 column chromatography, using a $CHCl_3$–MeOH gradient from 0 to 10% MeOH, to give **1** (54.8 mg), **2** (142.3 mg), and a mixture (164.0 mg) that was purified by reversed-phase column chromatography (MeOH–H_2O, 4:1) to obtain **3** (22.2 mg). Sixth fraction (216.3 mg) was BNT (**5**) in pure state.

3.3.1. Cohaerin C (1). $[\alpha]_D^{20}$ +3.7 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3425, 1784, 1717, 1637, 1530, 1458, 1380, 1188, 1066, 976; UV λ_{max} nm (log ε): 353 (4.1), 233 (3.9); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 354 (+3.0), 257 (−1.0), 230 (+1.0); FABMS *m/z* 505 $(M+Na)^+$; HRFABMS *m/z* 505.2206 [Calcd for $C_{28}H_{34}O_7Na$ $(M+Na)^+$, 505.2202]; ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.2. Cohaerin D (2). $[\alpha]_D^{20}$ −2.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3427, 1764, 1683, 1627, 1537, 1456, 1379, 1248, 1157, 1068, 754; UV λ_{max} nm (log ε): 341 (4.2), 269 (3.3); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 353 (+3.3), 280 (−2.8); FABMS *m/z* 503 $(M+Na)^+$; HRFABMS *m/z* 503.2031 [Calcd for $C_{28}H_{32}O_7Na$ $(M+Na)^+$, 503.2046]; ^1H and ^{13}C NMR (CD_3OD) data, Tables 2 and 3.

3.3.3. Cohaerin E (3). $[\alpha]_D^{20}$ −1.6 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3314, 1766, 1683, 1624, 1532, 1466, 1167, 1013, 875; UV λ_{max} nm (log ε): 344 (3.1), 267 (3.0); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 359 (+12.8), 291 (−6.4), 261 (−7.1); FABMS *m/z* 463 $(M+H)^+$; HRFABMS *m/z* 463.2148 [Calcd for $C_{28}H_{31}O_6$ $(M+H)^+$, 463.2121]; ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.4. Cohaerin F (4). $[\alpha]_D^{20}$ +1.2 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3415, 1711, 1678, 1615, 1548, 1456, 1379, 1167, 1070, 782; UV λ_{max} nm (log ε): 354 (3.0), 232 (2.9); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 370 (+1.0), 318 (+0.8), 254 (−0.7), 228 (+0.9); FABMS *m/z* 479 $(M+Na)^+$; HRFABMS *m/z* 479.2442 [Calcd for $C_{27}H_{36}O_6Na$ $(M+Na)^+$, 479.2410); ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.5. Bioassays. Inhibition of nitric oxide production of **1–4** in RAW 264.7 cells stimulated by lipopolysaccharide was evaluated by the same method as previously reported.[16]

Table 2. ^1H NMR data for cohaerins C–F (**1–4**)

Position	1	2	3	4
1	6.87 (s)	8.75 (s)	8.91 (d, 0.6)	6.83 (t, 1.4)
4	6.15 (s)	6.47 (s)	6.49 (s)	6.08 (s)
5	5.41 (d, 1.1)	5.39 (s)	5.42 (d, 1.1)	5.45 (d, 1.1)
8	3.85 (dt, 1.9, 7.4)			3.32 (td, 1.9, 9.9)
9	1.40 (s)	1.70 (s)	1.75 (s)	1.13 (s)
12	2.80 (m), 2.62 (m)	2.89 (dd, 4.1, 18.4), 2.61 (dd, 5.8, 18.4)	6.84 (d, 7.4)	2.62 (overlapped), 2.80 (overlapped)
13	4.39 (m)	4.29 (m)	7.24 (t, 8.0)	4.38 (m)
14	2.80 (m), 2.62 (m)	2.77 (dd, 3.6, 16.2), 2.56 (dd, 7.4, 16.2)	6.84 (d, 8.0)	2.80 (overlapped), 2.60 (overlapped)
16	2.06 (s)	2.09 (s)	2.31 (s)	2.05 (s)
18	4.13 (d, 12.6)			3.22 (dd, 1.9, 17.6), 2.79 (m)
20	3.22 (m)	3.56 (q, 6.6)	3.62 (m)	2.67 (q, 6.9)
21	1.76 (m), 1.34 (m)	1.58 (m), 1.33 (m)	1.55 (m), 1.28 (m)	1.34 (m), 1.68 (m)
22	1.28 (m), 1.20 (m)	1.13 (m), 1.02 (m)	1.12 (m), 1.18 (m)	1.22 (m), 1.27 (m)
23	1.24 (m)	1.30 (m)	1.25 (m)	1.25 (m)
24	1.22 (m)	1.11 (m)	1.15 (m)	1.24 (m)
25	1.23 (m)	1.16 (m)	1.19 (m)	1.26 (m)
26	0.87 (t, 7.1)	0.82 (t, 7.1)	0.81 (t, 7.1)	0.87 (t, 7.1)
27	1.23 (d, 7.1)	1.12 (d, 6.6)	0.88 (d, 7.4)	1.15 (d, 6.9)

Table 3. ^{13}C NMR data for cohaerins C–F (**1**–**4**)

Position	**1**	**2**	**3**	**4**
1	143.8	155.0	153.8	145.2
3	153.3	155.8	154.5	153.0
4	111.8	114.4	113.1	111.8
5	107.1	106.3	105.5	105.3
6	192.0	192.9	190.9	199.3
7	82.5	89.1	87.7	73.0
8	43.5	166.5	165.4	40.2
9	19.1	26.0	26.4	21.4
10	130.6	130.9	118.5	130.8
11	160.7	164.5	155.7	160.3
12	40.8	41.7	113.9	40.9
13	65.3	66.1	131.8	65.4
14	46.1	46.7	122.8	46.2
15	194.0	196.5	139.0	193.9
16	23.0	23.0	20.2	23.0
17	169.0	169.6	167.9	
18	51.2	125.7	124.2	40.2
19	206.0	202.1	201.4	213.3
20	45.7	44.7	43.7	46.3
21	31.7	34.6	33.4	32.9
22	27.0	27.7	26.9	27.2
23	29.3	30.3	29.1	29.3
24	31.6	32.8	31.6	31.6
25	22.5	23.5	22.4	22.6
26	14.0	14.4	14.0	14.0
27	17.2	15.2	14.8	16.8
4a	144.9	146.5	144.6	146.9
8a	116.3	112.5	111.4	120.5

Antimicrobial activities were tested as reported concurrently[17] (in slight modification of the method reported in Ref. 15).

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