Full Papers

Fluorescent Compounds from the Cultured Mycobiont of *Amygdalaria panaeola*

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New fluorescent compounds, named panaefluorolines D−H, were isolated from the cultured mycobiont of a lichen, *Amygdalaria panaeola*. The structures were elucidated on the basis of spectroscopic data, especially 2D-NMR. The relative configuration of panaefluoroline D was determined by means of X-ray crystallographic analysis.

Lichens are symbiotic associations of algal and fungal partners with worldwide distribution. They produce many characteristic phenolic compounds, such as depsides, depsidones, and dibenzofurans, which are considered to be biosynthesized by their fungal partners.[1] We have reported the structures of various secondary metabolites.[2−6] In addition, novel metabolites have been isolated from mycobionts of lichens cultured under stress conditions, for example under osmotic stress or in the presence of 10% sucrose.[7−12] Our studies of secondary metabolites of cultured mycobionts of lichens have uncovered new naphthoquinone derivatives[12] and a zealalenone derivative.[13] Moreover, we have isolated two bioactive isofuranonaphthoquinone derivatives, bostrycoidin and 8-*O*-methylbostrycoidin, from the cultured mycobiont of *Arthonia cinnabarina* (DC.) Wallr.[13]

We have isolated thallus fragment spores of *Amygdalaria panaeola* (Ach.) Hertel & Brodo, collected in Finland in 1990, and subsequently were able to culture the mycobiont on malt-yeast liquid medium. A fluorescent yellowish green coloration appeared after one week of cultivation. Culture was continued for 4 weeks, then the yellowish green-colored compounds were isolated chromatographically and their structures were determined. We have already reported three novel fluorescent compounds, named panaefluorolines A−C, from the culture filtrate of *A. panaeola*.[14] In the present paper, we report the isolation and structure elucidation of five new fluorescent compounds, named panaefluorolines D (**1**), E (**2**), F (**3**), G (**4**), and H (**5**). The relative configuration of **1** was determined by means of X-ray analysis.

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Results and Discussion

Panaefluoroline D (**1**) was obtained as yellow crystals from $CHCl_3$−MeOH−acetone, $[\alpha]_D^{22}$ +286.6° (*c* 0.61, MeOH), and the molecular formula $C_{18}H_{21}NO_5$ was determined from the positive HRFABMS data, *m/z* 332.1510 $[M + H]^+$ (calcd for $C_{18}H_{22}NO_5$, 332.1498). The UV spectrum of **1** was similar to that of panaefluoroline A.[14] The IR spectrum exhibited hydroxyl (3300 cm^{-1}) and carboxyl (1635 cm^{-1}) absorptions. The ^1H NMR spectrum (Table 1) of **1** showed three methyl singlets at δ 1.26, 1.38, and 2.81. The signal at δ 2.81 was assigned to a methyl group linked to an sp^2 carbon, on the basis of its chemical shift. The ^{13}C NMR spectrum and DEPT revealed the presence of three methyls, two methylenes, six methines, and seven quaternary carbons, including one carboxylic carbonyl carbon. These ^1H and ^{13}C NMR patterns are similar to those of panaefluorolines A−C,[14] except for the signals due to the side chain on the isoquinoline skeleton. All proton and carbon signals were assigned with the aid of HMQC, ^2J and ^3J HMBC, and DQF-COSY experiments. The side chain showed signals due to one methylene, one methine, and one carbonyl. The methine proton at δ 5.53 (1H, dd, J = 7.4, 3.5 Hz) had an HMQC correlation with the signal at δ 73.0 and showed HMBC correlations with the alcoholic methylene carbon at δ 63.6 and the carbonyl carbon at δ 170.0. Thus, the structure of the side chain was concluded to be HO−CH_2−CH−COO^-. The methine proton at δ 5.53 showed an HMBC correlation with a signal at δ 146.6 due to an aromatic methine carbon in the isoquinoline skeleton, and the methine carbon (δ 73.0) bearing this proton was considered to be linked to the nitrogen atom. These data suggested that the nitrogen was quaternary and the carboxylic acid was ionized. The proposed structure of **1** is consistent with the molecular formula, $C_{18}H_{21}NO_5$, and is supported by the positive HRFABMS data, positive FABMS *m/z* 332 $[M + H]^+$, and negative FABMS *m/z* 331 (M^-), as well as by the phase-sensitive NOESY correlations (Figure 1) and DQF-COSY correlations.

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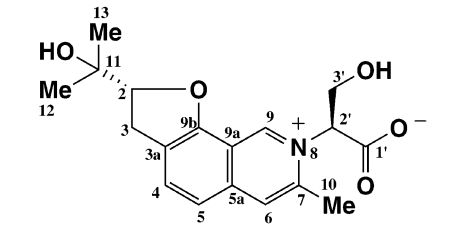

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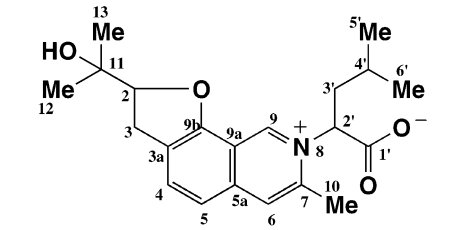

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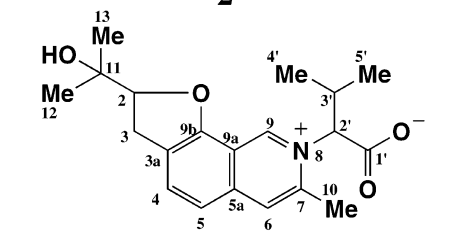

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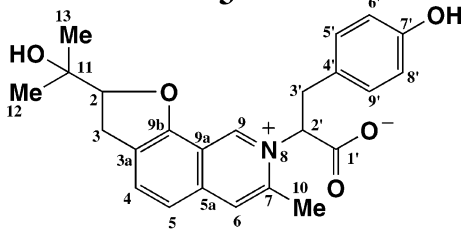

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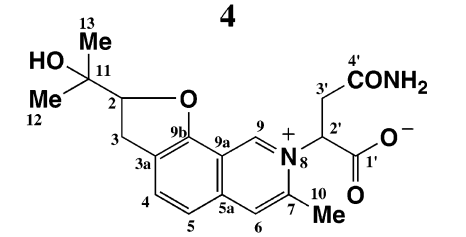

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Finally, single-crystal X-ray analysis unambiguously confirmed the structure of **1** (Figure 2). The crystal structure has three independent molecules in the asymmetric unit, together with 10 molecules of water. Therefore, the structure of panaefluoroline D was determined as shown in **1**, including the relative configuration.

Panaefluoroline E (**2**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{25}$ +197.4° (*c* 0.27, MeOH), and had the molecular formula $C_{21}H_{27}NO_4$, on the basis of the positive HRFABMS data, *m/z* 358.2021 $[M + H]^+$ (calcd for $C_{21}H_{28}NO_4$, 358.2018). The ^1H and ^{13}C NMR spectra (Table 1) were similar to those of **1**, except for the signals of the side chain moiety on nitrogen. This moiety of **2** exhibited methyl signals at δ 1.02 and 1.08 (each 3H, d, J = 6.6 Hz), which showed HMQC correlations with the methyl carbons at δ 22.5 and 23.0, respectively. The methylene protons, δ 2.21 (1H, m) and 2.40 (1H, m), showed HMQC correlations with the methylene carbon at δ 43.2, and the two methine protons at δ 1.70 (1H, m) and 5.38 (1H, dd, J = 8.4, 6.5 Hz) showed HMQC correlations with the methine carbons at δ 26.5 and 70.4, respectively. The methine proton at δ 5.38 showed HMBC correlations with the carbonyl carbon at δ 172.4 (C-1′) and the methine carbon at δ 145.1 (C-9). The methyl protons at δ 1.02 and 1.08 showed HMBC correlations with the methyl carbons at δ 22.5 and 23.0, respectively, as well as the methine carbon at δ 26.5 and the methylene carbon at δ 43.2. The methylene protons at δ 2.21 and 2.40 showed HMBC correlations with the carbonyl carbon at δ 172.4 (C-1′), the methine carbon at δ 26.5, and the methyl carbons at δ 23.0 and 22.5. In the DQF-COSY spectrum, the methyl protons at δ 1.02 and 1.08 were coupled with the methine proton at δ 1.70 (m), and the methine proton at δ 1.70 was coupled with the methylene protons at δ 2.21 and 2.40. The proposed structure of **2** was also supported by the phase-sensitive NOESY correlations, as in the case of **1**. Thus, the structure of panaefluoroline E was established as **2**.

Panaefluoroline F (**3**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{22}$ +228.9° (*c* 0.28, MeOH), and was assigned the molecular formula $C_{20}H_{25}NO_4$, on the basis of the positive HRFABMS data, *m/z* 344.1864 $[M + H]^+$ (calcd for $C_{20}H_{26}NO_4$, 344.1862). The ^1H and ^{13}C NMR spectra (Table 1) of compound **3** were similar to those of **2**, except that **3** lacked the signals of one methylene carbon and two protons in the nitrogen side chain moiety, compared with **2**. This moiety showed signals due to two methyl groups at δ 0.87 (3H, d, J = 6.6 Hz) and 1.30 (3H, d, J = 6.6 Hz), which exhibited HMQC correlations with the resonance at δ 19.2 and 20.2, and two methine resonances at δ 2.69 (1H, m) and 4.97 (1H, d, J = 10.2 Hz) that showed HMQC correlations with the resonances at δ 34.5 and 77.6 and the quaternary carbon at δ 171.8. These data suggest that the structure of the side chain is $(CH_3)_2$−CH−CH−COO^-. The structure of the side chain was supported by the following HMBC correlations: H-2′with C-1′ and C-5′ (CH_3); H-3′, H-4′, and H-5′ with C-2′. The NOESY correlations further supported the proposed structure. Moreover, H-2′ showed an HMBC correlation with the quaternary C-7 on the isoquinoline skeleton, and H-9 showed a similar correlation with C-2′. Therefore, the structure of panaefluoroline F was determined as shown in **3**.

Panaefluoroline G (**4**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{22}$ −110.3° (*c* 0.31, MeOH). The molecular formula $C_{24}H_{25}NO_5$ was suggested by the positive HRFABMS data, *m/z* 408.1816 $[M + H]^+$ (calcd for $C_{24}H_{26}NO_5$, 408.1811). The ^1H and ^{13}C NMR spectra of **4** (Table 1) were similar to those of **1**, **2**, and **3**, except for the signals due to the nitrogen side chain moiety. The side chain protons at δ 3.56 (1H, dd, J = 14.8, 10.0 Hz) and 3.75 (1H, dd, J = 14.8, 4.5 Hz) showed HMQC correlations with the methylene carbon at δ 39.8. The methine protons at δ 5.65 (1H, dd, J = 10.0, 4.5 Hz), 6.55 (1H, d, J = 8.4 Hz), and 6.90 (1H, d, J = 8.4 Hz) showed HMQC correlations with the methine carbons at δ 72.5, 116.7, and 131.1, respectively. The methine protons at δ 6.55 and 6.90, the two methine carbons at δ 116.7 and 131.1, and the quaternary carbons at δ 127.7 and 158.3 suggested a disubstituted phenolic moiety. The methine proton at δ 6.55 showed HMBC correlations with the quaternary carbons at δ 127.7 and 158.3, and the methine proton at δ 6.90 showed an HMBC correlation with the methylene carbon at δ 39.8. The methylene protons at δ 3.56 and 3.75 showed HMBC correlations with the methine carbon at δ 131.1 and the quaternary carbon at δ 127.7. Moreover, the methine proton at δ 5.65 showed HMBC correlations with the methine carbon at δ 146.6 (C-9), the quaternary carbon at δ 144.3 (C-7), the carbonyl carbon at δ 172.1 (C-1′), the methylene carbon at δ 39.8 (C-3′), and the quaternary

Table 1. ^{13}C and ^{1}H NMR Data for **1**−**5** in CD_3OD

	1		**2**		**3**		**4**		**5**	
position	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
2	94.0	5.03 (t, 9.0)	94.1	5.08 (t, 9.1)	94.1	5.07 (t, 9.0)	94.0	5.04 (t, 9.0)	94.1	4.96 (t, 9.0)
3	31.6	3.46 (d, 9.0)	31.6	3.48 (d, 9.1)	31.6	3.48 (d, 9.0)	31.7	3.46 (d, 9.0)	31.7	3.45 (d, 9.0)
3a	127.8		128.1		128.2		127.9		128.0	
4	136.1	7.98 (d, 8.1)	136.2	8.00 (d, 8.1)	136.4	8.01 (d, 8.3)	136.3	7.97 (d, 8.1)	136.5	8.00 (d, 8.1)
5	118.7	7.53 (d, 8.1)	118.8	7.56 (d, 8.1)	118.7	7.56 (d, 8.3)	118.7	7.49 (d, 8.1)	118.9	7.56 (d, 8.1)
5a	138.2		138.1		138.0		138.6		138.5	
6	126.3	8.12 (s)	127.0	8.17 (s)	126.7	8.16 (s)	126.3	8.00 (s)	126.8	8.15 (s)
7	144.6		144.2		144.2		144.3		145.2	
9	146.6	9.82 (s)	145.1	9.59 (s)	145.3	9.85 (s)	146.6	9.51 (s)	145.2	9.59 (s)
9a	114.6		114.8		114.8		114.3		114.9	
9b	159.5		159.4		159.5		159.8		159.5	
10	20.2	2.81 (s)	20.5	2.86 (s)	20.6	2.88 (s)	20.1	2.58 (s)	20.3	2.92 (s)
11	72.4		72.5		72.4		72.5		72.6	
12	25.0	1.26 (s)	24.7	1.27 (s)	24.9	1.27 (s)	25.1	1.36 (s)	24.7	1.24 (s)
13	25.6	1.38 (s)	25.8	1.41 (s)	25.9	1.43 (s)	25.2	1.30 (s)	25.7	1.36 (s)
1′	170.0		172.4		171.8		172.1		171.4	
2′	73.0	5.53 (dd, 7.4, 3.5)	70.4	5.38 (dd, 8.4, 6.5)	77.6	4.97 (d, 10.2)	72.5	5.65 (dd, 10.0, 4.5)	67.7	5.85 (dd, 10.7, 4.0)
3′	63.6	4.37 (dd, 12.6, 3.5)	43.2	2.21 (m)	34.5	2.69 (m)	39.8	3.56 (dd, 14.8, 10.0)	39.8	3.45 (dd, 10.7, 7.1)
		4.47 (dd, 12.6, 7.4)		2.40 (m)				3.75 (dd, 14.8, 4.5)		3.57 (dd, 7.1, 4.0)
4′			26.5	1.70 (m)	19.2	0.87 (d, 6.6)	127.7		173.7	
5′			22.5	1.02 (d, 6.6)	20.2	1.30 (d, 6.6)	131.1	6.90 (d, 8.4)		
6′			23.0	1.08 (d, 6.6)			116.7	6.55 (d, 8.4)		
7′							158.3			
8′							116.7	6.55 (d, 8.4)		
9′							131.1	6.90 (d, 8.4)		

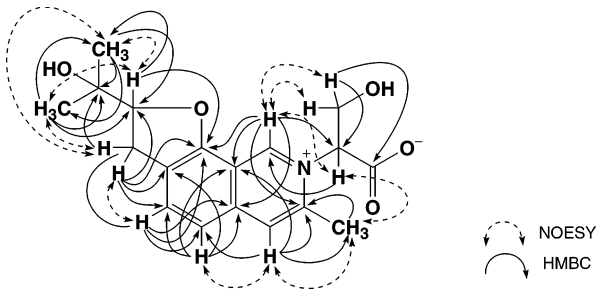

Figure 1. HMBC and phase-sensitive NOESY correlations for **1**.

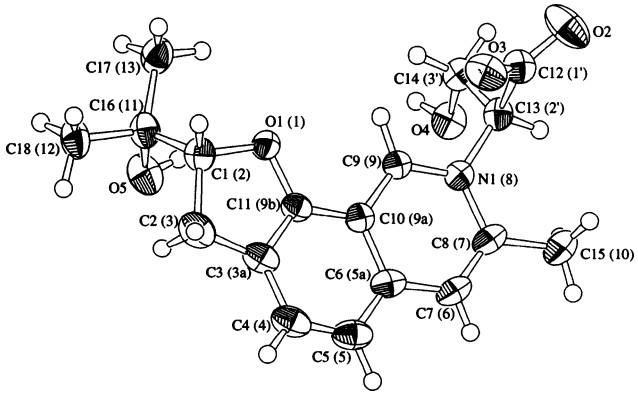

Figure 2. ORTEP drawing of one of the three independent molecules in the crystal unit of **1**. (Numbers in parentheses are position numbers.)

carbon at δ 127.7 (C-4′). Thus, the structure of panaefluoroline G was established as shown in **4**.

Panaefluoroline H (**5**) was obtained as a yellowish green amorphous solid, $[\alpha]_D^{25}$ +132.7° (*c* 0.22, MeOH), and was assigned the molecular formula $C_{19}H_{22}N_2O_5$, on the basis of the HRFABMS data, *m/z* 359.1617 $[M + H]^+$ (calcd for $C_{19}H_{23}N_2O_5$, 359.1607). The IR spectrum exhibited hydroxyl (3440 cm^{-1}) and carboxyl (1635, 1645 cm^{-1}) absorptions. The ^{1}H and ^{13}C NMR spectra (Table 1) were similar to those of **1**−**4**, except for the signals due to the nitrogen side chain moiety. The ^{1}H and ^{13}C NMR data and the molecular

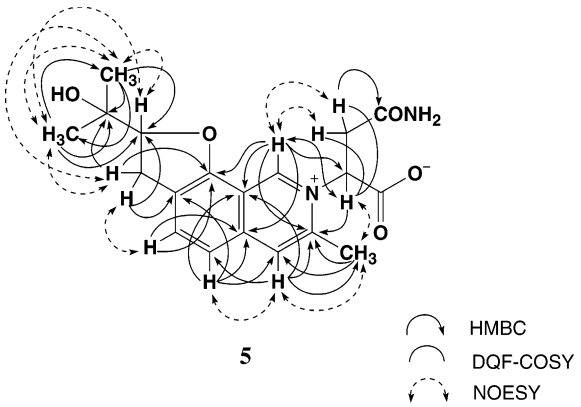

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Figure 3. HMBC, DQF-COSY, and phase-sensitive NOESY correlations for **5**.

formula indicated the structure $NH_2CO-CH_2-CH-COO^-$ for the side chain. This was supported by the DQF-COSY data (Figure 3): the methylene protons at δ 3.45 and 3.57 were coupled with the methine proton at δ 5.85 (1H, dd, *J* = 10.7, 4.0 Hz). The proton at δ 5.85 (H-2′) showed an HMBC correlation with the carbon at δ 145.2 (C-9), and H-9 at δ 9.59 (s) was correlated with C-2′ (δ 67.7). The proposed structure of **5** was supported by the HMBC and phase-sensitive NOESY data (Figure 3). Thus, the structure of panaefluoroline H was established as shown in **5**.

The isolated compounds (**1**−**5**) all possess a furo[2,3-*h*]-isoquinoline skeleton, which may be biosynthesized via the well-known polyketide route. We speculate that the nitrogen atom of the skeleton may be derived from serine, valine, tyrosine, leucine, and asparagine, respectively. Panaefluorolines A−C, reported in 2003,[14] have the same skeleton, and the nitrogen atom of these compounds may be derived from threonine, glycine, and alanine, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto MP micromelting point apparatus. UV spectra were recorded on a Shimadzu UV-240

spectrophotometer. The IR spectra were measured with a JASCO A-102 IR spectrophotometer or a JASCO IR Report-100 infrared spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM-AL-400 (^{1}H 400 and ^{13}C 100 MHz) and JEOL JNM-LA500 (^{1}H 500 and ^{13}C 125 MHz) spectrometers, using a CD_3OD solution with TMS as the internal standard. The $[\alpha]_D$ values were determined with a JASCO DIP-370 digital polarimeter. The MS spectra were obtained using a JEOL JMS-700. Column chromatography was carried out on 70−230 mesh silica gel (Merck). HPLC was performed with a JASCO PU 980 unit and a JASCO UV 970 (Gulliver) detector.

Materials and Culture. *Amygdalaria panaeola* thalli (collection no. f-181) were collected in July 1990 in Prov. Koillismaa, Kuusamo, Finland, by one of the authors (I.Y.). One week after collection, a piece was cut from the thallus. According to the Yamamoto method,[16] thallus fragments were homogenized in a mortar with sterilized H_2O, and small segments of 150−500 μm in size were selected by using a two-filter system. Each segment was inoculated onto an agar plate containing 5 mL of malt-yeast extract (MY) medium[17] and cultured at 15 °C in the dark. After 6 months, the mycelia derived from the small segment had grown to a colony that excreted fluorescent yellowish green material into the medium. An agar block bearing a mycobiont colony was cut out and transferred to fresh MY medium (5 mL) in a 60 mm diameter Petri dish. The mycobiont (strain no. 0049M) was grown in the dark at 15 °C and subcultured every 4 months in the culture collection of Akita Prefectural University.

The cell aggregates (ca. 1 g) of this mycobiont subcultured on the agar plate were homogenized in a mortar, suspended in the liquid MY medium (100 mL) in a 300 mL Erlenmeyer flask, and then cultured on a rotary shaker (120 rpm) at 20 °C in the dark for 4 weeks. After incubation, the liquid culture showed a fluorescent yellowish green color.

Extraction and Isolation. The medium was filtered, and the filtrate (1.36 L) was lyophilized and extracted with MeOH three times. The MeOH extract (10.83 g) was applied to a Diaion HP20 column, and the fluorescent compounds were eluted with MeOH. The MeOH eluate (1.23 g) was chromatographed on an ODS silica gel (Senshu Kagaku, 100 μm, Pegasil PREP ODS 7515-12A) column with an H_2O and 50−100% MeOH solvent system to yield crude fluorescent yellowish green pigments, fractions A−G. Fraction C was chromatographed on a silica gel column (Merck silica gel 230−400 mesh) with a gradient solvent system of $CHCl_3$−MeOH−H_2O (30/10/0.8, 30/12/1, 30/15/1, and 30/20/1) and purified by HPLC with $CHCl_3$−MeOH−H_2O (30/8/0.5) as the eluent (flow rate: 3.0 mL/min, detection at 365 nm) to yield compound **1** (20.6 mg). Fraction G was chromatographed on a silica gel column (Merck silica gel 230−400 mesh) with a $CHCl_3$−MeOH−H_2O (30/10/0.8) solvent system and purified by HPLC (Senshu Pak Pegasil silica 60-5 4251-N, 1 × 25 cm) with $CHCl_3$−MeOH−H_2O (30/6/0.3) as the eluent (flow rate: 3.0 mL/min, detection at 365 nm) to yield compound **2** (4.4 mg). Fraction F was chromatographed on a silica gel column (Merck silica gel 230−400 mesh) with a gradient solvent system of $CHCl_3$−MeOH−H_2O (30/7/0.5, 30/10/0.8, 30/12/1, 30/15/1, and 30/20/1) to give fractions F-1−F-7. Then, fraction F-3 was purified by HPLC (Senshu Pak Pegasil silica 60-5 4251-N, 1 × 25 cm) with $CHCl_3$−MeOH−H_2O (30/7/0.5) as the eluent (flow rate: 3.0 mL/min, detection at 365 nm) to yield compounds **3** (5.5 mg) and **4** (4.7 mg). Fraction B was chromatographed on a silica gel column (Merck silica gel 230−400 mesh) with a gradient solvent system of $CHCl_3$−MeOH−H_2O (30/10/0.8, 30/12/1, 30/15/1, and 30/20/1) to give fractions B-1−B-9. Then, fraction B-2 was purified by ODS-HPLC (Senshu Pak ODS-4251-N 60-5 4251N, 1 × 25 cm) with 30% MeOH as the eluent (flow rate: 1.0 mL/min, detection at 365 nm) to afford compound **5** (2.3 mg).

Panaefluoroline D (1): yellow crystals (from $CHCl_3$−MeOH−acetone); mp 152 °C (dec); $[\alpha]_D^{25}$ +286.6° (*c* 0.61, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.49), 258 (4.55), 304 (3.70), 315 (3.67), 412 (4.01); IR ν_{max} (KBr) cm^{-1} 3300, 1635, 1380, 1350, 1270, 1000; HRFABMS (pos) *m/z* 332.1510 [M + H]$^+$ (calcd for $C_{18}H_{22}NO_5$, 332.1498); FABMS (pos) *m/z* 332 ([M + H]$^+$); FABMS (neg) *m/z* 331 (M$^+$); *anal*. calcd for $C_{18}H_{21}NO_5 \cdot 1/3H_2O$, C, 55.23; H, 7.12; N, 3.58, found C, 55.21; H, 6.96; N, 3.34; ^{1}H and ^{13}C NMR, see Table 1.

X-ray Crystallographic Analysis of Panaefluoroline D (1). All measurements were made on a Rigaku AFC7S diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). Crystal data: Yellow prismatic crystal, monoclinic, $3C_{18}H_{21}NO_5 \cdot 10H_2O$ (M_r = 1174.25), space group $P2_1$ with a = 13.755(2) Å, b = 22.046(4) Å, c = 10.356(2) Å, β = 106.58(1)°, V = 3009.7(9) Å^3, Z = 2, and D_{calcd} = 1.296 g/cm^3. The structure was solved by direct methods (SIR97[18]) and expanded using Fourier techniques (DIRDIF94[19]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in calculated positions, except for those on the lattice water molecules, which were not refined. The final cycle of full-matrix least-squares refinement was based on 5621 unique reflections (2θ < 136.02°, R_{int} = 0.023) and 738 variable parameters and converged with unweighted and weighted agreement factors of R = 0.106, R_w = 0.204, and R_1 =0.064 for $I > 2.0\sigma(I)$ data. CCDC 236311 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Panaefluoroline E (2): yellowish green amorphous solid; $[\alpha]_D^{25}$ +197.4° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.20), 260 (4.03), 304 (3.26), 317 (3.16), 413 (3.42); IR ν_{max} (KBr) cm^{-1} 3400, 1650, 1380, 1350; HRFABMS (pos) *m/z* 358.2021 (calcd for $C_{21}H_{28}NO_4$, 358.2018); FABMS (pos) *m/z* (rel int) 358 ([M + H]$^+$), 244; FABMS (neg) *m/z* 357 (M$^-$); ^{1}H and ^{13}C NMR, see Table 1.

Panaefluoroline F (3): yellowish green amorphous solid; $[\alpha]_D^{25}$ +228.9° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.21), 248 (sh, 3.97), 263 (4.04), 305 (sh, 3.35), 317 (sh, 3.22), 415 (3.45); IR ν_{max} (KBr) cm^{-1} 3450, 1655, 1630, 1570, 1445, 1360, 1280, 1170, 1110, 1010; HRFABMS (pos) *m/z* 344.1864 (calcd 344.1862, $C_{20}H_{26}NO_4$, [M + H]$^+$); FABMS (pos) *m/z* 344 ([M + H]$^+$), 244; FABMS (neg) *m/z* 343 (M$^-$); ^{1}H and ^{13}C NMR, see Table 1.

Panaefluoroline G (4): yellowish green amorphous solid; $[\alpha]_D^{24}$ −110.3° (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.16), 258 (4.10), 313 (3.33), 414 (3.47); IR ν_{max} (KBr) cm^{-1} 3410, 1637, 1575, 1520, 1445, 1350, 1280, 1240, 1170, 1130; HRFABMS (pos) *m/z* 408.1816 [M + H]$^+$ (calcd for $C_{24}H_{26}NO_5$, 408.1811); FABMS (pos) *m/z* 408 ([M + H]$^+$), 316, 244; FABMS (neg) *m/z* 407 (M$^-$); ^{1}H and ^{13}C NMR, see Table 1.

Panaefluoroline H (5): yellowish green amorphous solid; $[\alpha]_D^{25}$ +132.7° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.27), 258 (4.26), 304 (sh, 3.35), 315 (sh, 3.30), 415 (3.62); IR ν_{max} (KBr) cm^{-1} 3440, 1675, 1650, 1645, 1635, 1445, 1360, 1280; HRFABMS (pos) *m/z* 359.1617 (calcd for $C_{19}H_{23}N_2O_5$, 359.1607); FABMS (pos) *m/z* 359 ([M + H]$^+$), 244; FABMS (neg) *m/z* 358 (M$^-$); ^{1}H and ^{13}C NMR, see Table 1.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Culberson, C. F. *Chemical and Botanical Guide to Lichen Products*; The University of North Carolina Press: Chapel-Hill, 1969.
(2) Kinoshita, K.; Matubara, H.; Koyama, K.; Takahashi, K.; Yoshimura, I.; Yamamoto, Y. *Bibl. Lichenol.* **1993**, *53*, 129−135.
(3) Kinoshita, K.; Matubara, H.; Koyama, K.; Takahashi, K.; Yoshimura, I.; Yamamoto, Y.; Miura, Y.; Kinoshita, Y.; Kawai, K. *J. Hattori Bot. Lab*. **1994**, *75*, 359−364.
(4) Kinoshita, K.; Matubara, H.; Koyama, K.; Takahashi, K.; Yoshimura, I.; Yamamoto, Y.; Miura, Y.; Kinoshita, Y.; Kawai, K. *J. Hattori Bot. Lab*. **1994**, *76*, 227−233.

(5) Matubara H.; Kinoshita K.; Koyama K.; Takahashi K.; Yoshimura I.; Yamamoto Y.; Kawai K. *Phytochemistry* **1994**, *37*, 1209−1210.
(6) Kinoshita, K.; Takatori, K.; Narui, T.; Culberson, C. F.; Hasumi, M.; Koyama, K.; Takahashi, K. *Heterocycles* **2004**, *63*, 1023−1026.
(7) Yamamoto, Y.; Matsubara, H.; Kinoshita, Y.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Ahmadjian, V.; Kurokawa, T.; Yoshimura, I. *Phytochemistry* **1996**, *43*, 1239−1242.
(8) Tanahashi, T.; Kuroishi, M.; Kuwahara, A.; Nagakura, N.; Hamada, N. *Chem. Pharm. Bull.* **1997**, *45*, 1183−1185.
(9) Miyagawa, H.; Hamada, N.; Ueno, T. *Phytochemistry* **1993**, *34*, 589−591.
(10) Miyagawa, H.; Hamada, N.; Sato, M.; Ueno, T. *Phytochemistry* **1994**, *36*, 1319−1322.
(11) Miyagawa, H.; Yamashita, M.; Hamada, N. *Phytochemistry* **1997**, *46,* 1289−1291.
(12) Kon, Y.; Kashiwadani, H.; Wardlaw, J. H.; Elix, J. A. *Symbiosis* **1997**, *23*, 97−106.
(13) Yamamoto, Y.; Kinoshita, Y.; Thor, G. R.; Hasumi, M.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Yoshimura, I. *Phytochemistry* **2002**, *60*, 741−745.
(14) Kinoshita, K.; Yamamoto, K.; Koyama, K.; Takahashi, K.; Yoshimura, I. *Tetrahedron Lett*. **2003**, *44*, 8009−8011
(15) Kohno, J.; Hiramatsu, H.; Nishio, M.; Sakurai, M.; Okuda, T.; Komatsubara, S. *Tetrahedron* **1999**, *55*, 11247−11252.
(16) Yamamoto, Y.; Mizuguchi, M.; Yamada, Y. *Agric. Biol. Chem.* **1985**, *49*, 3347−3348.
(17) Ahmadjian, V. *Bryologist* **1961**, *64*, 168−179.
(18) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(19) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Gelder, R.; Israel R.; Smits, J. M. M. *The DIRDIF-94 program system*, Technical Report of the Crystallography Laboratory; University of Nijmegen: The Netherlands, 1994.

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